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Article Name 4- **Lymphocytopenia as a Mortality Predictor in Non - HIV Pulmonary TB Patients**

Authors Seyed Mehdi Mirsaeidi 1, Saeid Zareiy 1, Seyed Davood Mansoori 1, Payam Tabarsi 1, Majid Valioallahpour Amiri 1, Mehdi Kazempour Dizaji 1, Mohammad Reza Masjedi 2, and Ali Akbar Velayati 3

Authors Specialization 1 Department of Infectious Diseases, 2 Department of Pulmonary Medicine, 3 Department of Pediatrics, NRITLD, Shaheed Beheshti University of Medical Sciences and Health Services, TEHRAN-IRAN

Introduction

Tuberculosis (TB) is among the top ten causes of global mortality and affects low-income countries in Correspondence to: Mirsaeidi SM Tel:+98-21-2296362 E-mail address: mmirsaeidi@nritld.ac.ir particular (1). Mortality from tuberculosis is high even among patients without multidrug resistance who are not known to be infected with HIV (2). TB is responsible for more than two million deaths per year worldwide (3). Decreased survival is significantly associated with HIV – seropositivity, old age, and failure to complete the full treatment regimen and a low CD4 T-lymphocyte counts (4). CD4+ cell counts were reported below 300 cells / mm3 in HIV-seronegative active pulmonary tuberculosis (5). They had a very poor prognosis during the first weeks of treatment (6). There are several case reports that show relation of T-lymphocytopenia and attribution to disseminated tuberculosis (7,8,9,10). Knowledge of mortality risk factors in TB patients may improve their survival. With regard to the importance of lowered CD4 cell counts in predicting a poor prognosis for TB patients and due to the lack of flow-cytometric devices in low–income, high- contamination countries, it seems logical to look for an available and simple alternative as a predictor. In this study, we demonstrate that lymphocytopenia detected in peripheral blood count may be a useful predictor of mortality in TB patients.

Material & Method

This study was conducted at a university-affiliated hospital that provides medical care to tuberculosis patients as a tertiary center. The study design was retrograde case- control. All deceased cases during March 2002 to February 2003 with a definite diagnosis of tuberculosis (positive cultures for Mycobacterium Tuberculosis) or those cases compatible with a diagnosis of TB (clinically and radiologically) (11) who had not been tested for mycobacteria due to the lack of sputum or a short period of hospitalization were included in the study. Mortality was defined as death from any cause during the hospitalization period with TB diagnosis. Following re-evaluation of the case group (deceased), equal number of cases were selected from documented pulmonary TB patients (positive culture for Mycobacterium Tuberculosis) who had been hospitalized and discharged during last year, having been matched for sex and age with the original case group, as controls. Demographic data

and hematologic indices of the first complete blood counts (CBC) of patients at the time of hospitalization were recorded in a specified questionnaire. Cases were classified based on two definitions for lymphocytopenia. Lymphocytopenia was defined as either less than 15% of the total white blood cell count (12) or a total lymphocyte count of less than 1000/ml (13,14,15) or 1500 / ml (16,17) (depending on different classifications). The data were analyzed, using non-parametric measures for quantitative variables and X2 for qualitative variables. To evaluate the behavior of response variables, logistic regression test was used with Wald method. Sampling was sequential for the deceased group (cases) and simple randomized following sex and age matching for the living group (controls).

Result

The frequency of lymphopenia according to both definitions is as follows: The frequency of lymphocyte counts below 15% was 21(67%) for the deceased group and only 3(9%) for the living group. Total lymphocyte counts below 1000 were detected in 11(37%) of the deceased group and 5(15%) of the living group. For the below 1500 definition, 20 (64%) and 8 (24%) cases were detected in the case and control groups, respectively. No statistically significant difference was found in the WBC count of two groups. However, there was a significant difference in the percent and the total count of lymphocytes (p=0.0001, p=0.02). Following results were obtained for lymphocytopenia after excluding HIV seropositive cases. For the below 15% definition, OR = 20 with a CI 95% of 4.8-81 was obtained. For the below 1000/ml total lymphocyte count definition, OR = 3.5 with a CI 95% of 1.07 - 11.68 was calculated, and for the total below 1500 definition, OR = 5.6 with a CI 95% of 1.9 - 16.7 was obtained.

The major protective immune response against intracellular bacteria such as mycobacterium tuberculosis is cell – mediated immunity (18). It has been well established that CD4+ T–cells are the dominant protective T cells (19,20). A cell- mediated immune (CMI) response of the Th1 type is essential to mount a protective immunity against M. tuberculosis. (21,22,23) Based on previous studies, it is evident that in a normal situation CD4+ cells comprise 40-65% (13,24,25) of peripheral blood lymphocyte counts, lymphocytopenic patients show a prominent decrease in this sub-population (13). Alterations in blood lymphocyte have been well-established in pulmonary TB patients (26,27,28). It has been shown that a decrease in the total lymphocyte counts may occur, particularly early in the diagnosis period, and gradually increases with proper treatment. Collazos et al. (17) showed that lymphocytopenia was present in 22% of TB patients before treatment, and it raised to a high percentage following chemotherapy after 27 weeks. Morris et al. (29) also reported lymphocytopenia in 17% of diagnosed patients. Despite known lymphocytopenia in TB patients, to our knowledge, it has not been reported to date a correlation similar to what we observed between lymphocytopenia and mortality. Although the reasons for the initial decline of lymphocytes are unclear, several arguments support a homeostatic mechanism. First, active tuberculosis has been related to apoptosis of circulating lymphocytes, (30) which can decrease the total number of these cells. Second, a complex network between stimulatory and inhibitory cytokines and other products such as products of Th1 activation (31) may produce this cytopenia. Third, a paralyzed immunity may occur due to over stimulation with mycobacteria poly - antigens, suppressing cell over production in the bone marrow. Fourth, although

Discussion

mycobacterial infections stimulate antibody responses, humoral immunity seems to play little part in the protection against tuberculosis (31). However, a decrease in B- lymphocyte counts in patients, at different stages of tuberculosis with respect to controls, has been reported (32,33) that indicates a global lymphoid involvement in tuberculosis. Due to the above reasons, we conclude that lymphocytic response to active tuberculosis involves T and non-T cells that make total circulatory lymphocyte pool. Although Wessels et al. (34) showed that full blood count has no diagnostic predictive value in diagnosis of childhood TB, we showed that full blood count has a good predictive value for mortality due to tuberculosis in adults. This study demonstrated that lymphocyte counts below 1000 were present in 11 (37%) of the deceased cases and in 5 (15%) of the living cases; the difference was significant (p= 0.038). A significant difference was also detected for the below 15% definition of lymphocytopenia which was evident in 21 (67%) and 3 (9%) of the case and controls respectively (p= 0.0001). By changing the definition of lymphocytopenia to counts below 1500/ml, such as what Collazos et al. (17) used as a definition, the difference between two groups becomes more significant (p= 0.02). Kony et al's study revealed that in the course of TB disease, CD4 counts below 300 correlate with the severity of symptoms. (5) Recently, authors have also reported a case of disseminated tuberculosis in association with idiopathic CD4 lymphocytopenia (35). It appears that a decline in CD4+ cell line and dissemination of disease along with high microbial burden of mycobacterium tuberculosis accounts for mortality in patients. Pilheu et al. (6) reported that CD4+ lymphopenia is associated with a poor prognosis in HIV- seronegative pulmonary TB patients.

1. Borgdorff MW, Floyd K, Broekmans JF. Interventions to reduce tuberculosis mortality and transmission in low-and middle- income countries. Bull World Health Organ 2002; 80(3): 217-27.
2. Pablos-Mendez A, Sterling TR, Frieden TR. The relationship between delayed or incomplete treatment and all-cause mortality in patients with tuberculosis. JAMA 1996, 16; 276(15): 1223-8.
3. Dolin PJ, Raviglione MC, Kochi A. Global tuberculosis incidence and mortality during 1990 – 2000. Bull World Health Organ 1994. 72(2): 213-20.
4. Garin B, Glaziou P, Kassa- Kelembho E, Yassibanda S, Mbelesso P, Morvan J. High mortality rates among patients with tuberculosis in Bangui, Central African Republic. Lancet 1997; 350(9087): 1298.
5. Kony SJ, Hane AA, Larouze B, Samb A, Cissoko S, Sow PS, et al. Tuberculosis-associated severe CD4+ T-lymphocytopenia in HIV-seronegative patients from Dakar. SIDAK Research Group. J Infect 2000; 41(2): 167-71.
6. Pilheu JA, De salvo MC, Gonzalez J, Rey D, Elias MC, Ruppi MC. CD4+ T-lymphocytopenia in severe pulmonary tuberculosis without evidence of human immunodeficiency virus infection. Int J Tuberc lung Dis 1997; 1 (5): 422-6.
7. Zaharatos GJ, Behr MA, Libman MD. Profound T- lymphocytopenia and cryotococcemia in a human immunodeficiency virus-seronegative patient with disseminated tuberculosis. Clin Infect Dis 2001; 33(11): E 125-8.
8. De Socio GV, Gerli R, Menichetti F. Disseminated tuberculosis and idiopathic CD4+ T- lymphocytopenia. Clin Microbiol Infect 1999; 5(10): 653-654.
9. Neukirch B, Kremer GJ. Disseminated extrapulmonary tuberculosis in

Refrences

idiopathic CD4 lymphocytopenia. Dtsch Med wochenschr. 1995; 120(1-2): 23-8.
10. Onorati P, Carfagna P, Palange P, Venditti M, Serra P. CD4(+) T-lymphocytopenia and pneumocystis carinii pneumonia in a patient with miliary tuberculosis. Eur J intern Med 2001; 12(2): 134-136.
11. Treatment of tuberculosis: Guidelines for national programmes. Geneva, World Health Organization, 2003 (document WHO / CDs/ TB/ 2003. 313).
12. Http://www.healthatoz.com/healthatoz/Atoz/ency/lymphocytopenia.htm
13. The Merck Manual of Diagnosis and therapy, Section , Ch. 135, leukopenia and lymphocytopenia accessed in www.merck.com / pubs/mmanual/ section II/ chapter 135 / 135 b.htm.
14. Miale JB. Laboratory Medicine, vol 15, Hematology, 6th ed, Mosby, st louis, 1982.
15. Thomas J. kipps, Lymphocytosis and lymphopenia, Williams Hematology, 6th ed, MC Graw Hill, 2001.
16. Slaurence A. Boxer, Disorders of phagocyte funcion, Text book of Medicine, 2/st ed. Saunders, 2000.
17. Collazos J, Martinez E, Mayo J, Rinon M. Response of lymphocyte subsets in patients under treatment for tuberculosis. Eur J Clin Microbiol Infect Dis 2000; 19(8): 623 – 6.
18. Kaufmann SH. Immunity to intracellular microbial pathogens. Immunol Today 1995; 16(7): 338 - 42.
19. Andersen P. Host responses and antigens involved in protective immunity to Mycobacterium tuberculosis. Scand J Immunol 1997; 45(2): 115- 31.
20. Cooper AM, Flynn JL. The protective immune response to Mycobacterium tuberculosis. Curr opin Immunol 1995; 7(4): 512-6.
21. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in interferon gamma gene- disrupted mice. J Exp Med 1993; 178(6): 2243-7.
22. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. J Exp Med 1993; 178(6): 2249-54.
23. Newport MJ, Huxley CM, Huston S,Hawrylowicz CM, Oostra BA, Williamson R, et al. A mutation in the interferon gamma-receptor gene and susceptibility to mycobacterial infection. N Engl J Med 1996; 335 (26): 1941-9.
24. Shah Gasempour S, Gerami M, Entezami Z. Enumeration of peripheral blood lymphocyte subsets in healthy Iranian papulation. Arch of Med 2001, 4(2): 80-3.
25. Mirsaeidi M, Shah Ghasempour, Amiri MV, et al. Lymphocyte sub-populations and lymphocyte Activation Markers in pulmonary TB patients, Tanaffos, 2002, 1(4), 37-44.
26. Ainslie GM, Solomon JA, Bateman ED. Lymphocyte and lymphocyte subset numbers in blood and in bronchoalveolar lavage and pleural fluid in various forms of human pulmonary tuberculosis at presentation and during recovery. Thorax 1992; 47(7): 513-8.
27. Onwubalili JK, Edwards AJ, Palmer L. T4 lymphopenia in human tuberculosis. Tubercle 1987; 68(3): 195-200.
28. Singh KJ, Ahluwalia G, Sharma SK, Saxena R, Chaudhary VP, Anant M. Significance of haematological manifestations in patients with tuberculosis. J Assoc physicians India 2001; 49: 788; 790-4.
29. Morris CD, Bird AR, Nell H. The haematological and biochemical changes in severe pulmonary tuberculosis. Q J Med 1989; 73(272): 1151-9.
30. Khomenko AG, Kovalchuk LV, Mishin VI, Pavliuk AS, Veselova AV. Increased apoptosis of immunocompetent cells as a possible mechanism

in the development of immunodeficiency in patients with acutely progressive tuberculosis. Probl Tuberk 1996; 6: 6-10.
31. Lai CK, Ho S, Chan CH, Chan J, Choy D, Leung R, Lai KN. Cytokine gene expression profile of circulating CD4+ T cells in active pulmonary tuberculosis. Chest 1997; 111(3): 606 - 11.
32. Dunlap NE, Briles DE. Immunology of tuberculosis. Medical clinics of North America 1993; 77: 1235 – 51.
33. Wada M. Flow cytometric analysis of peripheral T lymphocytes from patients with mycobacterial diseases. Kekkaku 1992; 67(5): 393 – 407.
34. Wessels G, Schaaf HS, Beyers N, Gie RP, Nel E, Donald PR. Haematological abnormalities in children with tuberculosis. J Trop pediatr. 1999; 45(5): 307 – 10.
35. Mirsaeidi SM, Amiri M, Jamaati HR, et al. Alveolar proteinosis, reccerrent VZV infection, TB lymphadenitis and aspergillus fumigatus arthritis in idiopathic CD4 T lymphocytopenia: A case report, European Respiratory Society (ESR), 2003 vienna. [Abstract].

Conclusion

Based on the results of this study it is possible to define lymphocytopenia as an appropriate measure to determine mortality risk during the course of tuberculosis. Meanwhile, it is necessary to precisely supervise and manage all TB patients with lymphocytopenia and maintain support of these patients until lymphocytopenia subsides. Prospective studies would help to confirm the evidence presented in this paper and to highlight this association.

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Images of Article

table 1.JPG , table 2.JPG