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## Sputum Adenosine Deaminase Activity in Patients with Pulmonary Tuberculosis and Lung Cancer

### Aktywność deaminazy adenozyiny w płwocinie pacjentów chorych na gruźlicę i raka płuca

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#### Abstract

**Objectives.** The aim was to evaluate sputum adenosine deaminase (ADA) activity in patients suffering from pulmonary tuberculosis (TB) compared with patients with lung cancer and elucidate the possible applicability of ADA activity in patients with a negative acid-fast bacilli (AFB) smear.

**Material and Methods.** Sputum samples were collected from 34 patients with pulmonary TB (21 with positive and 13 with negative AFB smear) and 33 patients with lung cancer. The samples were diluted 1 : 6 and homogenized with phosphate buffer (70 mM, 0.5 Mmol NaCl, pH = 6) and, after centrifugation, ADA was determined in the supernatant by the Giusti method.

**Results.** The mean ADA value in the TB group was  $18.29 \text{ IU/l} \pm 15.76$  and in the lung cancer group  $11.25 \text{ IU/l} \pm 8.38$  ( $p = 0.026$ ). ADA activity in the TB AFB-positive group was  $16.57 \text{ IU/l} \pm 13.23$  and in the TBC AFB-negative group  $20.31 \text{ IU/l} \pm 15.89$  ( $p = \text{ns.}$ ).

**Conclusions.** Patients suffering from pulmonary TB have higher sputum ADA activity than patients with lung cancer. Sputum ADA activity may be a useful tool for the diagnosis of pulmonary TB, especially in the detection of active AFB smear-negative TB cases (*Adv Clin Exp Med* 2007, 16, 4, 533–535).

**Key words:** tuberculosis, adenosine deaminase, sputum.

#### Streszczenie

**Cel pracy.** Oznaczenie aktywności deaminazy adenozyiny (ADA) u pacjentów chorych na gruźlicę płuca w porównaniu z pacjentami chorymi na raka płuca i ocena możliwości zastosowania testu aktywności ADA u pacjentów z AFB-ujemnym rozmazem płwociny.

**Materiał i metody.** Próbkę płwociny zebrano od 34 pacjentów chorych na gruźlicę płuca (21 – z dodatnim rozmazem i 13 – z ujemnym rozmazem AFB płwociny) i 33 pacjentów chorych na raka płuca. Próbkę rozcieńczono w proporcji 1 : 6 i homogenizowano za pomocą bufora fosforanowego (70 mM; 0,5 Mmol NaCl, pH = 6) i po odwirowaniu oznaczono aktywność ADA w nadsączu metodą Giustiego.

**Wyniki.** Średnia wartość ADA w grupie pacjentów chorych na gruźlicę płuca wynosiła  $18,28 \pm 15,76 \text{ IU/l}$ , a w grupie pacjentów chorych na raka płuca  $11,25 \pm 8,38 \text{ IU/l}$  ( $p = 0,026$ ). Aktywność ADA w grupie pacjentów chorych na gruźlicę płuca AFB-dodatnich wynosiła  $16,57 \pm 13,23 \text{ IU/l}$ , a w grupie pacjentów chorych na raka płuca AFB-ujemnych  $20,31 \pm 15,89 \text{ IU/l}$  ( $p = \text{ns.}$ ).

**Wnioski.** Aktywność ADA w płwocinie pacjentów chorych na gruźlicę płuca jest większa niż w płwocinie pacjentów chorych na raka płuca. Oznaczenie aktywności ADA w płwocinie może być użytecznym narzędziem do rozpoznania gruźlicy płuca, zwłaszcza przypadków AFB-dodatnich (*Adv Clin Exp Med* 2007, 16, 4, 533–535).

**Słowa kluczowe:** gruźlica, deaminaza adenozyiny, płwocina.

Tuberculosis (TB) remains the most widespread and deadly infectious disease worldwide and it has been recently declared a great emergency by the World Health Organization (WHO) [1]. The mainstay of successful treatment largely depends on early diagnosis, usually accomplished through the analysis of clinical and radiological findings. The most important diagnostic method is the bacteriological detection of *Mycobacterium tuberculosis*. However, new methods are being developed, since pulmonary tuberculosis quite often poses a diagnostic problem in verifying a confident and early diagnosis [2].

ADA is an enzyme that catalyzes the hydrolytic and irreversible deamination of adenosine to inosine as well as deoxyadenosine to deoxyinosine. The determination of ADA concentration in pleural fluid is currently used for the diagnosis of tuberculous pleurisy [3]. There are also studies showing high levels of ADA in serum and bronchoalveolar lavage (BAL) of patients with pulmonary tuberculosis [4]. On the other hand, there are only a small number of studies which have investigated the possible role of ADA activity in material obtained with non-invasive methods, such as sputum [5]. Even in the presence of the disease, sputum may be acid-fast bacilli (AFB) negative and quite often bronchoscopy cannot be carried out as many patients either have many contraindications or they cannot tolerate this procedure.

The aim of this study was to evaluate sputum ADA activity in patients with pulmonary TB compared with patients with lung cancer and to examine the possible usefulness of ADA activity in patients with negative AFB smear.

## Material and Methods

Thirty-four consecutive immunocompetent patients with pulmonary TB and 33 consecutive patients with histologically or cytologically confirmed bronchogenic carcinoma (22 patients with

non-small-cell lung cancer and 11 patients with small-cell lung cancer) were included in the study. Patients with pulmonary TB had suggestive clinical symptoms and/or radiological findings together with at least one of the following: 1) a positive Ziehl-Nielsen result, 2) a positive sputum culture 3), or histopathological findings that indicate TB. Sputum samples were obtained from all patients included in the study for microbiological and cytological examination. Ziehl-Nielsen staining and Löwenstein culture were performed according to the hospital protocol.

Sputum samples were diluted 1 : 6 and homogenized with 70 mmol phosphate buffer (pH 6.0) containing 0.5 Mmol NaCl, kept on a bench rocker for 12 hours at 4°C, and then centrifuged at 16,000 rpm for 30 minutes at -4°C. Total ADA activity was measured in the supernatant by the Giusti method [6]. The results were corrected by multiplying them with the dilution coefficient. Measurements were performed on the same day by the same investigator, who is very experienced in interpreting these results.

Statistical analysis was performed using non-parametric methods (Mann-Whitney) for comparison between tuberculosis and lung cancer patients. Statistical significance was set at  $p = 0.05$ . Receiver operating characteristics (ROC) curves were constructed in order to establish a sensitivity-specificity relationship.

## Results

The mean age of patients in the TB group was lower than that of patients in the lung cancer group, but the difference was not statistically significant ( $p = 0.246$ ). Of the 34 patients with pulmonary TB, 21 were smear positive and 13 smear negative. The mean value of sputum ADA in the TB group was 18.29 IU/l, while it was 11.25 IU/l in the lung cancer group ( $p = 0.026$ ). Using a cut-off level of 15 IU/l for TB, the sensitivity and specificity were

**Table 1.** Sputum ADA values of the patients in the two groups as well as of patients with pulmonary TB according to Ziehl-Nielsen staining

**Tabela 1.** Wartości ADA w płwocinie u pacjentów z dwóch grup i chorych na gruźlicę płuca wg skali Ziehla-Nielsen

	TB (Gruźlica)	Lung cancer (Rak płuc)	TB AFB (+)	TB AFB (-)
N (males)	34 (20)	33 (19)	21	13
ADA activity (Aktywność ADA) U/l	18.29 ( $\pm$ 15.76)	11.25 ( $\pm$ 8.38)	16.57 ( $\pm$ 13.23)	20.31 ( $\pm$ 15.89)
p	0.026		ns.	

60% and 92%, respectively. The mean value of sputum ADA activity in the TB subgroup of patients with positive AFB result was 16.57 IU/l and in patients with AFB-negative result it was 20.31 IU/l, with the difference not being statistically significant (Table 1).

## Discussion

ADA is an enzyme found in the majority of cells, particularly in lymphocytes and monocytes, and it has two isoenzymes, ADA1 and ADA2. High ADA activity in pleural fluid is valuable in the diagnosis of tuberculous pleurisy and it is increased in the BAL of patients with pulmonary tuberculosis compared with patients with lung cancer [3, 4].

ADA levels have been so far measured in material obtained using invasive methods such as bronchoscopy and thoracentesis, and very few studies have examined ADA activity on material

obtained by non-invasive methods. Sputum ADA activity of the TB group was significantly higher than in the lung cancer group, which is in accordance with the results of a first report by the present authors [7]. When the two subgroups of TB patients were examined separately, relying on the positive or negative result of Ziehl-Nielsen staining, it was found that sputum ADA was comparatively high in both subgroups. It seems that sputum ADA is a valuable marker for the diagnosis of pulmonary TB, although high values of ADA were reported in BAL of patients suffering from other respiratory infections such as chronic pneumonia and empyema thoracis [8]. The simplicity of obtaining material such as sputum makes this measurement a useful diagnostic tool, especially when the AFB smear is negative, although the sensitivity of this test is comparatively low. In this way, the determination of an increased ADA activity in smear-negative patients who are strongly suspected of having pulmonary tuberculosis may be helpful in approaching diagnosis and initiating treatment.

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