# DARK SPECKS AND DARK OVAL BODIES: TWO VALUABLE STRUCTURES IN TUBERCULOUS LYMPHADENOPATHY

**[1]HUMAIRAH MEDINA LIZA LUBIS, [2]RATNA AKBARI GANIE, [3]DELYUZAR, [4]PUTRI CHAIRANI EYANOER**

[1]Doctoral Programme, Universitas Sumatera Utara, Medan 20155, Sumatera Utara, Indonesia
[1*]Department of Anatomical Pathology, University of Muhammadiyah Sumatera Utara, Medan 20217, Sumatera Utara, Indonesia
[2]Department of Clinical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan 20155, Indonesia
[3]Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan 20155, Indonesia
[4]Department of Community Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan 20155, Indonesia
E-mail: [1]humairahmedina@umsu.ac.id, [2]ratnaakbariganie@usu.ac.id, [3]dr_delyuzar@yahoo.com, [4]putrice00@yahoo.com

**Abstract**: –
**Background** - In aspiration biopsy cytology of lymph node stained with May Grunewald Giemsa, diagnosis of tuberculosis is established based on finding of epitheloid cells and (or) Langhans cells. However there were cases that didn't show these two kinds of cells, should be considered as non-specific lymphadenitis, couldn't be treated with common antibiotics, but successful with anti-tuberculous drugs. The aspirates might show either of two structures: (1) dark specks in background of eosinophilic granular necrotic material (DS), (2)small dark shrunken oval bodies in clusters of macrophages (DOB). It is desired that DS and DOB could be used as indicators of tuberculosis, and both have relation with allergic process.
**Methods** - Immunocytochemical stain with rabbit polyclonal to Mycobacterium tuberculosis antibody (ab905), Abcam, was conducted to slides being previously diagnosed as suspected tuberculosis (by DS and DOB) and had been successfully treated with anti-tuberculous drugs. Expression of anti-mycobacterium tuberculosis antibody were examined on each of the 30 cases with DS and DOB. In addition, anti-interferon-γ (IFN-γ) and anti-interleukin-4 (IL-4) were also performed in 5 cases with DS, and in 3 with DOB.
**Results** - Expressions of anti-mycobacterium antibody were observed on 27 of 30 cases with DS, and 21 of 30 cases with DOB. There is no difference between the two structures in respect to their ability in diagnosing of tuberculous lymphadenopathy ($p>0.05$). All 5 cases with DS showed strong expression of IL-4, and weak expression of IFN-γ, while in one case of DOB, the expression of IFN-γ was more intense.
**Conclusions** - DS and DOB can be benefited as indicator of tuberculous lymphadenopathy. There was no difference in ability of DS and DOB in diagnosing tuberculous lymphadenopathy. Further research related to IFNγ and IL4 is needed to know whether DS and DOB have any relation with allergic process.

**Keywords** - Dark Specks, Dark Oval Bodies, Tuberculous, Lymphadenopathy

## I. INTRODUCTION

In developing countries like Indonesia, tuberculosis is still a national problem and is the fourth rank cause of death.[1] Although the lung is the major site of infection of tuberculosis (TB), but in Medan extrapulmonary TB including scrofula are common.

Tuberculosis is readily diagnosed cytologically if there are epitheloid cells or Langhan's giant cells in the aspirate.[1-7] In the case of not having these two cells we should make a culture or Ziehl-Neelsen staining. But culture could take at least six weeks to obtain a diagnosis, so that the patients are delayed from getting treatment. Eventhough failed to show these two kinds of cells, in some cases especially with purulent aspirate, the material could grow tuberculous bacilli when cultured. In aspiration biopsy cytology of neck lymph node stained with May Grunewald Giemsa, we noticed two structures: (1). necrotic tissue in the form of structure of dark specks in background of eosinophilic granular material (DS), (2). small dark shrunken round oval bodies in clusters of macrophages (DOB) (Figure 1 and 2). The cases couldn't be treated with common antibiotics, however successful with anti-tuberculous drugs. Our previous work of culturing the aspirate of DS, yielded growth of tuberculous bacilli. We regarded these lesion as tuberculous lymphadenitis (scrofula).

The cases were of cytologically diagnosed as suspected tuberculous lymphadenopathy (with DS and DOB) and had been successfully treated with anti-tuberculous drugs (ATD) but failed with common antibiotics, were considered as TB, based on ex juvantibus diagnosis.

So far research related to cytokines of Th1 or Th2 response were directed to lung tuberculosis, or bronchoalveolar lavage (BAL), while to the lymph nodes was nearly none. Beside, the study of IFN-γ and IL4 which have been carried on pulmonary tuberculosis is an assessment of the levels of cytokines in the blood and the results of research on the Th2 response and IL-4 still controversial, contradictory to each other.

The aim of this study is investigate whether or not the DS and DOB could be use as a cytological diagnostic tools to establish diagnosis of tuberculosis and whether the both have relation with allergic process.

## II. MATERIAL AND METHODS

The study was cross sectional, using 30 aspirate smears of lymph nodes collected from the Anatomical Pathology Diagnostic Center of the Faculty of Medicine, Universitas Sumatra Utara, Medan, Indonesia.

Expression of anti-mycobacterium tuberculosis antibody were examined on each of the 30 cases with DS and DOB. Immunocytochemical stain with rabbit polyclonal to Mycobacterium tuberculosis antibody (ab905), Abcam, was conducted to specimens. In addition, anti-interferon-γ (IFN-γ) and anti-interleukin-4 (IL-4) were also performed in 5 cases with DS, and in 3 with DOB.

**Immunocytochemistry Examination**

After rinsing slides briefly in phosphate-buffered saline (PBS), the endogenous peroxidase activity was inhibited by incubating the sections with hydrogen peroxide for 30 minutes. After microwave antigen retrieval, blocking the stuff with 5% normal horse serum (NHS) for 15 minutes, the slides were then treated with primary antibodies – anti-mycobacterium tuberculosis antibody (ab905) (Abcam, London, UK) at 1/100 dilution overnight, then treated with Dako Real En Vision. Antigen was visualized with Diaminobenzidine (DAB) and counter-stained with haematoxylin.

In every works, positive control and negative controls were included. Staining was categorized as positive and negative assessment. Performance of staining to IFN-γ and IL-4 was the same as to Ab905.

## III. RESULTS

Expressions of anti-mycobacterium antibody were observed on 27 of 30 cases with DS, and 21 of 30 cases with DOB (Figure 1 and 2). There is no difference between the two structures in respect to their ability in diagnosing of tuberculous lymphadenopathy ($p>0.05$)

All 5 cases with DS showed strong expression of IL-4, and weak expression of IFN-γ, while in one case of DOB, the expression of IFN-γ was more intense (Figure 3-8).

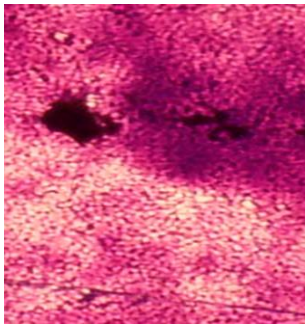


**Figure 1: Aspirates with dark specks (DS) with finely granular eosinophilic amorphous mass in the background (MGG, 400x)**

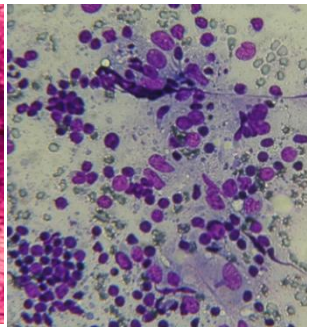


**Figure 2: Small dark oval body (DOB) inside grouped some macrophages (MGG, 400x)**

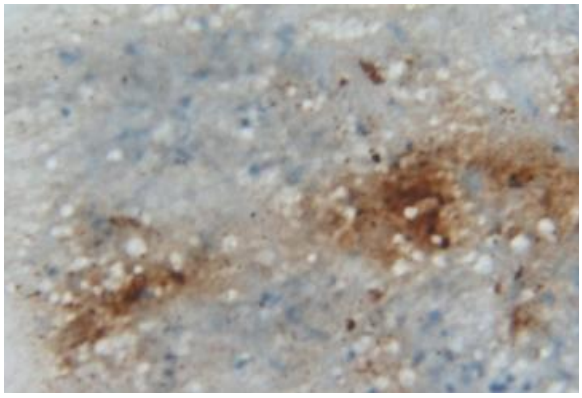


**Figure 3: Positive expression of Ab905 in DS (ICC, 400x)**

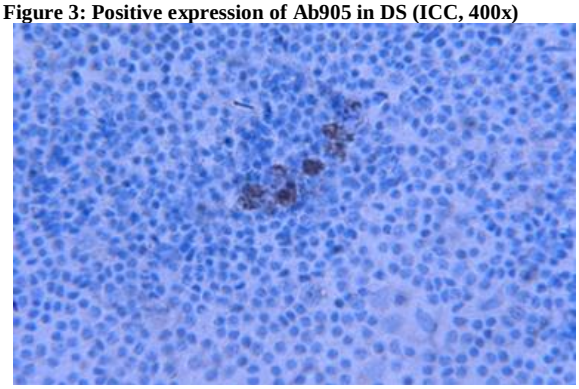


**Figure 4: Positive expression of Ab905 in DOB (ICC, 400x)**

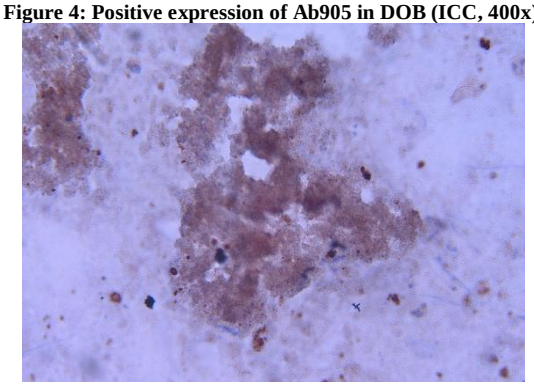


**Figure 5: Positive expression of IL4 in DS (ICC, 400x)**

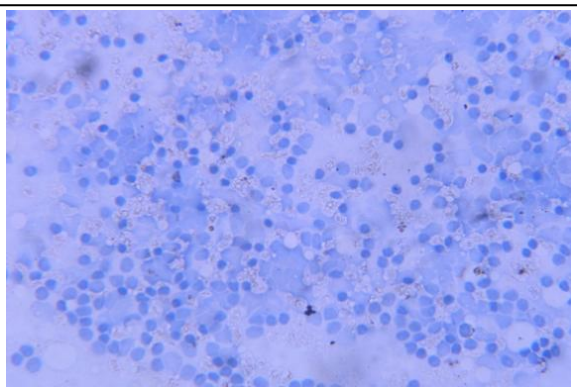


**Figure 6: Negative expression of IL-4 in DOB (ICC, 400x)**

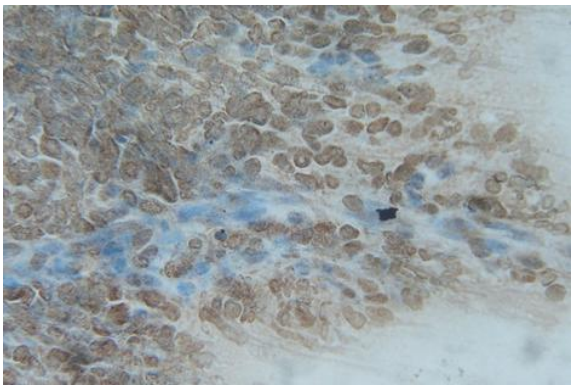


**Figure 7: Positive expression of IFN-γ in DOB (400x)**

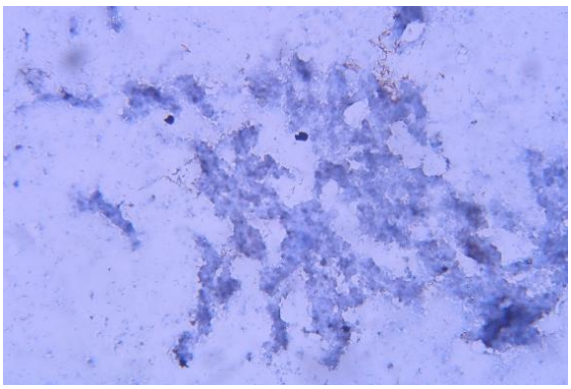


**Figure 8. Negative expression of IFN-γ in DS (400x)**

## IV. DISCUSSION

TB protein was expressed both in DS and DOB. There are two possibilities why the expression happen. First, DOB might be there are 2 possibilities: first, DOB is a preliminary lesion before DS occurs in other words DOB and DS may be in one spectrum, and second, cytologicaly DOB could be generated by atypical mycobacteria, as explained by Ab905 datasheet, that the antibody is reactive with other mycobacteria species including: M. avium, M. phlei, and M. parafortuitum.

Related to the nature of aspirate, aspirates of DS usually purulent that we often incorrectly diagnose as ordinary abscess, while the DOB aspirate is serous with or without blood similar to chronic non-specific inflammation. Dark specks more easily visible with May-Grunewald Giemsa staining compared to staining Papanicolaou and Hematoxylin-eosin.

Several studies have been conducted to prove that DS is an indication of TB. Lubis[8] related DS with culture, Delyuzar with PCR[9] and Balaji[10] correlated FNAC in 135 children with lymphadenitis with unsuccessful treatment after two weeks of usual antibiotic treatment, and Muyanja with histopathological confirmation.[11]

In treating some cases of scrofula with anti tuberculous drug (ATD), we got impression that patients with DS showed a faster response to treatment compared with those of DOB. But we have not been able to produce the reason. It could be the DOB that we supposed TB is lesion caused by atypical mycobacterium.

Although it is mentioned that the immunohistochemistry (ICC) is not all detected cytokines (eg IL-4), but we saw expression of this cytokine. This is according to information stating that IL-4 can be expressed on tissue.[11]

Necrosis of tuberculosis is considered a logical extension of the disease (with the formation of DS. The possibility is meant by Dheda, which, relating to a Th1 response is a Th2 response DS being associated with DOB. Interferon (IFN)-γ produced by Th1 cells evokes cell-mediated immunity and phagocyte-dependent inflammation, while interleukin 4 (IL-4) by Th2 cells evokes strong antibody responses are responsible for atopic allergic disorders.[13]

IL-4 produced by TH-2 cell play a role as a regulator of the cell or silencer of TH1 dangerous work, and have more related to allergic process. IL-4 would lead to a Th2 response which will resist protective cytokines with consequent loss of control of tuberculosis. Recognition of the role of these cytokines is expected that we can use it as a basis for consideration to provide anti- cytokine drugs, especially anti-IL-4 on sufferers who are resistant to anti tuberculous drug (ATD).

TH1 cells are marked with a product cytokine of IL-2, IFN-γ and TNF-β, without IL-4, IL-5, IL-9, IL-13, while the cells marked with the TH-2 IL-products 4, IL-5, IL-9 and IL-13 without IFN-γ and TNF-β. Cytokines produced by the cells Th1 role in the activation of macrophages and response to delayed-type hypersensitivity (DTH), while the cytokines produced by the cells TH2 play a role in the increased production of IgE antibodies, mast cell degranulation, activation of eosinophils and regulate development of TH1 cells and macrophage activity. Thus the cells TH2 cytokines play a role as a regulator of the cell or silencer TH1 dangerous work, and have more contact with the allergic process.[1]

Ashenafi suggest that in patients with active pulmonary tuberculosis was found decreased levels of IFN-γ, along with increased levels of IL-4 compared with controls. IFN-γ role in protection tuberculosis, while increased levels of IL-4 would lead to a Th2 response which will resist protective cytokines with consequent loss of control of tuberculosis.[14]

## V. CONCLUSIONS

Dark specks and dark shrunken oval bodies within collections of macrophages are two valuable structures that can be appeared in aspirate of tuberculous lymphadenopathy. The two structures can be benefited in diagnosing of tuberculous lymphadenopathy.

There was no difference between the ability of DS and DOB in diagnosing of tuberculous lymphadenopathy.
Further immunological research related to IFNγ and IL4 with more samples is needed, to know whether the existance of DS and DOB have any relation with allergic process.

## REFERENCES


[1] V. Kumar, R. S. Cotran, and S.L. Robbins SL. Basic Pathology. 7th. Edition. Philadelphia: Saunders; 2003.

[2] J. A. Linsk, S. Franzen. Clinical Aspiration Cytology. J.B. Lippincott Company; 1983.

[3] S. K. Lau, W. I. Wei, C. Hsu, and C. G. Engzell, "Fine needle aspiration biopsy of tuberculous cervical lymphadenopathy." Aus. N.Z. J. Surg., vol. 58, pp. 947-950. 1988.

[4] M. S. Metre, and S. Jayaram. Acid Fast Bacilli in Aspiration Smears from Tuberculous Lymph Nodes Analyis. Acta Cytol., vol. 31, pp: 17-19. 1987.

[5] S. R. Orell, G. F. Sterret, M. N-I. Walters, and D. Wjitaker. Manual and Atlas of Fine Needle Aspiration Cytology. Edinburg: Churchill Livingstone; 1986.

[6] D. S. Ridley, and M. J. Ridley. "Rationale for the histological spectrum of tuberculosis." Basis for Classification. Pathology., vol. 19, pp: 186-192. 1987.

[7] Minimum essentials of laboratory procedure for tuberculosis control. Japan international cooperation agency, The research institute of tuberculosis, Japan anti-tuberculosis association, 1987.

[8] H. M. D. Lubis, H. M. L. Lubis, Lisdine. and N. W. Hastuti. Dark specks and eosinophilic granular necrotic material as differentiating factors between tuberculous and nontuberculous abscess. Majalah Patologi., Vol: 2, pp: 49-52. 2008.

[9] Delyuzar. Korelasi antara massa eosinofilik dengan partikel coklat gelap dengan Mycobacterium tuberculosis pada sitologi biopsi aspirasi, Disertasi Program Studi Doktor (S3) Ilmu Kedokteran FK USU Medan, 2019.

[10] J. Balaji, S. S. Sundaram, S. N. Rathinam, P. A. Rajeswari, and M. L. V. Kumari. Fine needle aspiration cytology in childhood TB lymphadenitis. The Indian Journal of Pediatric., vol: 76:, pp: 1241–1246. 2009.

[11] D. Muyanja, R. Kalyesubula, E. Namukwaya, E. Othieno, and H. Mayanja-Kizza. Diagnostic accuracy of fine needle aspiration cytology in providing a diagnosis of ervical lymphadenopathy among HIV-infected patients. Afr Health Sci., vol: 5(1), pp:107–116. 2015. doi:10.4314/ahs.v15i1.15

[12] S. Romagnani. T-cell subsets (Th1 versus Th2). Asthma Immunology., vol: 85, pp: 9. 2000.

[13] K. Dheda, J. S. Chang, R. A. Breen, L. U. Kim, J. A. Haddock, J. F. Huggett, M. A. Johnson, G. A. Rook, and A. Zumla. In Vivo and in vitro studies of a novel cytokine, interleukin 4delta2, in pulmonary tuberculosis. Am J Respir Crit Care Med., vol: 172(4), pp: 501-8. 2015.

[14] S. Ashenafi, G. Adereye, A. Bekele, M. Zewdie, G. Aseffa, A. T. Hoang, B. Carrow, M. Habtamu, M. Wijkander, M. Rottenberg, A. Aseffa, J. Anderson, M. Svensson, and S. Brighenti. Progression of clinical tuberculosis is associated with a Th2 immune response signature in combination with elevated levels of SOCS3. Clin Immunol., vol: 151(2), pp: 84-99. doi: 10.1016/j.clim.2014.01.010. Epub 2014 Feb 9.


★★★