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Simple culture method for diagnosis of mycobacteriallymphadenitis on practising fine needle aspirationcytology: an evaluation for its valueand implementation

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Simple culture method for diagnosis of mycobacterial lymphadenitis on practising fine needle aspiration cytology: an evaluation for its value and implementation.

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The purpose of this study was to evaluate the value of mycobacterial culture and the simple collecitng procedure in fine needle aspiration biopsy service. Forty-four cases who presented with cervical node enlargement and were received fine needle aspiration biopsy during the period of January 1993 - February 1995 were chosen. These patients had complete data on cytologic features, Kinyoun-stained smears and culture results. After the aspirate was expelled on to a glass slide, it was transferred by a swab and was inoculated in a non-selective egg-based media, then incubated at 37°C. With this simple technique, thirty five cases (80%) were successfully cultivated. The ratio of classical and atypical mycobacteria was 4.8 : 1. Two-three months were required for the organisms to develop into a detectable colony except for rapid growers which usually

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grew within one week. To assess the value of culture, three complementary parts, namely cytologic feature, acid-fast stain and culture, are separately considered. Suggestive cytologic features were present in 70 % of studied cases; acid fast bacilli were detected in 45 %; and culture yielded positivity in 80 %. A small percentage of cases (7%) that failed by cytologic criteria and negative acid-fast stain was verified by culture. Hence, it was apparent that culture not only the means that permitted identification of various species of mycobacteria, but also added to diagnostic yield. However, for clinical utility, suitable media and methods will have to employ to meet rapid detection.

Key words: *FNA, Mycobacterial lymphadenitis, Tuberculous lymphadenitis, Culture.*

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พิเชฐ สัมปทานุกุล, นิพนธ์ อุดมสันติสุข, รสสุคนธ์ ศรีพัฒนาวัฒน์. วิธีการเพาะเชื้อแบบง่ายเพื่อวินิจฉัยโรคติดเชื้อไมโคแบคทีเรียของต่อมน้ำเหลืองในการเจาะดูดด้วยเข็มขนาดเล็กทางเซลล์วิทยา: การประเมินคุณค่าและการใช้งาน. จุฬาลงกรณ์เวชสาร 2539 พฤษภาคม; 40(5): 383-391

การศึกษานี้มีวัตถุประสงค์เพื่อประเมินคุณค่าและวิธีเก็บตัวอย่างอย่างง่ายๆ ในการเพาะเชื้อไมโคแบคทีเรียจากสิ่งที่เจาะดูดได้ด้วยเข็มขนาดเล็กที่ใช้ในเซลล์วินิจฉัย โดยเลือกผู้ป่วยที่มาพบแพทย์เพื่อรับการตรวจโดยเจาะดูดต่อมน้ำเหลืองที่คอโต ระหว่างเดือนมกราคม พ.ศ. 2536 ถึงกุมภาพันธ์ พ.ศ. 2538 จำนวน 44 ราย โดยทั้งหมดมีองค์ประกอบของการตรวจครบทั้ง 3 ประเภท คือ รายละเอียดทางเซลล์วิทยา, ผลการตรวจเชื้อในสเมียร์โดยวิธีดินหย่วน, และผลการเพาะเชื้อ วิธีการเก็บตัวอย่างกระทำโดยใช้ไม้พันสำลีป้ายตัวอย่างที่เจาะดูดได้ซึ่งถูกเป่าออกมาจากหัวเข็ม อยู่บนสไลด์แก้วที่สะอาด ลงในอาหารเลี้ยงเชื้อชนิดไม่จำเพาะที่มีไข่ขาวเป็นองค์ประกอบหลัก การเพาะเลี้ยงเชื้อกระทำที่อุณหภูมิ 37 องศาเซลเซียส โดยเทคนิคนี้ตัวอย่างจำนวน 35 ราย หรือคิดเป็นร้อยละ 80 มีเชื้อไมโคแบคทีเรียงอกขึ้นมา คิดเป็นสัดส่วนระหว่างชนิดไมโคแบคทีเรียม ทูเบอ-คูโลซิส และชนิดไม่ใช่ไมโคแบคทีเรียม ทูเบอคูโลซิส เท่ากับ 4.8 : 1 โดยส่วนใหญ่จะใช้เวลาเจริญราว 2-3 เดือน คงมีแต่กลุ่มเชื้อไมโคแบคทีเรียมชนิดโตเร็วที่จะงอกภายใน 1 สัปดาห์ การประเมินคุณค่าของการเพาะเชื้อพบว่า เมื่อแยกพิจารณาแต่ละองค์ประกอบ ลักษณะทางเซลล์วิทยาช่วยการวินิจฉัยได้ร้อยละ70 เชื้อโรคตรวจพบในสเมียร์ได้ร้อยละ 45 ขณะที่การเพาะเชื้อให้ผลบวกร้อยละ 80 มีผู้ป่วยจำนวนน้อย (ร้อยละ 7) วินิจฉัยได้โดยการเพาะเชื้ออย่างเดียว ซึ่งแสดงให้เห็นว่านอกจากการเพาะเชื้อจะเป็นวิธีการเดียวที่ใช้แยกเชื้อชนิดต่างๆ ของไมโคแบคทีเรียได้ ยังมีส่วนช่วยในการวินิจฉัยโรคที่ไม่สามารถวินิจฉัยโดยลักษณะทางเซลล์วิทยาร่วมกับการตรวจเชื้อโรคในสเมียร์ แต่การนำมาใช้เพื่อวินิจฉัยโรคในทางคลินิกนั้น คงต้องพัฒนาอาหารเลี้ยงเชื้อตลอดจนวิธีการเพาะเชื้อที่จะให้ผลได้รวดเร็ว

Fine needle aspiration (FNA) biopsy is a simple technique that is gaining increasing popularity because it gives an accurate diagnosis of most palpable lumps.[1,2] The small needle (external diameter = 0.7 mm) used in the process usually gets about 0.2-0.5 ml of specimen for a solid lesion. It is surprising that with such a tiny sample size can be implemented for culturing in case of suspected mycobacterial infection.

Mycobacteria are slow growing, aerobic, non-spore forming bacilli. The high lipid content in their cell walls confers upon them the distinctive property of acid-fastness. There are two groups of mycobacteria. Tubercle bacilli,or members of the *Mycobacterium tuberculosis* complex (MTBC), cause human tuberculosis. Atypical mycobacteria, or mycobacteria other than tubercle bacilli (MOTT), are those mycobacteria that have characteristics distinct from those of *M.tuberculosis.*[3] Both MTBC and MOTT can contribute to pulmonary infections and extrapulmonary diseases. Cervical node enlargement is the most common manifestation for mycobacterial infection outside the lung.[4] To distinguish tubercle bacilli from atypical mycobacteria, a culture is needed.[3] Practically, all specimens that are suspected for mycobacterial diseases will stain to search for acid-fast bacilli (AFB) with carbolfuchsin stains (the classic Ziehl-Neelsen stain which requires heating, and the cold Kinyoun's stain).[3] Unfortunately, both morphology and microbial stain cannot characterize and separate *M.tuberculosis* from all the other species of mycobacteria. Culture should be planned from the begining as it cannot follow if the specimen has been put into fixatives. Culture, acid-fast stain and cytologic features are three pertinent parts in the diagnosis of mycobacterial infection in a palpable lymph node by FNA. Subject to the amount of aspirated material, sometimes it is not sufficient to perform all three means. In this study, we evaluated a simple procedure to make a culture from lymph node aspirates in order to evaluate its effectiveness.

Materials and Methods

Routine FNA work at Chulalongkorn Hospital during the period Jan 1, 1993 to Feb 22, 1995 provided 44 clinically and laboratory supported cases of mycobacterial infection in cervical lymph nodes for which cytologic smears, acid-fast stain and culture results were available. The male to female ratio was 1:3. The average age was 32.9 years (range 16-69). All patients complained of neck node enlargement. Aspiration biopsy procedure was employed as described elsewhere.[1] In short, we used a 22 or 23 gauge needle attached to a 10 ml syringe, A brisk, to-and-forth movement,like cutting inside the mass under negative pressure, was used. According to handling the specimen, culture is processed as the following: The aspirates were expressed onto a clean glass slides, then a sterile swab was taken to transfer the aspirates inoculating on the surface of tubed solid Ogawa media (egg-based media). Thereafter, culture was simply incubated at 37°C until colonies development or within 3 months. We made simple conventional methods for identification of mycobacteria that included observation of rate of growth, colony morphology and pigmentation. Cytologic smears were wet-fixed and stained according to Papaniculaou technique. Descriptive features were rendered as

previously explained.[5] Smears and stains for AFB were prepared by air dried and fulfilled the method of Kinyoun's.

Result

Thirty-five cases were successfully cultivated. Of the nine negative cases, the acidfast organisms were readily detected on two cases. Seven others were clinically and cytologically proved. Three cases of them had histopathology support as well. The substantial cytologic features showed either microscopic caseating material or epithelioid cell aggregates or both. Of the culture positive cases, six samples grew atypical mycobacteria whereas 29 cases turned out to be tuberculosis. This made the ratio of MOTT : MTBC equal to 1 : 4.8. Incubation period usually was in the range of 2-3 months. Atypical mycobacteria of the rapid grower group developed colonies within one week. A summary of patients and pertinent laboratory findings has been provided in Table1. Cytology + Acid-fast stain + Culture complementary took a 100% sensitivity of the diagnoses. Cytology alone (epithelioid cell aggregates and/or microscopic caseating material) gave the suggestive host reaction in 31 out of 44 cases (70%). Acid-fast stain picked up the organisms in 20 cases (45%). Culture yielded in 35 cases (80%). Cytology + acid-fast stain could determine or helped suggestion of mycobacterial infection in 93% of the cases. Three cases (7%) were only detected by positive culture.

Table 1. Results of culture, AFB stain, and cytologic features of the studied cases.

no	sex	age	host status	culture	AFB	cytologic features*
1	f	46	HIV +	t.b.	p	necrotizing pattern
2	f	26		t.b.	n	epithelioid cell aggre.
3	f	28		t.b.	n	necrotizing pattern
4	f	16		t.b.	p	necrotizing pattern
5	f	36		atyp	p	purulent pattern
6	f	64		t.b.	n	epithelioid cell aggre.
7	f	36		n	p	necrotizing pattern
8	f	28		t.b.	n	necrotizing pattern
9	f	20		t.b.	n	necrotizing pattern
10	m	18		n	n	epithelioid cell aggre.
11	f	23		atyp	p	necrotizing pattern
12	f	69		atyp	p	epithelioid cell aggre.
13	f	39		atyp	p	epithelioid cell aggre.
14	m	23		t.b.	n	caseating material
15	f	30	HIV +	n	n	caseating material
16	f	56		t.b.	n	epithelioid cell aggre.

no	sex	age	host status	culture	AFB	cytologic features*
17	f	45		t.b.	p	epithelioid cell aggre.
18	m	22	HIV +	t.b.	p	necrotizing pattern
19	f	31	thalassemia	atyp	n	caseating material
20	m	34		t.b.	n	caseating material
21	f	24		n	n	epithelioid cell aggre.
22	f	31		t.b.	n	epithelioid cell aggre.
23	f	61		n	n	epithelioid cell aggre.
24	f	31		t.b.	p	epithelioid cell aggre.
25	m	28	HIV +	n	p	necrotinizing pattern
26	f	37		t.b.	p	caseating material
27	f	36		t.b.	n	epithelioid cell aggre.
28	f	20		t.b.	n	caseating material
29	f	38		t.b.	n	epithelioid cell aggre.
30	f	28	HIV +	t.b.	p	necrotizing pattern
31	m	31	HIV +	t.b.	p	epithelioid cell aggre.
32	f	25		t.b.	n	caseating material
33	f	30		t.b.	p	epithelioid cell aggre.
34	m	43		t.b.	p	caseating material
35	f	33		n	n	epithelioid cell aggre.
36	m	37		t.b.	n	epithelioid cell aggre.
37	m	35	HIV +	t.b.	p	necrotizing pattern
38	m	33	HIV +	t.b.	p	necrotizing pattern
39	f	31		n	n	caseating material
40	f	39		atyp	p	epithelioid cell aggre.
41	f	27		n	n	epithelioid cell aggre.
42	f	23		t.b.	n	caseating material
43	m	17		t.b.	n	epithelioid cell aggre.
44	f	19		t.b.	p	caseating material

Abbreviations : f= female; m=male; atyp= atypical mycobacteria; t.b.= tubercle bacilli; p= positive; n= negative;epithelioid cell aggre.= epithelioid cell aggregates.

* The terms for cytologic features herein were shortened. Epithelioid cell aggregates were chosen when smears comprised epithelioid cells in aggregation with or without associated caseous material. Caseating material was denoted when smears displayed small and large eosinophilic patches of caseous necrosis without apparent epithelioid cells. Necrotizing pattern implied necrotic cells combined with sparse and inconsistent distribution of mixed inflammatory cells in the smeared background. Purulent pattern meant abundant neutrophils.

Discussion

Tuberculous lymphadenitis, occurring predominantly in the cervical region, is the most common manifestation of non-respiratory tuberculosis worldwide.[4] Female cases preponderate over males with a ratio of 3:1,both in this study and in others as well.[6,7] The average age in our group of patients was 32.9 years. Fine needle aspiration is an indispensable part of modern diagnosis that obviates surgical intervention in many cases.[8] It is particularly advantageous in cases of tuberculous lymphadenitis as it leaves no scars. Nevertheless, from our previous study[5] and also from others,[9,10] it is remarkable that the diagnosis of tuberculous lymphadenitis cannot depend upon cytological features alone. Some cases, especially in those with necrotizing patterns, air dried smears for acid-fast stain are very helpful.[11] Culture, in addition, adds to the diagnostic yield and also permits the specific identification of acid-fast bacilli and the determination of drug susceptibility. Therefore, we attempted to cultivate the organisms from lymph node aspirates.

The simple procedure we employed proved to be successfully implemented. Thirty-five cases (80%) were successfully cultivated and nine cases failed. We speculate that the failures were due to inadequacy of the samples. Culturing was verified in this study as a more powerful diagnostic means than carbol-fuchsin stain. Seventeen cases were diagnosed by culture while acid-fast stain gave negative results. This can be partly explained by our recent finding that the smears in those days were made too thin. We currently use a thick smear to enable better detection of the organisms. However, it is generally accepted that the number and types of specimens are major influencing factors in the detection of the organisms. Although the smear is not as sensitive as culture techniques and requires approximately 10^4 bacilli per milliliter of sputum to be positive, smear examination provides an easy, rapid, presumptive diagnosis of mycobacterial disease.[12] Acid-fast bacilli positivity in aspiration smears of tuberculous lymphadenitis is between 40.6 and 56.4.[11] Ours was 45 %. Moreover, culture is the only means to discriminate between atypical mycobacteria and tubercle bacilli. In our study, the ratio was 4.8:1 in favor of *M.tuberculosis*. Knowledge of these different types will assist in follow-up and treatment, as well as for the epidemiologic ground.[13,14] Atypical mycobacterial infections in children and HIV-positive patients are frequent [14,15] but in our small sample we had no children. Six out of eight cases of HIV-positive patients was found to be tuberculosis, whereas the other two cases did not grow organisms. There was a case of thalassemia afflicted with recurrent lymphadenitis that by culture, on the recurrence, revealed a rapid grower. Four other atypical mycobacterial adenitis cases occurred in immunocompetent hosts of which there were 35 cases. The sole disadvantage of conventional culture is that it takes time. It requires about one week for rapid growers and approximately two to three months for other mycobacteria to develop into a detectable colony. Being practical,the treatment should not wait for the outcome of the culture. Cytology,coupled with acid-fast stain, can detect the majority of cases and enables diagnosis to be done within an hour. The incubation period

greatly depends upon the type of media.[3,12,16] The inexpensive,non-selective Ogawa media was used in our study. Radiometric systems utilizing broth media provides rapid detection of mycobacterial growth in an average period of 5 to 12 days.[3] Moreover, molecular technology combined with polymerase chain reaction is under intense development for widespread application.[16] These methods will have beneficial clinical roles over conventional cultures to determine a small percentage of cases undiagnosed by routine cytology and acid-fast stain. Nevertheless, the price is high.

In conclusion,our study showed that a simple procedure to cultivate mycobacteria from aspirates of diseased nodes can be implemented and can get a highly positive yield rate. Culture adds to diagnostic yield rather than cytologic feature and acid-fast smear in a small percentage. The one drawback of conventional culture is that it is time-consuming. Significantly, culture will discriminate between atypical and classical mycobactria, and permits sensitivity tests for the organisms. It is, therefore, recommended that for all patients with suspected tuberculous lymphadenitis and who undergo fine needle aspiration, culture should be conducted if possible.

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