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Multinucleate giant cells in sublabial salivary gland tissue in Sjögren's Syndrome

A diagnostic pitfall

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Summary. The presence of multinucleate giant cells in the sublabial salivary gland tissue in Sjögren's syndrome is an unusual phenomenon which can give rise to differential diagnostic problems. We found in 4 cases of 55 patients with Sjögren's syndrome multinucleate giant cells. In 2 of these 4 patients epimyoepithelial islands were also present. The combination of both multinucleate giant cells as epimyoepithelial islands can mimic the histological picture of a non-caseating granulomatous disease.

To discriminate between an epimyoepithelial island and an epithelioid granuloma the immunoperoxidase technique with antibodies directed against muramidase appeared an useful tool. The epithelioid cells contain muramidase whereas the cells in the epimyoepithelial island do not contain this enzyme.

Thus, multinucleate giant cells are a rare phenomenon in Sjögren's syndrome, therefore restricting its diagnostic significance. When they occur in Sjögren's syndrome staining for muramidase can be of help to avoid a false positive diagnosis of diseases in which non-caseating granulomatous inflammation occur, such as in sarcoidosis.

Key words: Multinucleate giant cells – Sjögren's syndrome – Epimyoepithelial islands – Sarcoidosis – Immunoperoxidase technique – Muramidase

Introduction

The term sicca syndrome comprises the clinical picture of keratoconjunctivitis sicca (KCS) and xerostomia independent of underlying disease. Several clinicopathological entities such as Sjögren's syndrome, sarcoidosis, amyloidosis, haemochromatosis and lipomatosis can give origin to sicca syndrome (Hené et al. 1979).

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The term Sjögren's syndrome (SS) is used to describe a chronic autoimmune disease characterized by lymphocytic infiltration and destruction of salivary and lacrimal gland tissue resulting in a sicca syndrome (Fischbach et al. 1980). Moutsopoulos et al. (1980) introduced the classification of primary and secondary SS. Primary SS is definied as SS without other concomitant disease, while the term secondary SS is used in association of SS with a connective tissue disease. An enumeration of the concomitant connective tissue diseases can be find in the literature (Fischbach et al. 1980; Moutsopoulos et al. 1980; Manthorpe et al. 1981). In literature the term sicca syndrome is often considered synonymous with primary SS. We have suggested to reserve the term sicca syndrome to describe the clinical picture (Hené et al. 1983).

Chisholm and Mason (1968) described the significance of the sublabial salivary gland biopsy as a valuable aid in the diagnosis of SS. Other investigations have confirmed these findings and detailed descriptions of the histopathological alterations in the sublabial salivary glands can be found in literature (Davies et al. 1973, Greenspan et al. 1974; Tarpley et al. 1974; Daniels et al. 1975; Friedman et al. 1979; Chomette et al. 1981).

The presence of multinucleate giant cells (MNGC) in sublabial salivary glands in SS may form a diagnostic pitfall. This may distract the unawary observer from considering the possibility of SS, by suggesting a diagnosis of sarcoidosis, cheilitis granulomatosa or even tuberculosis. Indeed we have been faced with this problem, in which a patient had been unnecessary subjected to extensive investigative procedures.

This diagnostic error may also be due to the scarcity of reports on MNGC in salivary gland tissue in SS. For example, apart from Akin et al. (1975) none of the above cited detailed histopathological descriptions mentioned this phenomenon.

Therefore we studied the occurrence of MNGC in the sublabial salivary gland biopsies from patients with SS. In addition the usefulness of muramidase (=lysozym) presence was investigated as a discriminator between epithelioid granulomas and epimyoepithelial islands to prevent confusion of a non-caseating granulomatous disease and SS.

Material and methods

Patients. Our study included 291 sublabial salivary gland biopsies performed for diagnostic purposes from patients suffering from sicca syndrome. Confirming to the criteria formulated in recent literature we selected 55 patients with a primary or secondary SS (Fischbach et al. 1980; Fox et al. 1982; Shillitoe et al. 1982). Shortly these criteria are:

1. A local lymphocytic sialadenitis of grade 4 according to Chisholm and Mason (1968), which implies a focusscoring greater than 1 focus per 4 mm^2. A focus is defined as an aggregate of 50 or more lymphocytes and histiocytes, usually with a few plasma cells placed peripherally (Waterhouse and Doniach 1966).

2. The presence of KCS by a complete ophthalmological evaluation, that includes the slitlamp biomicroscopic appearance after staining with both Rose-Bengal and fluorescein of the cornea and conjunctivae, a decreased tear make up time, reduced tear production as measured by the Schirmer test (Manthorpe et al. 1981). In evaluation of KCS we always use the lysozyme activity test; mostly the Schirmer test was also employed (Van Bijsterveld 1969).

Sublabial salivary gland tissue of 4 patients showing the characteristic radiographic features of sarcoidosis (bilateral hilar lymphadenopathy) were included for sake of comparison.

Sublabial biopsies obtained from 75 patients that underwent intra-oral surgery for non-related diseases served as a control group.

Tissue processing. The sublabial salivary glands of the control group and a part of the patient group were fixed in a formol-sublimate solution according to Bosman et al. (1977). The remainder of the biopsies were fixed in 4% formol. In case of formol-sublimate fixation the mercury pigment was removed before embedding in paraffin by immersion in Lugol's iodine. Sections of 5 μm were stained by hematoxylin-eosin.

Focusscoring. The focusscoring was performed on 5 μm HE sections. The area of the sublabial salivary gland tissue was measured with a point-counting method using a calibrated coherent 100 points double test grid (Weibel 1979). The measurements were performed with a drawing tube at a magnification of 40×.

Immunohistochemistry. Muramidase (=lysozym) was demonstrated by the unlabelled peroxidase-antiperoxidase (PAP) procedure (Mason and Taylor 1975). The PAP-procedure with antibodies against muramidase can be applied on 4% formol fixed as well as on formol-sublimate fixed tissue. The sera were used at the following dilutions: rabbit anti-muramidase 1:50, swine anti-rabbit serum 1:50 and PAP 1:40. Controls were performed by substituting the primary antibody by phosphate buffered saline (PBS). All antisera were obtained from DAKO, Denmark.

Results

Light microscopy. The histopathological findings are summarized in Table 1. MNGC are found in the sublabial salivary glands in 4 out of the 55 patients with SS. The salivary glands tissue of these 4 patients exhibits an extensive

Table 1. A summary of the histopathological features

Patient	Sex	Age (years)	I	II	III	IV	V	VI	VII	VIII	IX	X	Diagnosis
1	F	40	23	18.5	5	+	+	−	+	+	+	−	primary SS
2	F	24	31	24.3	5.1	+	+	−	−	+	+	−	primary SS
3	F	37	9	5.3	6.7	+	+	−	−	−	+	−	primary SS
4	M	34	13	15.0	3.5	+	+	−	+	−	+	−	secondary SS[a]
5	M	24	1	2.6	1.5	+	+	+	+	−	+	+	sarcoidosis
6	F	57	0	5.0	0	+	+	−	+	−	−	+	sarcoidosis
7	F	23	1	6.1	0.7	+	+	−	−	−	+	+	sarcoidosis
8	M	25	0	16.3	0	+	+	−	+	−	+	+	sarcoidosis

I = number of lymphocytic foci
II = area of sublabial salivary gland tissue in mm^2
III = number of lymphocytic foci per 4 mm^2
IV = + presence of diffuse lympho- and plasmacytic infiltrate
V = + atrophy of acinar parenchym
VI = + presence of intralobular fibrosis
VII = + presence of ductectasia
VIII = + presence of epimyoepithelial islands
IX = + presence of MNGC
X = + presence of epithelioid granulomas

[a] This patient is also suffering from polymyositis and diabetes insipidus due to interstitial nephritis

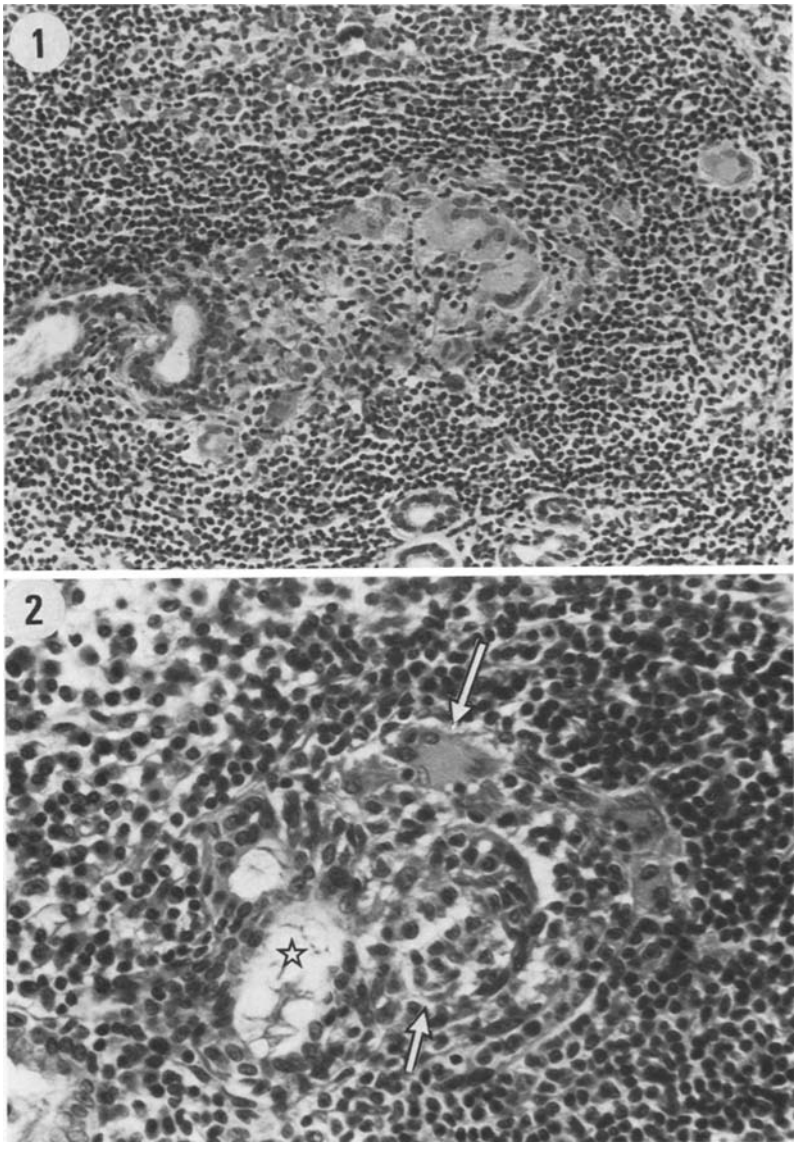

Fig. 1. A large lymphocytic focus containing 2 MNGC and 1 MNGC in the periphery of the focus. (Haematoxylin-eosin; ×96)

Fig. 2. A duct (*asterisk in lumen*) with proliferating ductal cells resulting in a duct related epimyoepithelial island (*short arrow*) with a MNGC of Langhans type (*long arrow*). (Haematoxylin-eosin; ×210)

loss of functional parenchym, which is replaced by large lymphocytic infiltrates. The MNGC are found invariably in the immediate vicinity of the lymphocytic foci (Figs. 1 and 2). In 2 of these 4 patients epimyoepithelial islands, first described by Morgan and Castleman (1953), were also present in the sublabial glands. MNGC are juxtaposed to some of these islands

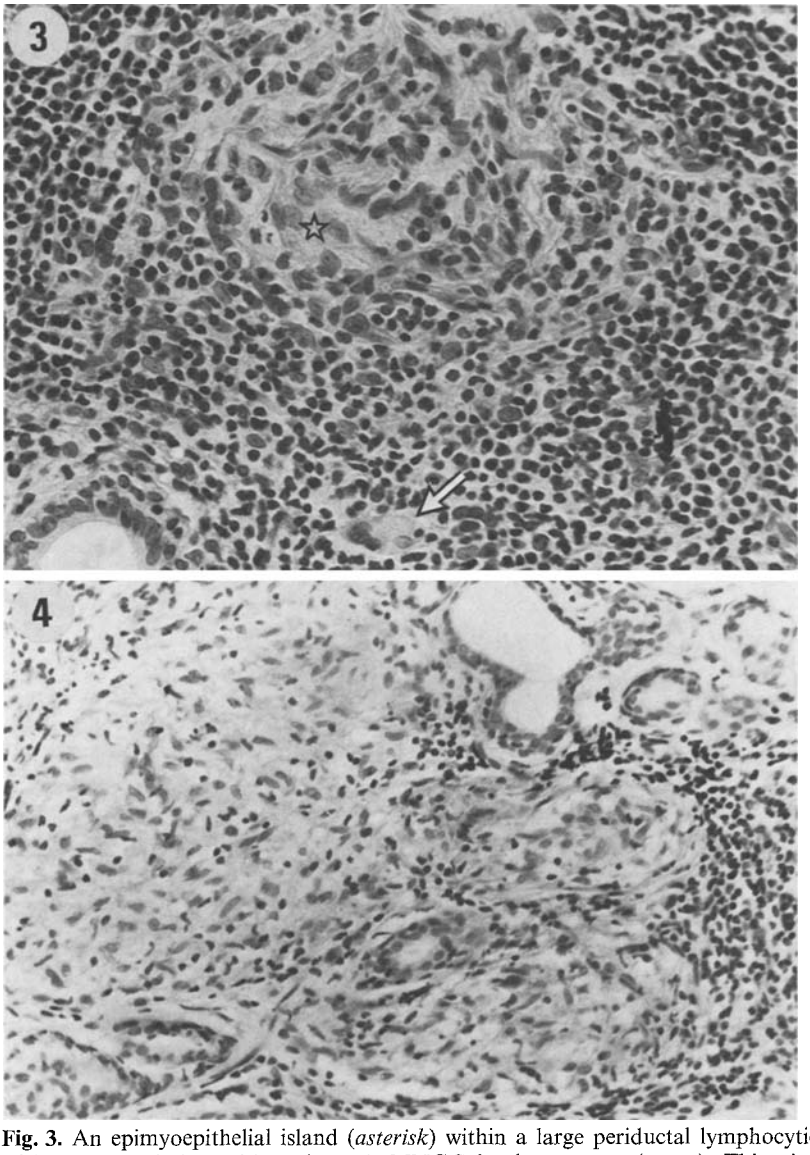

Fig. 3. An epimyoepithelial island (*asterisk*) within a large periductal lymphocytic focus and without any relation with a duct. A MNGC is also present (*arrow*). This picture mimics an epithelioid granuloma. (Haematoxylin-eosin; ×210)

Fig. 4. An epithelioid granuloma in sublabial salivary gland tissue of a patient with sarcoidosis. Lymphocytic rimming does not surround the entire granuloma as is seen in Fig. 2–3. (Haematoxylin-eosin; ×96)

(Figs. 2 and 3). The epimyoepithelial islands are always situated in the larger lymphocytic foci. These epimyoepithelial islands can resemble the non-caseating epithelioid granuloma as are seen in some granulomatous diseases (Fig. 3). Some of the epimyoepithelial islands are associated with ducts (Fig. 2), but in other cases no relation with ducts is present (Fig. 3). In

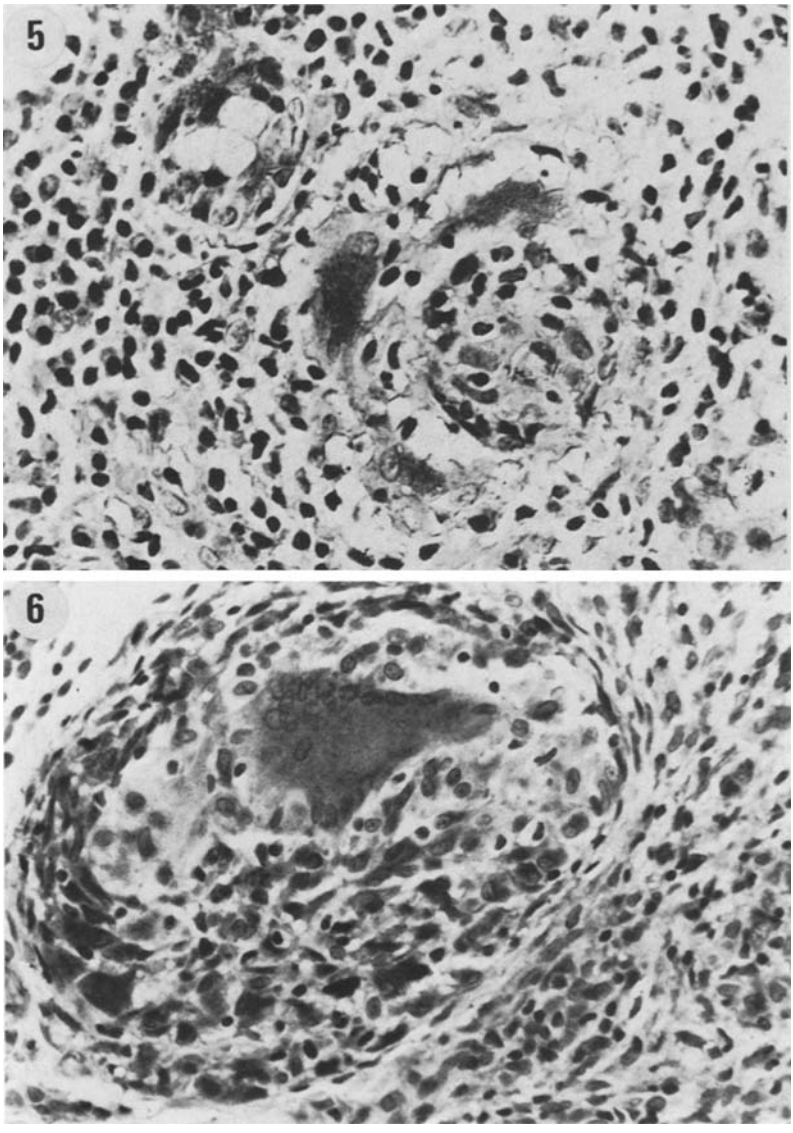

Fig. 5. A muramidase negative epimyoepithelial island surrounded by 3 muramidase positive MNGC. (Muramidase antiserum; PAP technique; DAB/haematoxylin; ×210)

Fig. 6. Muramidase positive MNGC within an epithelioid cell granuloma that is composed of muramidase positive epithelioid cells and histiocytic cells. (Muramidase antiserum; PAP technique; DAB/haematoxylin; ×210)

all the 4 patients there is a high focusscoring (Table 1). In none of these 4 patients there is any evidence for the existence of a systemic granulomatous disease.

In 3 of the 4 patients with sarcoidosis a local loss of acini is present in one or more sublabial salivary glands, while other glands of the same

Table 2. Most striking differences between SS and sarcoidosis

SS	Sarcoidosis
1. High focusscoring	1. Low focusscoring
2. Epimyoepithelial islands in large lymphocytic foci, with great density of lymphocytes	2. Epithelioid granuloma rimmed by small numbers of lymphocytes, sometimes the lymphocyte rimming is absent
3. Epimyoepithelial islands are muramidase negative, only a few muramidase positive cells may be present.	3. Epithelioid granuloma contains many muramidase positive epithelioid and histiocytic cells.

patient do not exhibit pathological alterations. This local atrophy is connected to a granulomatous inflammatory process (Fig. 4). In the 4th patient 2 out of the 4 sublabial salivary glands were completely replaced by noncaseating granulomatous tissue, one of these glands was strongly enlarged. The granulomas consist of epithelioid- and histiocytic cells mostly admixed with MNGC and they are often surrounded by a narrow rim of lymphocytes. In other granulomas there is a partly or complete lack of lymphocytic rimming (Fig. 4). In 2 out of the 4 patients with sarcoidosis exhibited an isolated lymphocytic focus in the preexisting gland tissue. The focusscoring of 1.5 in patient 6 (see Table 1) must be attributed to the small area of the sublabial salivary gland. Both in SS and in sarcoidosis the MNGC are of Langhans as well of foreign body type.

In the control group no MNGC are found in the sublabial salivary glands.

Immunohistochemistry. MNGC in SS as well as in sarcoidosis exhibit a positive reaction of variable intensity in the cytoplasm after staining for muramidase. (Figs. 5 and 6). In sarcoid granulomata the histiocytic and epithelioid cells are positive for muramidase whereas in SS only an occasional muramidase positive histiocytic cell is encountered (Fig. 6). The cells in the epimyoepithelial islands fail to reveal muramidase positivity (Fig. 5). Serous cells of the salivary gland tissue serve as positive controls for the muramidase-PAP-technique.
In Table 2 the most striking differences in histological and immunohistochemical respects are summarized.

Discussion

As has appeared from this study MNGC in SS is a rare phenomenon. Only in 4 out of 55 patients with SS we found MNGC in the sublabial salivary glands. MNGC do occur in granulomatous lesions in the oral cavity, the lips and salivary glands (Seifert 1980a and 1980b) but only one paper makes mention about MNGC in the sublabial salivary glands in SS (Akin et al. 1975). Bloch et al. (1965) at first described MNGC in the major salivary glands. The simultaneous occurrence of MNGC and epimyoepithelial islands in the sublabial salivary gland tissue was only observed in 2 of

55 patients with SS. The combination of these two rare phenomena in the accessory salivary glands was never described in literature.

MNGC in SS is not a finding by chance because no MNGC were found in our control group consisting of 75 patients and moreover, other authors (Chisholm and Mason 1968; Chisholm et al. 1970; Davies et al. 1973; Greenspan et al. 1974; Friedman et al. 1979) also do not make mention of this phenomenon in their control studies. Could the occurrence of MNGC in the major salivary glands, by Bloch et al. (1965) interpreted as a foreign body reaction due to previous sialography also be an expression of the underlying process. The present findings of MNGC in the sublabial salivary gland tissue strongly suggest this second possibility. Muramidase positive MNGC in the sublabial salivary glands in SS is also an indication against a foreign body reaction, because most MNGC in a foreign body reaction are muramidase negative (Blennerhassett and Papadimitriou 1981).

On account of the low incidence the presence of MNGC in SS has no diagnostic significance. On the contrary MNGC in the patients with a sicca syndrome give rise to a diagnostic pitfall, certainly in combination with epimyoepithelial islands. The simultaneous occurrence of MNGC and fully developed epimyoepithelial islands can mimic the histopathological picture of a non caseous epithelioid granuloma as can be seen in some granulomatous disease, i.e. sarcoidosis.

A comparative study of the histopathological alterations in the sublabial salivary gland tissue in SS and sarcoidosis exhibited the differences between these diseases. In sarcoidosis we saw: marked acinar replacement, mostly local, by discrete granulomas of epithelioid cells; often the presence of MNGC in or near the granulomas; no central necrosis; a small diffuse lymphocytic infiltrate toward the periphery of the granulomas; a low focusscoring. These histological findings are in conformity with the observations in foregoing studies of sarcoidosis in the sublabial salivary glands (Chisholm et al. 1971; Tarpley et al. 1972; Tannenbaum et al. 1974; Nessan and Jacoway 1979). In the sublabial salivary glands of our patients with SS with MNGC and epimyoepithelial islands we found: extended acinar atrophy due to focal lymphocytic sialadenitis; a high focusscoring; MNGC within or around the lymphocytic foci; epimyoepithelial islands which were situated within the large lymphocytic foci.

Additional and more striking differences between SS and sarcoidosis were observed with the immunoperoxidase technique using antibodies directed against muramidase. In sarcoidosis many of the histiocytic- and epithelioid cells are muramidase positive, which is in accordance with the observations done by other investigators (Mason and Taylor 1975; Motoi et al. 1980; Blennerhassett and Papadimitriou 1981). On the contrary the cells in the epimyoepithelial islands are muramidase negative, with the exception of one island wherein a few muramidase positive histiocytic cells were present. This latter finding agrees with the ultrastructural observations of Donath and Seifert (1972) who found histiocytic cells and lymphocytes within the epimyoepithelial islands. Other electron microscopic studies did not make mention about histiocytic cells in these islands (Boquist et al. 1970; Kahn 1979).

In addition to the differential diagnostic aid the muramidase staining sheds some light on the origin of the MNGC and their low incidence in SS. It is likely that the muramidase positive MNGC in sarcoidosis as well as in SS originate from the muramidase synthetizing histiocytic cells by fusion (Murch et al. 1982). Therefore the low incidence of MNGC in SS and the scarcely distribution of muramidase positive histiocytic cells in the epimyoepithelial islands can be clarified by our observations that only low numbers of muramidase positive histiocytic cells were present in the inflammatory infiltrates in SS.

In summary, it is shown that in sublabial salivary gland tissue the differentiation between sarcoidosis and SS with MNGC and epimyoepithelial islands mostly can be made on routine histopathological sections but that in doubtfull cases, immunoperoxidase staining for muramidase may be extremely helpfull.

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