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Tangles in Smears of Granulomatous Lymphadenitis – A Clue to the Diagnosis

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ABSTRACT

Objective: To assess the significance and nature of tangles in fine needle aspiration biopsy (FNAB) smears of granulomatous lymphadenitis.

Methods: The study group included 45 cases of clinically suspected granulomatous lymphadenitis particularly of tuberculous origin in which a cytologic diagnosis of granulomatous lymphadenitis (GL) was made on FNAB material. Smears of 21 lymph node aspirates without cytologic evidence of granulomatous disease (NGL) were included as a control group. One case with double pathology was excluded. All smears were fixed in 95% alcohol and stained with haematoxylin and eosin. Tangles were defined as a meshwork of haematoxyphilic string-like material occurring in a tangle in an otherwise well preserved smear. A score was given for the distribution (0 – 3) and density (1 – 3) of the tangles on the count of 10 high power fields for each aspirate. Statistical analysis was done by the Mann Whitney U test.

Results: Tangles were present in 41 of 45 (91%) GL and 7 of 21 (33%) NGL. The differences were statistically significant (p = 0.0008 and 0.0001 for distribution and density respectively). Six of the seven (84%) NGL with tangles were either cytologically diagnostic or suspicious of malignancy with 5 (71%) showing features of non-Hodgkin's lymphoma. The tangles were positive for nuclear stains (Feulgen) and originated in the nuclei of both lymphocytes and epithelioid cells, probably due to easy fragility of altered nuclear material.

Conclusion: The presence of tangles in smears should raise the possibility of GL in the absence of a malignancy, specifically a lymphoma. This becomes an important diagnostic clue especially in situations where epithelioid histiocytes, the hall mark of GL, are sparse or absent in cytologic material.

Keywords: tangles, smears, granulomatous lymphadenitis

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INTRODUCTION

Cytological diagnosis of granulomatous lymphadenitis (GL) is based on the presence of epithelioid histiocytes forming granulomata in smears (Fig 1)[1]. The aetiology of granulomatous lymphadenitis include infections such as mycobacteriosis, mycoses, and certain parasitic diseases and non-infective causes such as sarcoidosis, and reaction to tumour, among others. Caseous necrosis and Langhans giant cells are additional features. Recognition of an infective cause is important, as infections such as tuberculosis which is not uncommon in Asia, can be successfully treated if a specific diagnosis is made. The specific diagnosis of an infective process relies on demonstration of organisms such as acid fast bacilli, parasites, hooklets of cysticercosis, fungi, or others in aspirated material[2-5]. The tests for demonstration of a specific infective agent can be performed in the patient or even in aspirated material if a diagnosis of GL is made or at least suspected. It is well known that cytologic assessment of FNAB is reliable in detecting GL[1-3,6]. The diagnosis is based on the detection of epthelioid cells forming granulomata in smears which may be difficult at times, due to scarcity or the epithelioid cells being masked by other cells and necrosis. Therefore it is not surprising that attempts to search for an extension of diagnostic criteria of granulomatous lymphadenitis are still being made. Recently, the presence of eosinophilic structures as a diagnostic criterion for granulomatous lymphadenitis, most probably due to tuberculosis, was documented[6]. We have observed the presence of well formed nuclear tangles (a complex meshwork of string-like haematoxyphilic material) in smears of

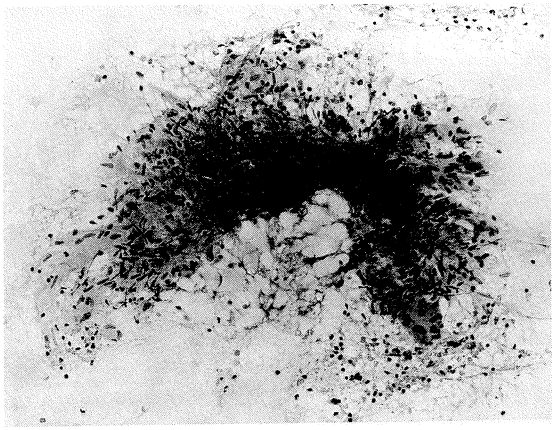

Fig 1 – A granuloma in a smear with epithelioid cells showing "foot print" nuclei and abundant cytoplasm (H & E x 100).

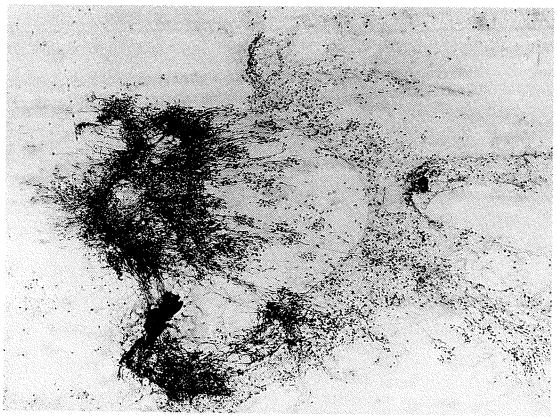

Fig 2 – Dense nuclear tangles (H & E x 40).

granulomatous lymphadenitis (Fig 2). Although there have been numerous publications on granulomatous lesions specifically of tuberculous origin only, one refers to this observation in spermatic granulomata[7].

Objective

The objectives of this study were to assess the significance and the nature of tangles or string-like haematoxyphilic material in the diagnosis of granulomatous lymphadenitis in fine needle aspiration biopsy smears.

METHODS

Forty-five lymph node aspirates with cytologic features of GL analysed over a period of 16 months, from September 1995 to January 1997, were included in the study group. Most cases were clinically suspected to be tuberculous lymphadenitis and who were either routinely referred for FNAB to the first author or channelled to be included in the study group. Twenty-one cases without clinical evidence of granulomatous lymphadenitis and cytologically diagnosed as NGL during the same period, were selected as the control group. Follow-up with histologic or microbiologic tests was traced for those available. One case with a double pathology and metastatic carcinoma with a granulomatous reaction was excluded.

All smears were of aspirates obtained by the standard techniques of fine needle sampling and smearing and fixed with 95% alcohol and stained with haematoxylin and eosin (H & E). Exclusion criteria were poor preservation, faulty smearing or severe crush artefact. Some smears with tangles were destained and restained with Feulgen stain, a special histochemical stain for nucleic acids.

The presence of tangles was assessed in terms of distribution and density. Tangles in smears is defined as a complex meshwork of string-like haematoxyphilic material. A score was given for each of the above qualities of the tangles by both authors. Of all smears and controls, 10 highest cellular fields were selected and scored for: 1) Percentage distribution (0 – 3), and 2) Density (1 – 3) of tangles under high power. Therefore the final score was out of 30 for each parameter, per smear. Percentage distribution was assessed on the area of microscopic field covered by tangles. Density of the tangles was assessed on the quality of string-like material forming the meshwork.

1) Percentage distribution of the tangles per field
   - negligible = 0 (< 25% of the field)
   - sparse = 1 (25% – 50% of the field)
   - moderate = 2 (50% – 75% of the field)
   - abundant = 3 (> 75% of the field)

2) Density of the tangles per field
   - low = 1 (a few strings widely separated from each other so that the spaces in the meshwork are prominent and easily seen)
   - high = 3 (numerous strings, closely aggregated so that the spaces in the meshwork are barely visible)
   - moderate = 2 (in between low and high)

The scores for percentage distribution and density of tangles for the 2 groups were compared and statistically analysed by the Mann Whitney U test. Distribution and the density of tangles in the control group were assessed according to the diagnosis.

RESULTS

All 45 cases included in the study group were clinically suspected to be GL, particularly due to tuberculosis and were confirmed as GL cytologically. Further follow-up was available for 17 of 45 cases. In four cases, the diagnosis was confirmed histologically. In 12 cases, clinical features and microbiological tests indicated mycobacterial disease; 8 patients had Mantoux positivity with or without high ESR and in 3 aspirates culture and/or direct smear and in another 2, polymerase chain reaction (PCR) was positive for mycobacteria. Histologic follow-up was available for 10 of the 21 controls. In all 10, the diagnosis of NGL was confirmed; NHL (n = 4), metastatic carcinoma (n = 4), reactive lymphadenitis (n = 2).

Tangles which were positive with Feulgen stain, confirmed the presence of nuclear material (Fig 3). They were observed to originate in the nuclei of both lymphocytes and epithelioid cells (Figs 4 & 5).

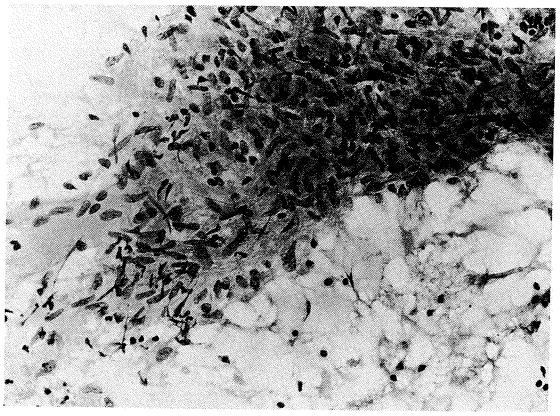

Fig 3 – Tangles showing purplish blue positivity, confirms presence of nuclear material (Feulgen stain x 100).

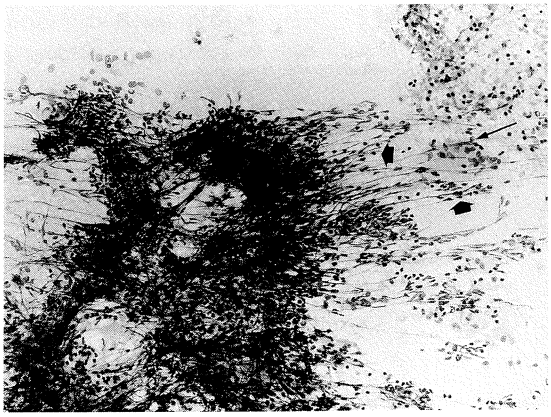

Fig 4 – Early nuclear streaks in a granuloma (H & E x 400). Tangles originating in nuclei of epithelioid cells (thin arrow) and lymphocytes (thick arrows).

Table I shows the comparison of tangles (distribution and density) for cases (GL) and controls (NGL) with the p values. Tangles were present in 41 (91%) GL and 7 NGL (33%). The tangles in GL were of high density (median = 24) while in NGL, they were of low density (median = 10). The presence of "tangles" was significantly higher in GL than in NGL both in terms of distribution (p = 0.0008) and density (p = 0.0001).

Table II shows the distribution and density of tangles in the control group (n = 21) which included reactive lymphadenitis, 8 metastatic deposits and 8 suspected or diagnosed as non-Hodgkin's lymphomas. Seven of the 21 showed tangles while six of seven NGL (84%) with tangles were either diagnostic or suspicious of malignancy, with 5 (71%) showing features of non-Hodgkin's lymphomas (NHL). Four of the latter five were histologically confirmed. In the NHL group, the tangles were abundant and dense. One each of reactive nodes and metastases showed moderately dense, moderate and low density and sparse tangles respectively.

Table I – Comparison of tangles (score per 10 fields) in cases and controls: the median score, range (within brackets) and p value

	Control (NGL)	Cases (GL)	P value
Distribution	0 (0 – 30)	18 (0 – 30)	0.0008
Density	10 (0 – 30)	24 (0 – 30)	0.0001

Table II – The distribution and density of tangles in the control group (n = 21) according to the diagnosis

Diagnosis	Distribution				Density			Number positive for tangles
	0	1	2	3	1	2	3	
Reactive	4	-	1	-	-	1	-	1/5 (20.0%)
Metastases	7	1	-	-	1	-	-	1/8 (12.5%)
NHL group	3	-	-	5	-	1	4	5/8 (62.5%)

(Distribution 0 = < 25%, 1 = 25% – 50%, 2 = 50% – 75%, 3 = > 75% and density, 1 = low, 2 = moderate, 3 = high)

DISCUSSION

Tangles showed smudged or streaked nuclei when they were positive for Feulgen stain in addition to being haematoxyphilic. Careful search proved the tangles to have originated from the nuclei of lymphocytes and epithelioid histiocytes (Figs 3 & 4). Tangles of high density (median = 24) occurred frequently (41%) in GL. Tangles were rarely present in NGL of reactive (20%) or metastatic (12.5%) nature. When present, they were neither abundant nor highly dense. In contrast, abundant to moderate to high density tangles were seen in 62.5% of NHL group. Perhaps the difference between the controls and cases might have been even more significant if the percentage of NHL group was less in the controls. Therefore in the absence of non-Hodgkin's lymphoma, the presence of high density tangles is a good clue to the diagnosis of GL, as shown in our results.

The presence of tangles becomes an important feature especially when epithelioid granulomata are sparse. The presence of tangles in a lymph node aspirate without evidence of a lymphoma should certainly prompt meticulous search for hidden granulomata. In the event of strong clinical suspicion of a specific GL, the presence of nuclear tangles may warrant even repeat aspirations to obtain more material for special stains or microbiological studies. Further investigation of the patient for confirmation of a granulomatous disease and to determine the aetiology may also be indicated.

The presence of nuclear tangles have been observed in spermatic granulomata by Guillemo et al [7]. They suggested the tangles to be lymphocytic debris and degenerated smeared nuclei. Nuclear tangles have also been described by Gurada et al in Hashimoto's thyroiditis[8]. They concluded that these are distorted, delicate lymphoid cells forming diagnostic tangles. A similar phenomenon of extension artefacts of lymphocytes ("rope-like") has been described in Hashimoto's thyroiditis by Bibo[9]. In our cases, tangles were observed to have originated in the nuclei of both lymphocytes and epithelioid cells. Both tangles and extension artefacts probably occurred due to an altered property of cells resulting in easy nuclear fragility. Activated or transformed T-lymphocytes are found in auto-immune thyroiditis and in granulomatous lesions; the latter also contain epithelioid cells which are transformed mononuclear phagocytes and are associated with a delayed hypersensitivity reaction. A defect in organ specific suppressor T-cells with emergence of helper T-lymphocytes, together with an abnormality resulting in presentation of self antigens to activated lymphocytes, is believed to be the basis of auto-immune thyroiditis.

Nuclear fragility also occurs due to malignant change. This feature has been observed even in histologic sections in certain malignancies such as small cell carcinoma of the lung[10] and non-Hodgkin's lymphomas. Crushed streaked nuclei though poses a diagnostic difficulty, also provides a clue to the cytologic diagnosis of small cell carcinoma[1]. It is significant that all five of 7 NGL showing moderately

or abundantly high density tangles were either diagnostic or suspicious of non-Hodgkin's lymphoma. This group contributed 33% of tangles in the controls.

True tangles due to nuclear fragility can be appreciated only in otherwise well preserved smears without undue crushing artefact due to poor smearing. Good preservation of the background cells is a good indicator of overall preservation. Undue crushing and forceful smearing can cause traumatic streaking of any cell in a smear. The numbers of GL and NGL included in the study and control groups do not reflect the true prevalence of the disease pattern as authors actively recruited patients clinically suspected to have tuberculous lymphadenitis for the study purpose.

In conclusion, although "tangles" are not pathognomonic of GL, presence of abundantly dense tangles is a significant clue to its diagnosis. In the absence of a non-Hodgkin's lymphoma, their presence should prompt the diagnostician to search for granulomata in a smear. In the appropriate clinical setting of a strong clinical suspicion of granulomatous disease, further investigations or microbiological studies may be performed to determine the specific aetiology.

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