NEUTROPHILS' MOVEMENT *IN VITRO*

Anna Korzyńska

Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, ul. Trojdena 4, 02-109 Warsaw, Poland

Ineffectiveness of neutrophils' motility is observed in some immunodeficiency' disorders. There is a need to find an adequate description and evaluation of the neutrophils' activity. Early studies describe an individual cell's behaviour by the Cell Motility Index (*CMI)* vector with three components: penetration, directionality and expansivenesses. Movement quantification has been improved by extending the cell's activity description to two stages of classification. In the first stage, one minute of each cell's behaviour has been classified into one of five classes of the cell's behaviour, using the k-NN method. In the second stage, the sample level, which corresponds to the final classification, the Shapiro-Francio test is used. The proposed new method has been used to describe differences between normal children and the Chediak-Higashi syndrome patients.

INTRODUCTION

Neutrophils' granulocytes, also called polymorphonuclear leukocytes, act as essential elements of the human immune system. Neutrophils demonstrate an active motility in response to inflammatory stimuli: they crawl towards the infected tissue and phagocyte or damaged bacterial cells. If their movement becomes ineffective, a patient suffers from recurrent, severe, pyogenic infections[1]. Immunodeficiency disorders diagnosis depends on indirect examination of neutrophils' samples. Their activity is evaluated using parameters describing population of cells in two static states. This quantification entails loss of information about the individual cell's behaviour and the dynamics of the process.

The development of image analysis methods, combined with improvements and reduction of the costs of imaging technology (quality, resolution and enlargement of microscopes), recording methods (low costs and high performances of video sensors), as well as power and speed of computers, have made real the direct examination of individual cell's behaviour in medical diagnostics[2-7].

A method for describing one cell's behaviour in time using the $CMI_{<\Delta t>}$ vector has been proposed in earlier studies[1,5,6]. This vector consists of three components (CMI^p, CMI^d, CMI^e), which correspond with three aspects of activity: the intensity of limited area *p*enetration, the *d*irectionality and the *e*xpansiveness of movement. CMI^p quantifies the intensity of penetration and describes how compactly or densely the cell path covers a given area. CMI^d quantifies the directionality, which is a measure of how effectively the cell covers a distance, and how much of its path is different from the straight line. CMI^e quantifies the expansiveness, as a measure of the area on which the cell executes its duty. The previous method[5] leads to the description of the single cell's behaviour by a sequence of numbers. These numbers are the sums of values of all the three aspects calculated for Δt, the minutes of examination $[CMI_{\Delta t,i}]_{i=1..q}$. The mean value of the time sequence and its standard deviation have been used to describe the single cell's activity during the whole examination time.

The new procedure involves classification of the cell's behaviour in Δt into one of the cell's behaviour classes in the 3D space of certain features. Thus, in this new approach, a single cell's behaviour becomes a time sequence of the classes' codes $[K_{\Delta t,i}]_{i=1..q}$. Then, the sample level of classification is performed, based on the distribution analysis of all cells' behaviour code in one sample. The classification criteria consider the fraction of the cells without any restriction of movement ability in the sample.

CLASSES OF CELLS' BEHAVIOUR

Three types of neutrophils' behaviour have been described[7]: chaotic, formal and directed (Fig. 1 with a 2 sec time increments sequence of elliptic models of the cell's contour) and some hybrid types of cell's behaviour in groups of normal subjects and patients with the cells' ineffectiveness of movement. The chaotic movement is correlated with a high value of the penetration index and with a middle or high value of expansiveness. The directed movement is correlated with a high value of directionality and with a middle or high value of expansiveness. The formal movement is correlated with a low value of expansiveness and with a middle value of the penetration index or directionality.

Detailed examination of the experimental material shows that, in the studied normal subjects, most of neutrophils' movement is more efficient in covering a field or a distance and is quicker and more expansive than in patients with a deficiency in the mechanism of crawling. Some of neutrophils move with a temporary restriction in their movement ability. All neutrophiles from patients with immunodeficiency connected with dysfunction of movement abilities, e.g. the Chediak-Higashi syndrome patients, exhibit restricted movement abilities. The distributions of codes of cells' behaviour differ in the two studied cases.

It is proposed to divide the 3D space of features into five classes (Fig. 2). CLASS I - cells showing expansiveness typical only of normal cells. CLASS II - cells showing an average expansiveness and a high penetration. CLASS III - an average expansiveness and a high directionality. CLASS IV - cells showing restricted movement ability. Lastly, CLASS V - for unclassified objects, which do not correspond to any of the other classes. The Nearest Neighbours Method (k-NN) is proposed to classify the examined cells' behaviour according to the above classification.

The distribution of cells' behaviour codes has been used as a background for the new method of the final classification on the sample level. Because the distribution of code of neutrophiles' in normal subjects is normally distributed, the Shapiro-Francio statistical test is proposed to examine whether the distribution of cells' behaviour codes is different from the Gaussian distribution.

These proposed methods of classification could easily be adjusted for a particular immunodeficiency, connected with the neutrophils' movement ineffectiveness, given a learning set containing observations. For example, the distributions of the behaviour of neutrophils, taken from a Chediak-Higashi syndrome patient, exhibit characteristics of this immunodeficiency bias.

MATERIALS & METHODS

To examine the proposed methods of classification, two specially constructed sets have been used: learning and testing sets. Each set contains description of one-minute cell's behaviour as the vector *CMI* with three components evaluated, using cell monitoring system[4,8]. Learning sets, consisting of 25 movement sequences, come from the experimental material collected from normal subjects[5] (Fig. 1). The testing set consists of the previously registered experimental material coming from healthy children and a Chediak-Higashi syndrome patient[1]. These sequences of neutrophils' behaviour have been compared to classification, which has been done by the operator during the video film examination.

RESULTS

Reclassification error of the k-NN method has the lowest value (8%) for five nearest neighbours examined during the learning test. But classification error, calculated on the testing set in comparison to classification made by an operator, had a higher value of 22%. The error of one time unit of the cells behaviour examination should fall in the next step of classification, where the distribution of neutrophils' behaviour of all cells in the sample is considered. The sample level of the classification has not been verified because of a small number of the experimentally collected samples. However, all single cell's behaviour classification was different from the operator's classifications in 15% cases.

Two samples, one from healthy normal children and one from the Chediak-Higashi syndrome patient, were examined and both showed results similar to those obtained in the previous investigations.

CONCLUSION

The proposed methods of classification are independent of the type of the neutrophil's ineffectiveness of in

movement. They can be adjusted to the particular syndrome, according to the introduction of experimental studies. The methods can be also adjusted to the changes in time unit of behaviour examination.

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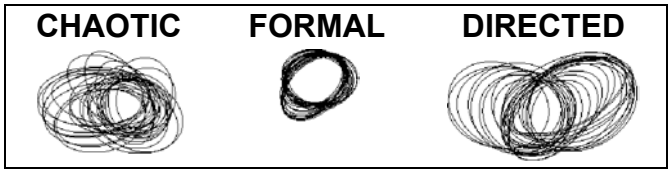

Figure 1: Basic Types of neutrophils' behaviour

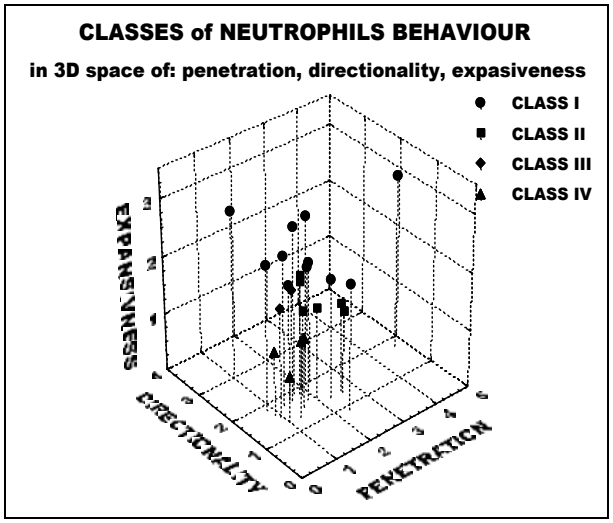

Figure 2: Classes of neutrophils' behaviour; learning set used in 5-nearest neighbours method of cell's one minute behaviour characterization