DIFFERENCES IN BAL MACROPHAGES CYTOLOGY DETERMINED BY MORPHOMETRIC ANALYSIS IN PATIENTS WITH SARCOIDOSIS

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BACKGROUND: Morphometric and image cytometric DNA analysis of the macrophages nuclei in BAL fluid of patients with sarcoidosis have been investigated in order to establish their diagnostic and clinical significance. In sarcoidosis macrophages are antigen presenting cells responsible for granuloma formation, however also neoplastic T cells, IL-15, IL-15, IL-17 and different growth factors. Morphological and image cytometric studies are larger than macrophages in healthy controls and possess different ultrastructural characteristics. They are more uniform in appearance than in hyperplasia preferentia and lack feature characteristics. Recently, three types of macrophages in sarcoidosis have been recognized ultrastructurally, 70% among them with signs of increased activity and cytokine release. Sarcoidosis onset can be acute or chronic with different outcomes.

MATERIAL AND METHODS: Seventy-three patients were included in the investigation (Table 1). BAL fluid (BALF) was obtained during diagnostic flexible bronchoscopy. Inclusion criteria for all patients with interstitial lung disease were lymphoma, bronchiolitis, sarcoidosis, IL-15, IL-17 and different growth factors. Morphometrical and DNA image analysis were performed on macrophages nuclei in cytological specimens of BALF. Archived original MOG stained slides were stained with Feulgen method for morphometric and DNA image analysis of macrophages nuclei. Random sampling was performed by systematic measurement of cells under microscope. Cells selection started at point inside a region with cellular material evenly distributed, processed cell by cell in one field, and continued to the next fields. Light microscopic analysis under immersion ½× was applied and analysis was conducted in one focal plane. In each case 100 or more nuclei were analyzed, and processed with an image analyzing software, FORM software for digital image analysis (FORMTEC, Zagreb). Objects contours were marked with special tools, interactive, by mouse selection (Figure 1). DNA image cytometric analysis of ploidy status was performed simultaneously as other morphometric measurements. Nuclei of neoplastic granulocytes were used as internal controls on the slide as investigated macrophages. DNA content was measured indirectly after quantitative DNA staining with Feulgen. IOD (integrated optical density) represents cytometric equivalent of DNA content, rescaling of the IOD values by comparison with IOD values of cells with known DNA content was necessary for quantification of nuclear DNA content of measured cells. Formulas of DNA content of investigated cell nuclei were included in Table 2. Statistical analysis was performed in Statistics for Windows version 6.0 (StatSoft, Inc., Tulsa, OK). Mean, median, standard deviation and minimum and maximum value was calculated for all morphometric parameters. Mean, standard deviation and 95% confidence interval (95% CI) were used for description of variables and variance analysis was used for comparison between the groups. X2-test was used for comparison of variables categories distribution among groups. Variables categories were represented as frequency (%). Classification criteria (diagnostic threshold values) were calculated with discriminant function analysis. Backward step-wise method in multivariate discriminant function analysis was performed because of sample size and complexity.

RESULTS:

Score that is largest among groups allows classification of macrophages population in that particular group of diseases and correct classification of sarcoidosis with acute or chronic onset.

REFERENCES: