

Conjunctival Biopsy in Sarcoidosis

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Background: Conjunctival biopsy is considered to be a simple, safe and specific diagnostic procedure for sarcoidosis. This study was designed to determine the value of this procedure in Taiwan.

Methods: This study was conducted from December 2003 to April 2005 at the uveitis clinic of Taipei Veterans General Hospital. Blind sampling was conducted, obtaining a biopsy sample measuring 1 cm long by 3 mm wide from both lower fornices. A positive result was defined as the presence of non-caseating granuloma when other granuloma-forming processes had been excluded.

Results: Twenty-nine patients (7 men, 22 women) were enrolled. Mean age at diagnosis was 47.8 ± 12.4 years. The most common initial symptom was eye-related problems in 19 (65.5%) patients. Of 58 biopsies, 15 (25.9%) specimens in 11 (37.9%) patients proved to be positive. Four patients experienced bilateral involvement; 7 patients had unilateral involvement. No prominent conjunctival nodules or follicles were noted. Gender, age, presence of uveitis, initial symptoms, and chest condition comparisons revealed no association between positive and negative conjunctival biopsies.

Conclusion: Blind and bilateral conjunctival biopsy, due to its ease, safety and specificity, could be the first biopsy in patients with clinical or chest X-ray abnormalities suggesting sarcoidosis. None of our patients with positive biopsy had nodular lesions. [*J Chin Med Assoc* 2006;69(10):472-477]

Key Words: Chinese, conjunctiva, granuloma, sarcoidosis, uveitis

Introduction

Sarcoidosis is a multisystem disease of unknown origin, characterized by widespread, non-caseating epithelioid cell granuloma. The condition may affect the chest, skin, eyes, liver, spleen, lymph nodes, glands, heart, renal system, bones, joints, central nervous system and other organs or tissues. Diagnosis of sarcoidosis is based on histologic evidence together with compatible clinical findings.^{1,2} Clinicopathologic correlation is essential, and the presence of non-caseating granuloma with negative staining for acid fast organisms and fungi is suggestive of the diagnosis.

Transbronchial and mediastinal biopsy are the most common tissue sites tested for sarcoidosis. Other biopsy sites include cutaneous lesions, the liver, and extrapulmonary peripheral lymph nodes.³ Conjunctival biopsy was described in the 1950s, and is considered to be a simple, safe and specific diagnostic procedure

for sarcoidosis.⁴⁻⁷ Nonetheless, it is still considered controversial and is not a popular choice for biopsy. This study was designed to determine the value of random, bilateral conjunctival biopsy in the diagnosis of sarcoidosis.

Methods

Diagnosis of sarcoidosis was based on the presence of non-caseating granuloma on tissue biopsy along with compatible clinical findings. Other granuloma-forming processes, such as tuberculosis, fungal infection and various foreign bodies, were excluded.^{1,2}

This study was prospectively conducted from December 2003 to April 2005 in the uveitis clinic of Taipei Veterans General Hospital. All patients with peculiar uveitis features indicative of sarcoidosis⁸ or those with a definitive diagnosis of sarcoidosis who

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E-mail: ym_chang@vghtpe.gov.tw • Received: February 27, 2006 • Accepted: September 7, 2006

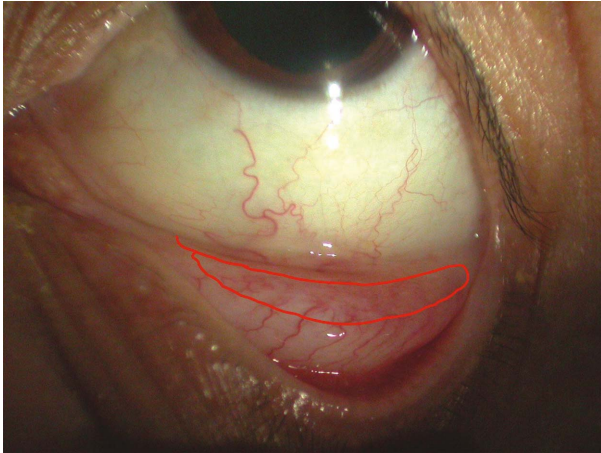


Figure 1. Conjunctival biopsy about 1 cm in length and 0.3 cm in width is taken from the inferior fornix, with no suturing.

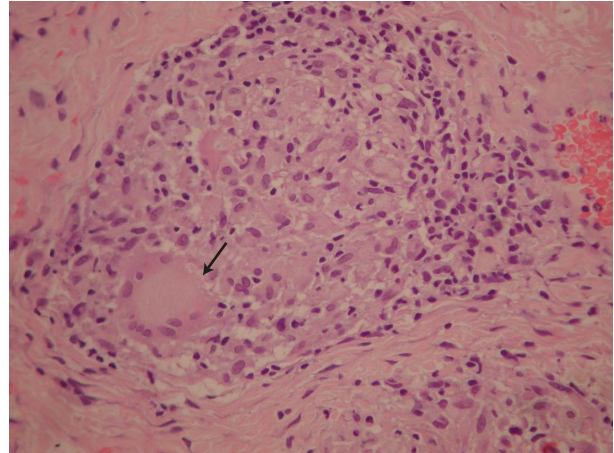


Figure 2. Conjunctival biopsy specimen shows non-caseating granulomas; the arrow indicates a multinucleate giant cell (hematoxylin & eosin, 200 \times).

were referred for evaluation of ocular involvement were suggested to receive conjunctival biopsy. Patients who were subsequently diagnosed with sarcoidosis as well as those with their diagnosis established before conjunctival biopsy were enrolled into this study.

The conjunctival biopsy was performed under subconjunctival anesthesia with 1–2 mL 2% lidocaine with 1:100,000 epinephrine. The lower eyelid was retracted, and a sample of conjunctiva measuring approximately 1 cm in length and 0.3 cm in width was excised from the inferior fornix with Westcott scissors (Figure 1). Hemostasis was achieved by applying pressure to the eye for 3 minutes. Prophylactic antibiotic ointment was then instilled into the lower fornix. All patients underwent bilateral biopsies.

The excised conjunctival tissue was fixed in 10% formalin and embedded in paraffin blocks. The embedded tissue was sectioned at 5 μ m at multiple levels, then stained with hematoxylin and eosin. A positive result was defined as the presence of non-caseating granuloma. To exclude other granuloma-forming processes, stains such as Kinyoun stain for acid-fast bacilli and Gomori methenamine silver and Periodic Acid–Schiff stain for fungi were performed (Figure 2).

The modified Scadding classification of lung involvement based on chest radiograph findings has been widely accepted. Stage 0 means no abnormality; Stage I, lymph node enlargement; Stage II, lymph node enlargement accompanied by pulmonary infiltration; Stage III, pulmonary infiltration with no lymph node enlargement; and Stage IV, evidence of pulmonary fibrosis with retraction of the hilar areas cephalad, cystic changes and bullae.¹

Data regarding age at diagnosis, gender, initial symptoms, tissue proof sites, disease stage by chest

X-ray and high-resolution computed tomography (CT), presence of uveitis, and conjunctival biopsy results were collected and analyzed. To assess the differences between patients with positive and negative biopsy results, categorical variables were analyzed using either the χ^2 test or Fisher's exact test. Goodman and Kruskal's Gamma test was used when ordinal variables were to be compared. Values of $p < 0.05$ were considered statistically significant.

Results

A total of 32 cases of sarcoidosis were examined, and 29 patients received conjunctival biopsy. Of these 29 patients, 22 had peculiar uveitis manifestations suggestive of sarcoidosis. Seven patients referred by pulmonologists for evaluation of ocular involvement of sarcoidosis were enrolled in this study (Table 1). All of the 29 patients (7 men, 22 women) were Chinese living in Taiwan. The mean age at diagnosis was 47.8 ± 12.4 years (range, 22–76 years). The most common initial symptom was eye-related problems in 19 (65.5%) cases. Ocular problems included blurred vision and floaters, and all patients were referred with the diagnosis of uveitis. Abnormal chest X-ray film on physical examination, respiratory problems and skin lesions were noted in 4 (13.8%), 3 (10.3%), and 2 (6.9%) patients, respectively. One patient presented with unexplained body weight loss.

The diagnosis of sarcoidosis was established before the time of conjunctival biopsy in 10 patients. The tissue proof sites were a mediastinal lymph node in 4 cases, lung parenchyma in 4, skin in 4, and supraclavical lymph node in 1 case. Conjunctival biopsy was

Table 1. Demographic and clinical characteristics of 29 patients with sarcoidosis who received conjunctival biopsy

No.	Age at diagnosis (yr)	Sex	Initial symptoms	Diagnosis of sarcoidosis before conjunctival biopsy	Tissue proof sites	Chest X-ray	Chest CT	Uveitis	Conjunctival biopsy	
									Right side	Left side
1	22	M	Eye	No	L	0	0	+	-	-
2	49	F	Eye	No	C	0	2	+	-	+
3	28	F	Chest tightness	No	MLN	1	1	+	-	-
4	50	F	Eye	No	C	0	1	+	+	-
5	56	F	Eye	Yes	MLN	1	1	+	-	-
6	50	F	Eye	No	MLN, C	3	2	+	+	-
7	42	M	Routine	No	MLN	1	1	-	-	-
8	64	F	Eye	No	L	3	3	+	-	-
9	40	F	Eye	No	MLN	1	1	+	-	-
10	32	M	Cough	Yes	C, L	3	2	-	-	+
11	23	F	Eye	No	MLN	1	2	+	-	-
12	55	F	Eye	Yes	C, L, S	3	2	+	+	+
13	29	M	Routine	Yes	L	0	1	-	-	-
14	51	F	Eye	No	C	0	2	+	+	-
15	50	F	Eye	No	C	0	0	+	+	+
16	53	F	Eye	No	C	0	1	+	+	-
17	55	F	Eye	No	L	0	3	+	-	-
18	40	M	Routine	Yes	MLN	1	1	-	-	-
19	44	F	Body weight loss	Yes	MLN, S, SLN	1	2	+	-	-
20	46	F	Cough	Yes	MLN	1	2	-	-	-
21	46	F	Eye	No	MLN	0	1	+	-	-
22	67	F	Eye	No	MLN	2	2	+	-	-
23	53	F	Skin	Yes	S	0	2	+	-	-
24	56	F	Eye	No	MLN	1	1	+	-	-
25	51	F	Eye	No	C	1	1	+	+	-
26	54	M	Eye	No	MLN, C	1	2	+	+	+
27	49	F	Skin	Yes	S, C	0	1	-	+	+
28	76	M	Eye	No	MLN	0	1	+	-	-
29	56	F	Routine	Yes	L	1	1	-	-	-

+ = positive; - = negative; C = conjunctiva; CT = computed tomography; L = lung parenchyma; MLN = mediastinal lymph node; S = skin; SLN = supraclavical lymph node.

positive in 3 of these 10 patients and 8 of the other 19 patients in whom sarcoidosis was suspected on the basis of clinical presentation. The conjunctiva was the sole site of tissue proof in 6 patients. These 6 were all female, and presented with typical features of sarcoid uveitis. In the 11 patients with negative conjunctival biopsy results, sarcoidosis was subsequently confirmed by biopsy of the mediastinal lymph node in 8 patients and lung parenchyma in 3. The chest conditions at the time of conjunctival biopsy were stage 0 in 12 patients, stage 1 in 12, stage 2 in 1, and stage 3 in the other 4 patients on chest X-ray examination. Chest CT revealed more advanced disease stage with stage 0 for 2 patients, stage 1 for 14 patients, stage 2 for 11 patients, and stage 3 for 2 patients. One particular case involved a 22-year-old man (case 1) referred with recurrent uveitis lasting

1 year. Ocular presentation with choroidal nodules and retinal vasculitis in both eyes strongly suggested sarcoidosis. Although chest CT and X-ray findings were negative, bronchoalveolar lavage revealed marked lymphocytosis with an increased ratio of CD4/CD8 and decreased peripheral blood. The patient received another bronchoscopic examination 3 months later, and non-caseating epithelioid granulomas were demonstrated on transbronchial lung biopsy.

Of 58 biopsy specimens from 29 patients, 15 (25.9%) specimens in 11 (37.9%) patients proved to be positive. Of the positive biopsy specimens, 4 (13.8%) patients had bilateral involvement; the other 7 (24.1%) had unilateral involvement.

Regarding the appearance of the conjunctiva prior to biopsy, prominent nodules only were found in

Table 2. Comparison of variables between positive ($n = 11$) and negative ($n = 18$) conjunctival biopsy results

	Biopsy result		<i>p</i>
	Positive, <i>n</i> (%)	Negative, <i>n</i> (%)	
Gender			0.678*
Male	2 (28.6)	5 (71.4)	
Female	9 (40.9)	13 (59.1)	
Age at diagnosis (yr)			0.334 [†]
21–30	0 (0)	4 (100)	
31–40	1 (33.3)	2 (66.7)	
41–50	5 (55.6)	4 (44.4)	
51–60	5 (50.0)	5 (50.0)	
> 60	0 (0)	3 (100)	
Uveitis			0.677*
With	9 (40.9)	13 (59.1)	
Without	2 (28.6)	5 (71.4)	
Initial symptoms			0.234*
Ocular	9 (47.4)	10 (52.6)	
Extraocular	2 (20.0)	8 (80.0)	
Disease stage by CXR			0.317 [†]
Stage 0	6 (50.0)	6 (50.0)	
Stage 1	2 (16.7)	10 (83.3)	
Stage 2	0 (0)	1 (100)	
Stage 3	3 (75.0)	1 (25.0)	
Disease stage by chest CT			0.373 [†]
Stage 0	1 (50.0)	1 (50.0)	
Stage 1	4 (28.6)	10 (71.4)	
Stage 2	6 (54.5)	5 (45.5)	
Stage 3	0 (0)	2 (100)	

*Fisher's exact test; [†]Goodman and Kruskal's Gamma test. CXR = chest X-ray; CT = computed tomography.

1 patient, but revealed a negative result. No prominent nodular or follicular changes were observed in the other 28 (96.6%) patients. Generally, a swollen conjunctiva with subtle fibrotic appearance revealed a high probability of positive results.

Comparisons of patient profiles, including gender, age at diagnosis by decade, presence of uveitis, initial symptoms, and chest conditions examined by chest X-ray and CT, revealed no significance between positive and negative conjunctival biopsy results (Table 2).

No biopsy-related complications or morbidity occurred in this study.

Discussion

Epidemiology studies indicate that sarcoidosis is rarely seen among Chinese.^{9,10} The most common ocular manifestation in sarcoidosis is uveitis.^{2,11} The pattern of uveitis in a given population is strongly influenced by

various genetic, geographic and environmental factors. Uveitis with sarcoidosis occurs in about 20% of uveitis patients in many geographic areas, including the United States, Japan and the cities of Amsterdam and Rotterdam.¹² In a large study conducted in China, sarcoidosis was absent in 1,752 uveitis patients.¹³ As noted in our previous report, uveitis with sarcoidosis is extremely rare, occurring in only 1 of 240 cases of uveitis from 1984 to 1986 in this clinic.¹⁴ However, there has been a notable increase in the number of patients with uveitis that consequently led to the diagnosis of sarcoidosis.¹⁵

Granulomas are usually widely distributed, so the biopsy site should be determined by risk assessment versus the likelihood of success. A study by Bornstein et al⁵ evaluated conjunctival biopsy in sarcoidosis in 3 patient groups. Group 1 consisted of 52 patients with biopsy-confirmed sarcoidosis in whom tissue other than conjunctiva yielded non-caseating granulomas. Group 2 consisted of 12 patients suspected of having

sarcoidosis. These patients had clinical manifestations either typical or strongly suggestive of sarcoidosis. Except for conjunctival biopsy, a biopsy procedure was either not performed, or, if performed, was negative for granuloma. In both study groups, intensive and repeated investigations were unsuccessful in disclosing an infectious or chemical etiologic agent. Group 3 consisted of 28 patients without sarcoidosis. Conjunctival biopsy was performed because sarcoidosis entered into the differential diagnosis during the course of the illness. Eleven patients had infectious granulomatous disease, either tuberculosis or histoplasmosis. The remaining cases consisted of varied medical diseases. The researchers concluded that false-positive reactions were not observed in patients with varied disease other than sarcoidosis. In another study conducted by Khan et al,¹⁶ 100 conjunctival biopsies were performed on patients with histologically confirmed sarcoidosis (60 biopsies), pulmonary tuberculosis (25 biopsies), and several non-specific inflammatory diseases of the lung and eye (15 biopsies). A positive biopsy was present in 33% of the patients with sarcoidosis, but in none of the patients with other disease processes. Khan et al¹⁶ suggested that this procedure, due to its ease, safety and specificity, should be the first biopsy performed in patients with clinical or chest X-ray abnormalities suggesting sarcoidosis. Saer et al¹⁷ obtained 58 postmortem conjunctival biopsies from 30 patients without sarcoidosis. Although aggregates of foreign body giant cells were observed in 3 specimens (5.2%), no distinct, non-caseating granuloma formation was seen. Therefore, the researchers concluded that non-caseating granuloma, which is used as the diagnostic feature of sarcoidosis, does not appear to be a hindrance in the diagnosis of sarcoidosis based on the presence of granuloma in the conjunctival biopsy specimen.

Cost-effectiveness evaluation of biopsy sites revealed that conjunctival biopsy performed in the outpatient setting was less expensive than any of the other biopsy procedures. In fact, 10 conjunctival biopsies could be performed for the same cost as 1 mediastinoscopy, and 5 conjunctival biopsies for the same cost as 1 transbronchial biopsy.¹⁸

Nodular lesion is the most common manifestation in sarcoidosis with conjunctival involvement.^{2,11,19,20} Multiple or solitary yellow nodules are the most frequently seen lesions.¹¹ In a series of sarcoidosis patients followed at the Massachusetts Eye and Ear Infirmary, conjunctival nodules were seen more frequently in patients younger than 35 years of age. Thirty percent of eyes in patients younger than 35 years old developed conjunctival nodules versus 5% of eyes in patients older than 35 years.²¹ On the other hand, the rate of

Table 3. Yield of conjunctival biopsy in the diagnosis of sarcoidosis

	Total number of patients	Positive, n (%)
Crick et al ²²	43	16 (35)
Bornstein et al ⁵	52	14 (27)
Khan et al ¹⁶	60	20 (33)
Nichols et al ²³	55	30 (55)
Spaide et al ⁷	47	19 (40)
Leavitt et al ¹⁸	41	21 (51)
Present study	29	11 (38)

positive biopsy was almost no different (24% vs. 20%) in either group presenting with conjunctival nodules. However, over half of the patients without conjunctival lesions had a positive biopsy.¹⁸ As in the study by Khan et al, the Massachusetts Eye and Ear Infirmary study found that it was not possible to predict which patient would have a positive conjunctival biopsy.¹⁶ In our study, we found that none of the patients with positive results had prominent nodular or follicular changes. To a certain degree, a swollen, subtle fibrotic appearance revealed relatively positive results in our series. However, this finding does not predict the real situation. So, a blind method of evaluation is recommended.

The results regarding the positive rate upon conjunctival biopsy vary among studies. Table 3 summarizes the incidence of positive conjunctival biopsies in sarcoidosis patients, which range from 27% to 55%, with a mean of 37.4%.^{5,7,16,18,22,23} Our incidence of 37.9% positive biopsy was nearly the same as those of other ethnic groups.

There was no association between the presence of uveitis and a positive conjunctival biopsy for sarcoidosis in this study. This tendency had also been observed in previous studies.^{16,23} Furthermore, there is also no significant association with gender, age at diagnosis, initial symptoms, and chest condition.

In conclusion, conjunctival biopsy may provide a simple and rapid means of distinguishing uveitis due to sarcoidosis. This biopsy may further confirm the diagnosis of sarcoidosis when other biopsies have failed to do so or cannot be performed. Furthermore, blinded bilateral conjunctival biopsy should be considered as a diagnostic tool before other invasive methods.

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