Diagnostic Values Of Neopterin In Serum And Bronchoalveolar Lavage Samples In Pulmonary Sarcoidosis

Deniz Dogan, Canturk Tascı, Seyfettin Gumus, Ergun Ucar, Arzu Balkan, Omer Deniz, Ergun Tozkoparan, Metin Ozkan, Halil Yaman, Hayati Bilgic

Gulhane Military Academy of Medicine, Department of Pulmonary Medicine,
06018, Ankara, Turkey,
dr_denizz@yahoo.com, ctasci@gata.edu.tr, sgumus@gata.edu.tr, eucar@gata.edu.tr, abalkan@gata.edu.tr, odeniz@gata.edu.tr, etozkoparan@gata.edu.tr, moazkan@gata.edu.tr, hbilgic@gata.edu.tr

Gulhane Military Academy of Medicine, Department of Biochemistry,
06018, Ankara, Turkey,
hyaman@gata.edu.tr

Abstract: Neopterin (NP), an marker of cellular immunity, is emitted from monocyte and macrophages through interferon-γ stimulation. Sarcoidosis is a systemic granulomatous disease and mainly involves lungs and lymphoid tissue. In this study we aimed to evaluate the diagnostic value of serum and Bronchoalveolar lavage (BAL) NP levels in lung sarcoidosis and its correlation through clinical parameters. Serum measurements were performed in 26 patients with sarcoidosis and 16 healty patients. Serum and BAL NP levels were significantly higher in the group with sarcoidosis (17.42±6.91 nmol/L and 1.50±1.46 mcmol/g-prt) than the group with non-sarcoidosis lung disease (12.38±7.46 nmol/L and 0.62±0.75 mcmol/g-prt) and the healthy group (8.12±4.20 nmol/L 0.42±0.22 mcmol/g-prt). In sarcoidosis BAL NP measurement was determined as 92%, 35%, 75% and 68% in means of specifity, sensitivity, PPD and NPD, respectively. In assessing sarcoidosis and tuberculosis differential diagnosis, BAL NP measurements were detected as 89%, 53%, 90% and 50% in means of specifity, sensitivity, PPD and NPD, respectively. BAL NP levels expressively proved correlation with radiological stage (r=0.879), FVC (r=-0.645), DLCO (r=-0.611) and DLCO/VA (r=-0.615). Serum NP levels just expressively showed correlation with FVC (r=-0.481) and FEV1 (r=0.507) values. It has been concluded that serum and BAL NP levels increased in sarcoidosis cases compared to healthy individuals and the other disease cases and these parameters are correlated with radiological stage, FVC and DLCO parameters, which are fundamental determinants in pulmonary sarcoidosis prognosis and treatment decision. We consider high BAL and serum NP levels may support sarcoidosis diagnosis in case invasive diagnostic applications cannot be made.

Keywords: bronchoalveolar lavage, neopterin, sarcoidosis, tuberculosis.

1. Introduction

Sarcoidosis is a kind of disease mainly involving the respiratory system with unknown etiology. It is characterized with granulomatous inflammations constituted by immune system in affected organs. A lot of chronical granulomatous diseases such as tuberculosis, wegener's granulomatosis, hypersensivity pneumonia can involve the lungs as well as sarcoidosis. This makes excluding other possible diagnosis difficult in sarcoidosis diagnosis. This difficulty has leaded researchers to conduct new studies in order to suggest markers which are specific in sarcoidosis diagnosis for a long time. Among these markers these are the ones mostly studied; angiotensin converting enzyme (ACE), interleukin–1 (IL1), interleukin-2 (IL2), interleukin-6 (IL6), interferon gamma (INF-γ), tumor necrosis factor alpha (TNF-α) and granulocyte monocyte colony-stimulating factor (GMCSF) [1]-[4]. However, no markers which are specific to sarcoidosis diagnosis have been proved in the studies made till now. Neopterin (NP) has been a pteridin derivative and has been used in diagnosis of lots of diseases and/or in determining their activities for nearly thirty years [5]-[7]. In the literature, there has been a limited number of studies searching NP levels in diagnosis of sarcoidosis and determining its activities [1], [3], [5]. In most of these studies, the number of cases is less than twenty and usually NP levels have been compared with healthy individuals. In addition, in most of these studies, NP levels have been evaluated just in serum and there has been a limited number of studies studying BAL NP level in sarcoidosis cases. In our study, we aimed to evaluate the diagnostic effectiveness of serum and BAL NP levels in untreated newly diagnosed pulmonary sarcoidosis cases and the relationship between the clinical, radiological and respiratory function parameters of the disease.

2. Materials & methods

2.1 Study Population

Having been approved by local ethics committee, total 86 cases followed by sarcoidosis prediagnosis in the Pulmonology Department of Gulhane Military Medical Academy between July 2009 and May 2010 were included to the study. 26 of these cases were diagnosed as having sarcoidosis histopathologically. FOB (Fiberoptic Bronchoscopy) was planned as the first process for sarcoidosis diagnosis. The diagnosis of cases not proving diagnostical results as a result of FOB process and/or rejecting FOB process was made through mediastinoscopy and/or thoracotomy. Sixteen healthy cases were recruited in the control arm.

2.2 Fiberoptic Bronchoscopy (FOB)

In FOB process firstly Bronchoalveolar lavage (BAL) samples were taken from all patients. BAL sampling was performed from the parts suitable lung lobe and segment where lung parenchyma lesions are highly observed in High Resolution Computed Tomography (HRCT), in patients not having radiologically explicit parenchymal lesion, sampling

was made from right lung middle lobe segments or left lung lingula segments. For BAL sampling, segment whose bronchoscope lead is determined beforehand was placed so that it could occlude bronchus lumen (wedge position), but this lumen can also be seen. Totally 100 ml (5X20 ml) sterile serum was given by physiological injector through a bronchoscope working channel and it was aspired with the same injector.

2.3 Biochemical Tests

Complete blood count, erythrocyte sedimentation rate, routine biochemistry, serum calcium, 24-hour urine calcium and arterial blood gas examination, Respiratory Function Test and Carbon monoxide diffusion (DLCO) tests were made on all patients with sarcoidosis prediagnosis taking place in this study. 10 ml venous blood sample was taken from all patients immediately after FOB process. Serums were separated from BAL and venous blood samples in 10 minutes through 3000 circulation/min. centrifuge and kept out of sun beam at -80 °C. In serum and BAL, NP measurements were made through high performance liquid chromatography (HPLC). For serum NP levels nmol/L, protein levels were measured through Lowry method as well as NP measurements in BAL material and BAL NP levels were explained as mcmol/g-proetin.

2.4 Radiological Assessment

Posteroanterior chest X-ray was taken for all patients and in phasing according to chest X-ray Siltzbach phasing was used [8], [9].

2.5 Statistical Assessment

All statistical analyses were made through SPSS 16.0 package program. All data were expressed as average±standard deviation unless otherwise specified. Averages showing parametrical distribution among groups were compared through student t test and the ones showing nonparametrical distribution were compared through Mann Whitney test. p values under 0.05 was based for statistical significance. To examine the correlation among data, for the ones showing parametrical distribution Pearson, for the ones showing nonparametrical distribution Spearman test was used. Correlation coefficients were expressed as r value. Chi-square test with Yates correction was applied to compare intergroup sex difference.

3. Results

26 of 86 cases which were followed with sarcoidosis prediagnosis were diagnosed histopathologically sarcoidosis. 16 cases for which bronchoscopy was made for hemoptysis etiology and foreign body aspiration suspicion but at which no pathology was determined for pulmonary and/or extrapulmonary according to clinical, radiological and bronchoscopic findings were taken into healthy control group. Demographical and clinical characteristics of study population was displayed in Table 1. Mean age in sarcoidosis cases was significantly higher than healthy control group. Men/Women ratios were statistically significant between healthy control group and sarcoidosis. 9 of sarcoidosis cases were diagnosed in the recent six months but not treated; therefore, BAL samples of these cases were not taken. BAL samples from totally 17 sarcoidosis cases were examined. Serum and BAL NP levels were significantly high in sarcoidosis group compared to healthy control group (p=0.006). Serum and BAL NP levels of study population were displayed in Table 2. When we evaluate the diagnostic effectiveness of serum and BAL NP levels between control group and sarcoidosis cases through ROC analysis and assessing the other diagnostic parameters, clinically and statistically significant diagnostic values were obtained only with BAL NP levels. In evaluating the relation between NP levels and respiratory function parameters, serum NP levels proved a negative correlation at a medium level with FVC and FEV1 values (p=0.034) while BAL NP levels proved a significant negative correlation with FVC, DLCO and DLCO/VA (DLCO for alveolar volume) values (p=0.021). Also, BAL NP levels showed strong correlation with radiological stage of sarcoidosis cases (R=0.879, p<0.001) (Figure 1) and significantly weak correlation (at the limit) with serum calcium levels (R=0.347, p=0.043). For other respiratory function parameters and biochemical parameters no significant correlation was found between serum and BAL NP levels.

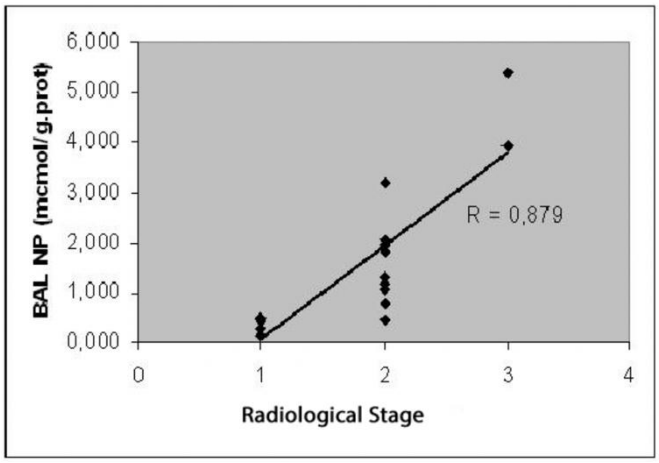

Figure 1: *The correlation graphic between BAL NP levels and radiologic stage*

Table 1: *Demographic characteristics of the study population*

Population	n	Age (years)	Gender (M/F)
Sarcoidosis	26	36.7±16.1[a,b,c]	(18/8)[a,b,c]
Control	16	26.8±9.7[c]	(16/0)[c]
p value		**a**, p=0.001 **b**, p<0.001 **c**, p=0.005	**a**, p=NS **b**, p=NS **c**, p=0.039

Table 2: *Serum BAL and NP levels in the study group*

Population	n	Serum NP (nmol/L)	BAL NP[1] (mcmol/g-prt)
Sarcoidosis	26	17.42±6.91[a,b,c]	1.50±1.46[a,b,c]
Control	16	8.12±4.20[c]	0.42±0.22[c]
p value		**a**, p=0.001 **b**, p=NS **c**, p<0.001	**a**, p=0.006 **b**, p=0.011 **c**, p=0.006

4. Discussion

If you are using Word, use either the Microsoft Equation Editor or the Math Type add-on (http://www.mathtype.com) for equations in your paper (Insert | Object | Create New | Microsoft Equation or Math Type Equation). "Float over text" should not be selected. Number equations consecutively with equation numbers in parentheses flush with the right margin, as in [1]. First use the equation editor to create the equation. Then select the "Equation" markup style. Press the tab key and write the equation number in parentheses. In this study, we have indicated that serum and BAL NP levels have increased significantly in sarcoidosis cases compared to healthy ones. Moreover, the most striking finding in our study has been the fact that BAL NP levels proved highly significant correlation with radiological stage of the disease in sarcoidosis cases. In examining the respiratory function parameters, BAL NP levels showed significant correlation with FVC, DLCO and DLCO/VA and serum NP levels showed significant correlation with FVC and FEV1. The biggest restriction in our study has been the fact that BAL samples could not be collected from all cases. For that reason, though the results show statistically significance, they should be confirmed with more comprehensive case series. In theory, another restriction of the data obtained from this study can be the fact that demographical characteristics among the groups show statistically significantly difference. Mean age value of sarcoidosis cases was nearly 10 years more than the healthy control group. Moreover, while eight of the sarcoidosis cases was women in sarcoidosis group, all patients were men in healthy group and this difference was statistically significant. There had been studies indicating slight changes in the NP serum levels with age according to creatine clearance. But, Turgan et al. declared there has been no variability in NP levels and age in Turkish population [12]. Moreover, Baganha et. al. have suggested age and NP concentration did not vary in accordance with sex. [13]. Based on these findings, we consider age and sex differences in the study population have negligible influence on serum and BAL NP levels. In sarcoidosis cases, there are not many studies evaluating inflammatory mediators in BAL. In a study it has been shown that IL8, a potent neutrophil chemotactic factor, has increased in BAL liquid [13]. In an older study, despite the number of cases was a few (n=10), it is shown that BAL NP levels were higher in sarcoidosis cases in comparison to healthy control and pneumonia cases [6]. Ziegenhan et al. have found that neutrophilia over 3% and eosinophilia over 1% in BAL cell count were indicative for a progressive disease [3]. These authors have indicated that BAL neutrophil levels increased not only in radiologically advanced stage cases (stage 3 and 4) but also in early stage (stage1 and 2) cases and this increase was correlated with systemic steroid necessity. It has been known that NP is a marker of increased monocyte/macrophage cell series. In previous studies, it has been suggested that serum and urine NP levels increased in active sarcoidosis and is an indirect marker of Th1 and INF-γ activity [12], [14]. Planck et al. have declared that serum NP levels which were high in pre-treatment period in ten sarcoidosis cases came back to normal in all patients apart from one patient after the illness remitted and became inactive [4]. One of the most important results obtained from our study is that BAL NP levels showed high correlation with radiological stage, FVC, DLCO and DLCO/VA which are the most significant parameters in clinical follow-up and in relation with the disease severity and prognosis in pulmonary sarcoidosis. While serum NP levels show correlation with FVC and FEV1, it doesn't show any correlation with other parameters, which can be deemed as clinically significant. According to our literature review, there is no other study which examines the relation of BAL NP levels with radiological stage in sarcoidosis and respiratory function test parameters. In our study, long-term follow-up findings of cases with sarcoidosis diagnosis have not taken place and, for that reason, prognostic role of serum and BAL NP levels in the disease has not been commented. However, it shows correlation with radiological prevalence and respiratory functions which have significantly determinative characteristic in prognosis and treatment decision, we think especially BAL and partly serum NP levels are effective in prognosis and treatment decision of sarcoidosis. As known, first line medication in the treatment of pulmonary sarcoidosis is corticosteroids. However, there is still no consensus regarding when to start the treatment. Comparing serum and BAL NP levels to findings in long-term follow-up, determining the cases requiring treatment may be clinically helpful.

References

[1] C. Prior, RA. Knight, M. Herold, G. Ott, MA. Spiteri, "Pulmonary sarcoidosis: patterns of cytokine release in vitro," European Respiratory Journal, IX (1), pp. 47-53.1996.

[2] P. Beirne, P. Pantelidis, P. Charles, AU. Wells, DJ. Abraham, CP. Denton, KI. Welsh, PL. Shah, RM. du Bois, P. Kelleher, "Multiplex immune serum biomarker profiling in sarcoidosis and systemic sclerosis," European Respiratory Journal XXXIV (6), pp. 1376-1382, 2009.

[3] MW. Ziegenhagen, ME. Rothe, M. Schlaak, J. Muller-Quernheim, "Bronchoalveolar and serological parameters reflecting the severity of sarcoidosis," European Respiratory Journal XXI (3), pp. 407-413, 2003.

[4] A. Planck, A. Eklund, J. Grunewald, "Markers of activity in clinically recovered human leukocyte antigen-DR17-positive sarcoidosis patients," European Respiratory Journal XXI (1), pp. 52-57, 2003.

[5] A. Berdowska, K. Zwirska-Korczala, "Neopterin measurement in clinical diagnosis," Journal of Clinical Pharmacy and Therapeutics," XXVI (5), pp. 319-329, 2001.

[6] JL. Dhondt, A. Darras, P. Mulliez, JM. Hayte, J. Crinquette, "Unconjugated pteridines in bronchoalveolar lavage as indicators of alveolar macrophage activation," Chest Journal XCV (2), pp. 348-351, 1989.

[7] KH. Mohamed, AA. Mobasher, AR. Yousef, A. Salah, IZ. El-Naggar, AH. Ghoneim, RW. Light, "BAL neopterin: a novel marker for cell-mediated immunity in patients with pulmonary tuberculosis and lung cancer," Chest Journal CXIX (3) pp. 776-780, 2001.

[8] N. Turgan, S. Habif, Z. Parildar, D. Ozmen, I. Mutaf, D. Erdener, O. Bayindir, "Association between homocysteine and neopterin in healthy subjects measured by a simple HPLC-fluorometric method," Journal of Clinical Biochemistry," XXXIV (4), pp. 271-275, 2001.

[9] MF. Baganha, A. Mota-Pinto, MA. Pego, MA. Marques, MA. Rosa, AJ. Cordeiro, "Neopterin in tuberculous and neoplastic pleural fluids. Lung CLXX (2), pp. 155–161, 1992.

[10] J. Muller-Quernheim, "Sarcoidosis: immunopathogenetic concepts and their clinical application," European Respiratory Journal, XXII (3), pp. 716-738, 1998.

[11] MW. Ziegenhagen, S. Schrum, G. Zissel, PF. Zipfel, M. Schlaak, J. Muller-Quernheim, "Increased expression of proinflammatory chemokines in bronchoalveolar lavage cells of patients with progressing idiopathic pulmonary fibrozis and sarcoidosis," Journal of Investigative Medicine, XLVI (5), pp. 223-231, 1998.

[12] BD. Car, F. Meloni, M. Luisetti, G. Semenzato, G. Gialdroni-Grassi, A. Walz, "Elevated IL-8 and MCP-1 in the bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrozis and pulmonary sarcoidosis," American Journal of Respiratory and Critical Care Medicine, CXLIX (3), pp. 655-659, 1994.

[13] M. Drent, JA. Jacobs, J. de Vries, RJ. Lamers, IH. Liem, EF. Wouters, "Does the cellular bronchoalveolar lavage fluid profile reflect the severity of sarcoidosis?," European Respiratory Journal XIII (6), pp. 1338-1344, 1999.

[14] A. Eklund, E. Blaschke, "Elevated serum neopterin levels in sarcoidosis," Lung, CLXV (6), pp. 325-332, 1986.

Author Profile

Author 1 D.D was awarded the title of Medical Doctor in 2002. He received specialty in Pulmonary Medicine at Gulhane Military Medical Academy between 2005-2010. He has been working as a chest diseases specialist since 2010.