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Morfometry of white blood cells

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Abstract - The interpretation of the microscopical images of white blood cells by visual subjective methods and by image analysis mediated objective methods both have their own limitiations and advantages. Although abnormal functioning of white blood cells can be reflected by abnormal microscopical images, other methods than visual or mathematical study of these images will often be needed to demonstrate abnormal functioning. In comparing images of single cells the human eye has advantages, whereas morphometry has the exclusive possibility to quantitate cellular constituents in images, and to compare characteristics of collections of cells, using statistical pattern recognition techniques for multiparameter analysis. This latter possibility can expand comparative morphology of cells to comparative morphometry of collections of cells. The quantitation of cellular constituents can expand the possibilities of cytochemistry.

AUTOMATED WHITE BLOOD CELL ANALYSIS

The vast quantity of white blood cell differential counts, that are performed all over the world, stimulated several producers of medical equipment to develop image analysis computers for that purpose. However, both the high cost of these appararatuses and the realization, that the impact of the information provided by the WBC differential on clinical decision making was limited (1), caused a less than expected interest for these machines. Nonetheless, their construction reached a high degree of technical perfection, and their performance seemed to satisfy most of their users. The competition of cytochemistry based flowcytometry systems for whole blood and white blood differential count analysis was strongly felt, and several image analysis computers for white blood cell analysis were withdrawn from the market: the Larc(Corning), ADC 500 (Abbott), and Diff 3 (Perkin-Elmer, later Coulter). At present two image analyzing white blood cell differentiators are still being marketed: the Hematrak (Geometric Data) and the Microx (Omron). Less complicated and less expensive methods to provide information on the composition of the circulating white blood cell pool have become possible, based on the differences in volume between the white blood cell classes. Both light scattering techniques and measurements of electrical impedance are used in flowcytometry systems to determine volume distributions of white blood cells. That means, that for the description of the circulating white blood cells at the moment several possibilities are available. In the first place the classical visual interpretation of the slide; and further next to image analysis of these slides, the flowcytometry methods based on cytochemistry and light scatter, on light scattering only, or on electrical impedance measurements. Which will be the place for image analysis computers for white blood cell differentiation is not yet clear.

APPLICATIONS OF MORPHOMETRY OF WHITE BLOOD CELLS BEYOND THE ROUTINE DIFFERENTIAL COUNT

Morphometry of white blood cells has the same limitiations as the classical visual and subjective morphology, where the results of both methods depend on the preparation of the sample. Flattening, drying, fixation and staining causes artifacts, which on the one hand are needed to allow interpretation of image, but on the other hand cause unwanted changes unrelated to relevant characteristics of the substrate.For studies comparing morphometrical parameters it is of critical importance that uniform preparation procedures are

used. Otherwise the data will inevitably concern the differences in preparation instead of, or at best mixed with,the differences meant to be measured. At that it has to be realized, that the information obtained from cellular images is limited,since not all abnormalities in function come to expression in morphology.

Classification of immature cells. Classification of immature myeloid cells differs essentially from classification of normal peripheral blood cells. Immature myeloid cells are subdivided according to their arbitrary stage in a process of continuous maturation, whereas the cells from the peripheral blood represent the end stages of maturation processes, without transitional forms between them. In a study of immature myeloid cells it was shown (2)that differences in morphometric parameters were present between the classes of immatures -especially promyelocytes- selected by two technologists working separately, although both used the same reference material. Which features contribute most to these differences,and which features are relevant for the classification of immatures can be concluded from studies of this type, which increase the objectivity of differentiation of immature cell classes.

Comparison of collections of cells. Visual comparison of images of single cells can be performed at present more accurately by a trained human than by image analysis, but the comparison of collections of cells is beyond the possibilities of the human brain. This can be performed, however by morphometrical image analysis, which allows to compare many parameters extracted from many cellular images in statistically reliable ways at varying levels of sophistication.
An application is discussed by Landeweerd et al. (3),who described shifts in morphometrical parameters of lymphocytes and monocytes in patients with Hodgkins disease in comparison to normals. A normal region is defined by the combination of the normal ranges of the morphometrical parameters, which can be represented as an area in a plane, on which is projected the cluster of normal data located in an imaginary multidimensional space (Fig. 1).When a similar projection is made of the morphometrical data of the Hodgkins disease patients, many appear to be projected outside this normal range (Fig. 2). The parameters contributing most to this abnormal result can be analyzed to translate the morphometrical abnormalities back to visual features,e.g. nucleus/cytoplasm ratio. The relation between morphometrical abnormalities and differences in function of circulating lymphocytes was not studied.

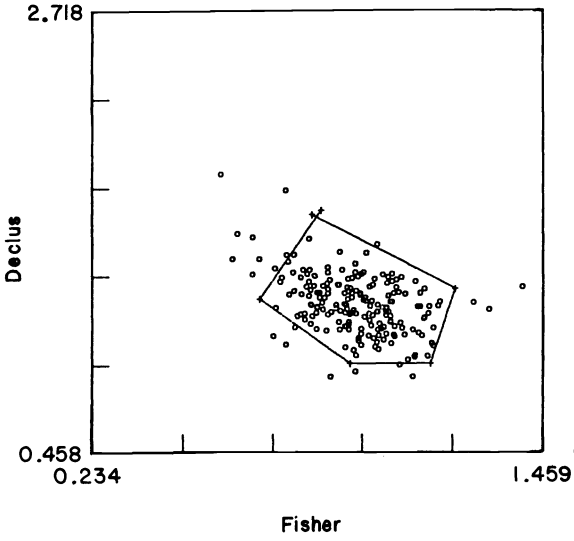

Fig. 1.

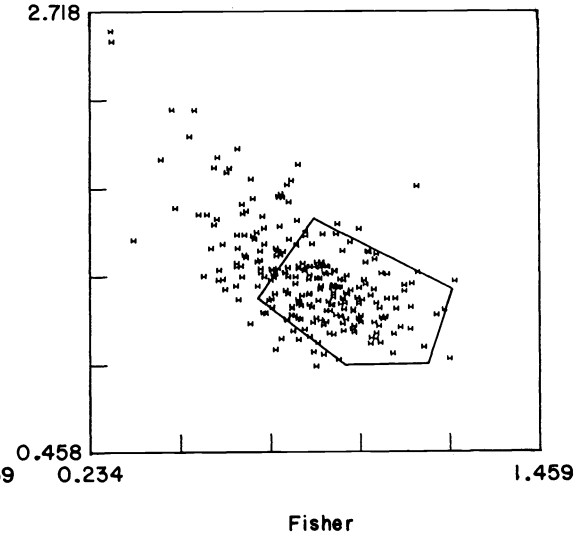

Fig. 2.

Fig. 1 Morphometrical parameters of lymphocytes of normal subjects, represented by the projection onto a plane of their cluster in a multidimensional parameter space. The normal region was defined interactively.

Fig. 2 Morphometrical parameters of lymphocytes of Hodgkin disease patients, shown in relation to the normal area defined in Fig. 1. Many lymphocytes appear to be projected outside the normal area.

Cytochemistry. The possibility to quantitate light absorption per picture point allows to determine the integrated light absorption in segments of an image, i.e. in cells or in parts of cells. This feature is very important for the enumeration of reticulocytes on a blood smear, which is performed routinely by the commercial image analyzers for WBC differentiation.
Image analysis of Feulgen stained cytological preparations can be used to quantitate the nuclear DNA. Although flowcytometry allows to determine the nuclear DNA in a much shorter time,the need to relate the nuclear DNA to morphology makes the image analysis method preferable in some cases (4). Much work on this subject has been done by Caspersson (5) already before the production of microprocessors made image analysis easier to handle.
Quantitation of the reaction products of cellular enzymes is possible in cytological preparations, Although data from practice are not available yet, in theory the quantitation of the reaction product of alkaline phosphatase in leukocytes must give much more accurate information than the L.A.P.-scoring by + to +++ (6).
Similarly the peroxidase reaction products,which proved to be of utmost importance in routine WBC analysis by flow systems, and in immunoperoxidase studies, can be quantitated together with morphometrical parameters in cytological preparations of WBC.
A related application of image analysis is its use in the evaluation and standardization of staining techniques, as described for the Azure-B-eosin stain (7). Differences in staining, not visible yet for the human eye,can be quantitated by image analysis systems.

Histological sections. Information on white blood cells not represented in the peripheral blood can be collected by analysis of imprint preparations, smears or histological sections of bone marrow or lymph nodes. In lymph nodes by far most information is obtained from histological sections, and several workers in the field of morphometry have tried to improve the accuracy of the evaluation of sections by image analysis (8). The abundance of factors influencing the measureremements in tissue sections poses many mathematical problems. However, in selected disease entities the information obtained by morphometry proved useful, e.g. in tumour grading (9) and in the description of lymphomas (10). For the investigation of white blood cells it is not to be expected, that morphometry of sections will be of practical value in the near future.
In conclusion, the development of image analysis systems dedicated to morphometry of white blood cells was undertaken for commercial reasons, but till now the commercial success was limited. The specific possibilities of these systems are suboptimally used, when serving only for cell by cell analysis. The comparison of collections of cells, and the quantitation of cellular constituents may be fruitful areas for further developments in morphometry of white blood cells.

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