MOBILITY OF NEUTROPHILS IN PATIENTS WITH INFECTED WOUNDS (EXPRESS ANALYSIS USING THE MAGISCAN IMAGE PROCESSOR)

A. A. Galkin, E. A. Tumanov, M. M. Filippov, A. M. Svetukhin, E. N. Timin, and A. A. Karelin

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The clinical use of a test of leukocyte mobility to assess the functional state of the nonspecific immunity system is restricted by the lack of any express method of recording and processing the data. In recent years semiautomatic systems have been developed for studying mobility of various types of cells [2, 6], and completely automated methods of recording and analysis of mobility also have been produced on the basis of image processing systems [1, 4]. Until now automatic methods have been used only in experimental research.

The investigation described below is the first attempt at clinical use of an automatic image processing system for the express analysis of leukocyte mobility in patients with wound infection.

EXPERIMENTAL METHOD

Leukocytes were isolated from a drop of blood by the known method [5, 9]. Several drops of blood, taken from the finger or the vein, were placed on a small coverslip and incubated at 37°C in a humid chamber for 10-15 min. After incubation the coverslip was washed to remove clots and erythrocytes by gentle rinsing in a small glass jar containing Hanks' solution. The coverslip with neutrophils adherent to it was transferred to a slide, on which a ridge of petrolatum had been applied beforehand around the perimeter of the small coverslip, filled with Hanks' medium, pH 7.3, containing additionally 0.5% of human serum albumin ("Fluka," Switzerland). The preparation was sealed above with a large coverslip so that an airtight nondrying chamber containing the cells for testing was formed.

The preparation was transferred to the heated stage of a "Jena-Mikroscop 250CF" microscope (East Germany), and heated for about 10 min to 37°C. With a ×6.3 objective and a frame measuring 512 × 512 μ, the number of cells in a field of vision must not exceed 70. The dark-field image was stored in a "Magiscan 2A" image processing system with interval of 1 min between frames. usually 20 frames were recorded to obtain a statistically significant picture of cell behavior. Subsequent mathematical analysis yielded information on the velocities of movement of the cells [1].

EXPERIMENTAL RESULTS

Trajectories of movement of neutrophils isolated from healthy human blood are given in Fig. 1a. The writers found previously [1] that the mean velocity of each neutrophil is constant for a long time (4-5 h), and that in each population cells are present with mobility ranging from 0 to 30 μ/min. Dependence of the trajectory of each neutrophil on time is shown in Fig. 1b. Dependence of the mean path traversed by all cells of the population on time is shown in Fig. 1c. Just as in [1], constancy of the velocity of the population as a whole and relative constancy of individual cell velocities will be seen. A histogram of displacements per minute (velocities) is given in Fig. 1d. Clearly the velocities of the neutrophils did not exceed 55 μ/min and lay most frequently between 5 and 10 μ/min.

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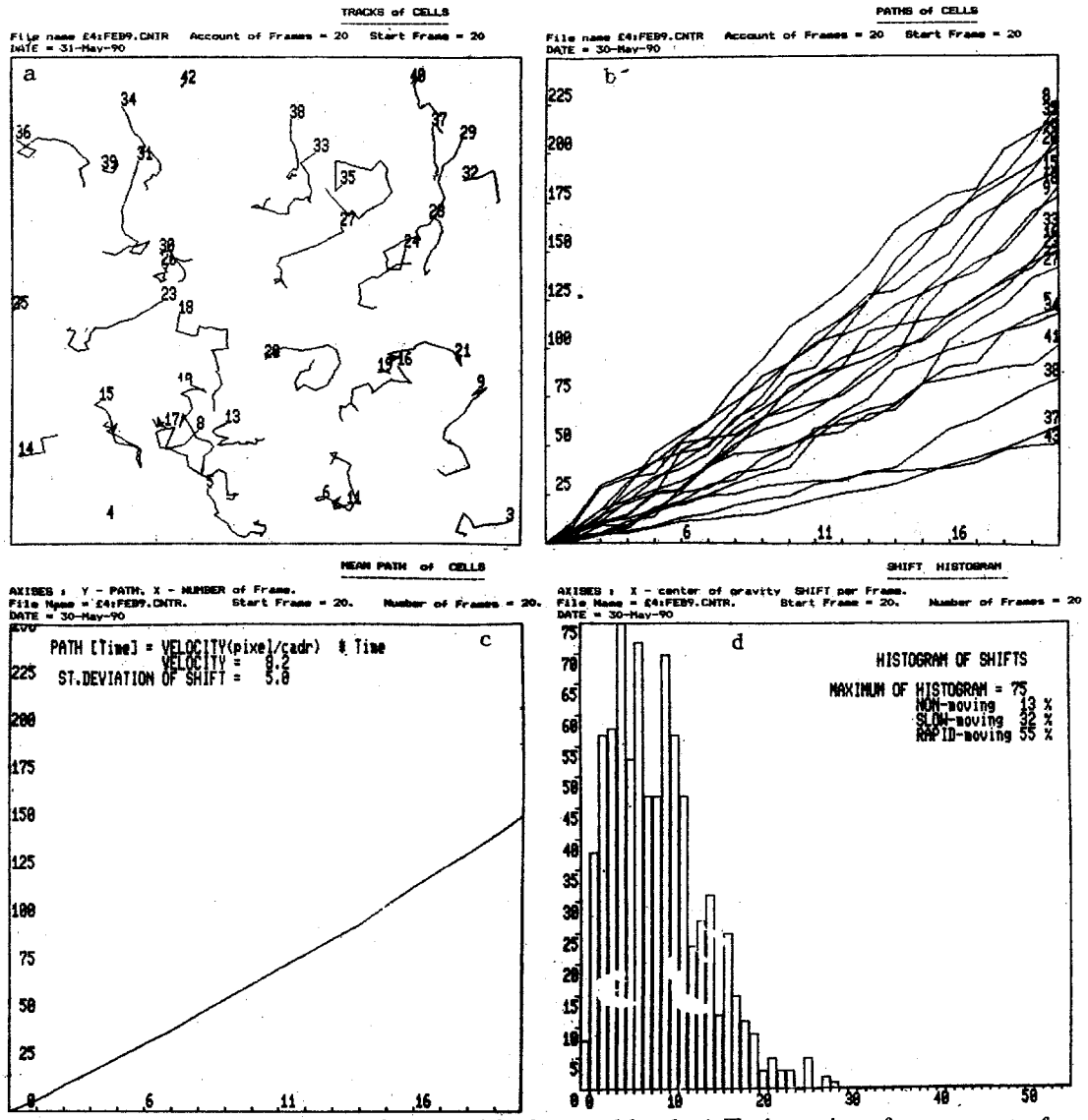

Fig. 1. Mobility of neutrophils isolated from healthy human blood. a) Trajectories of movement of neutrophils for 20 frames with interval of 1 min between frames, b) graph showing dependence of path traversed by each cell on time; X_{axis} — time (in min), Y_{axis} — path (in μ); c) mean path traversed by neutrophils as a function of time; d) distribution of minute shifts (velocities); X_{axis} — shifts (in μ); Y_{axis} — number of shifts.

Trajectories of movement of neutrophils isolated from the blood of a patient with severe wound infection are given in Fig. 2a, whereas individual paths of cells from the same preparation are shown in Fig. 2b. The average velocity in the preparation was much less than that of healthy donors (Fig. 2c). The histogram of velocities of neutrophils of a severely ill patient was shifted to the left (Fig. 2d). Comparison of the results given in Figs. 1 and 2 suggests that mobility of neutrophils can be used as an objective indicator of the severity of the state of patients with wound infection.

The mean velocity proved to be a stable parameter of mobility of the population: for instance, in the same sample, in different parts of the preparation, the mean population velocity for 30-70 cells did not differ by more than 0.7 μ/min.

To discover how characteristic was this decrease in neutrophil mobility in patients with wound infection, we tested two groups of patients, differing in the severity of their state.

Just as was done in [3], the whole cell population was divided into fast-moving, slow-moving, and nonmoving. The nonmoving cells were those which completed a shift of under 2 μ/min, slow-moving were cells with a speed of 2 to 7 μ/min, and fast-moving, cells with a speed of over 7 μ/min.

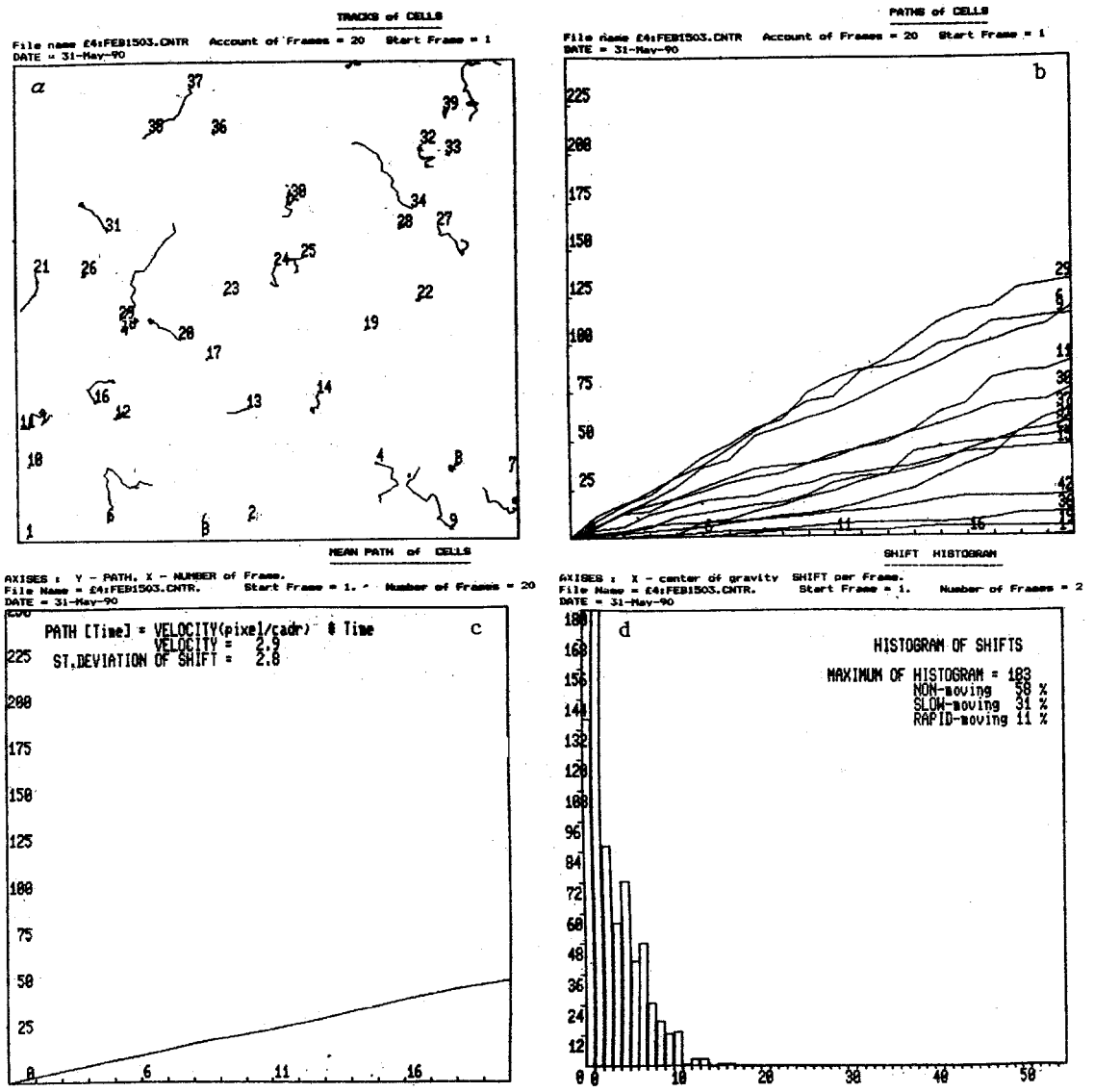

Fig. 2. Mobility of neutrophils isolated from blood of patient with severe wound infection: a) trajectories of movement of neutrophils for 20 frames; b) dependence of path traversed by each cell on time; X_{axis} — time (in min); Y_{axis} — path (in μ); c) dependence of mean path traversed by cells on time; d) distribution of minute shift (velocities); X_{axis} — amount of shift (in μ); Y_{axis} — number of shifts.

The control group consisted of 37 healthy blood donors. A histogram of the mean velocities in this group of donors is illustrated in Fig. 3a. Clearly the average velocities are distributed over the range from 7 to 11 μ/min, the mean value is 8.8 μ/min, and the average relative percentages of non-, slow-, and fast-moving neutrophils were 16, 28, and 56 respectively. A mean velocity of neutrophils of 7 μ/min was taken as the lower limit of normal, whereas a mean velocity below 7 μ/min was taken as a certain degree of pathology. For comparison with the norm, the mobility of neutrophils was studied in a group of 20 patients with severe wound infection. A histogram of the mean velocities of the neutrophils in this group is illustrated in Fig. 3b. It will be clear from Fig. 3b that the velocities were distributed over the range from 0 to 6 μ/min. The wide range of mean velocities of neutrophils in this group can be explained on the grounds that severely ill patients were subjected to the most intensive therapeutic procedures (surgical operations, hemoperfusion, plasmapheresis, and antibiotic therapy, etc.). The mean velocity of the neutrophils in this group was 3.4 μ/min and the relative percentages of non-, slow-, and fast-moving neutrophils were 53, 29, and 19, respectively. A histogram of the mean velocities of movement of neutrophils in a group of 26 patients with a state of average severity is illustrated in Fig. 3c. The velocities

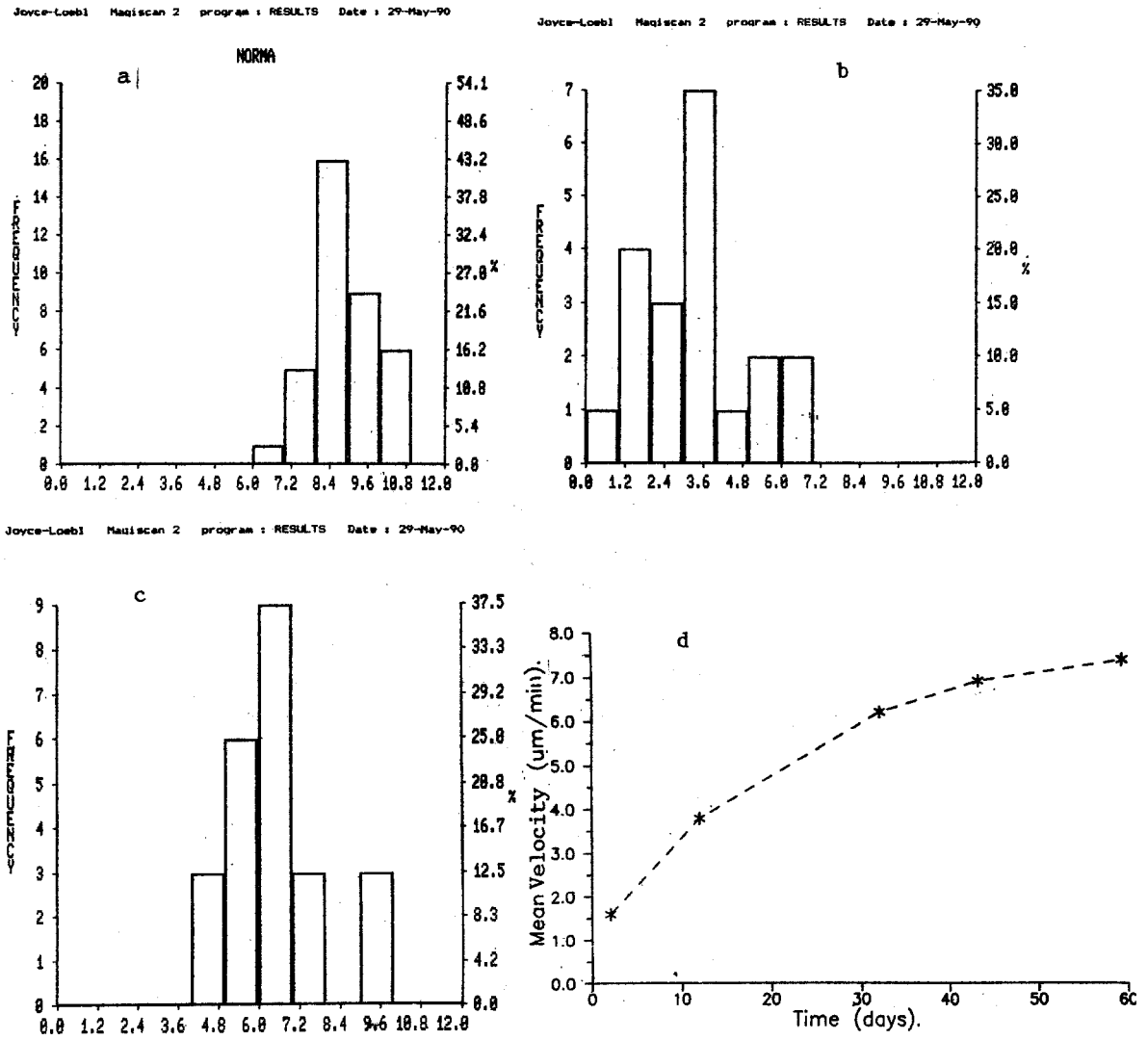

Fig. 3. Distribution of mean velocities of neutrophils in three groups of blood donors, differing in severity of their state. a) healthy donors; X_{axis} — average velocity (in μ); Y_{axis} — number of donors; b) patients with severe wound infection; c) patients with moderately severe wound infection; d) changes in neutrophil mobility of patient with severe infection during treatment; X_{axis} — time (days); Y_{axis} — mean velocity of movement of neutrophils (in μ).

are distributed within the range from 4 to 8 μ/min, the mean velocity for the group is 6.6 μ/min, and the relative percentages of non-, slow-, and fast-moving neutrophils are 26, 34, and 40 respectively. The data are summarized in Table 1.

The mean velocities of the neutrophils for groups of subjects differing in a general feature such as the severity of their condition differed statistically significantly by Student's test. Correlation between the velocity of movement of the neutrophils and severity of state for all three groups was 85%, whereas between groups of healthy individuals and patients with severe wound infection it was 90%, and between groups of patients with moderately severe infection and groups of healthy individuals and patients with severe infection it was 66 and 73% respectively. The overlapping of the distributions of velocities between groups can be explained, on the one hand, by the subjectivity of determination of severity of state, and on the other hand, by the character of therapeutic measures.

TABLE 1. Mean Velocity (in μ/min) and Composition of Neutrophils (in per cent) in Groups of Blood Donors

Group	Velocity, μ/min	Neutrophils		
		non-moving	slow-moving	fast-moving
Healthy	8,8±0,2	16	28	56
Patients with moderately severe infection	6,6±0,3	26	34	40
Patients with severe infection	3,4±0,4	53	29	19

Worsening of the patient's general state was accompanied by a decrease in the mean velocity of the neutrophils and in the number of fast-moving cells and an increase in the number of non-moving cells. On the basis of one arbitrarily taken blood sample, the state of the patient can be judged only with a certain degree of probability, but a velocity of under 3 μ/min undoubtedly signifies a patient with a severe infection, whereas a velocity of 5-6 μ/min corresponds highly probably to a state of average severity.

The time course of changes in neutrophil mobility during treatment of a patient with extremely severe wound infection is shown in Fig. 3d. Measurement of mobility, it will be seen, can be an objective indicator of the course of treatment. Daily testing of the patient enables the efficacy of pharmacotherapy to be evaluated.

We know from data in the literature [3, 7] that a decrease in neutrophil mobility is observed in diseases of varied etiology: bacterial infectious diseases, oncologic diseases, burns. To explain the decrease in motor activity of the blood neutrophils three hypotheses have been submitted. Two of them were based on the suggestion of a change in the subpopulation composition of the blood neutrophils or the escape of fast-moving cells into the tissues, or replacement of the blood by young, less mobile cells. The third hypothesis (alterative) explains the reduction of mobility by a direct inhibitory action of various serum factors or toxins on the neutrophils [8]. Our data show that the disturbances of mobility are stable and persist for several hours of observation on the cells. Support for the alterative hypothesis is given by an experiment in which cells from a healthy blood donor, with an initial mean velocity of 9 μ/min were added to the blood serum of a patient with severe wound infection. After only a few minutes the healthy cells were immobilized and their mean velocity fell to 3.7 μ/min, whereas in serum from a healthy blood donor, inhibition of mobility was not observed.

A test of the toxicity of the blood serum has been used for a long time in clinical practice. This test consists of determining the length of survival of freely-living infusorians (*Parameocium*; the Parameocium time test) in patients' blood serum. The method of express analysis of neutrophil mobility which we have developed can be used at the same time as a test of serum toxicity.

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