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Aspects of quantitative cytometry: an overview

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Abstract. In a short overview the state of the art and connected future possibilities in quantiative cytometry are given. Some aspects are presented in more detail by other papers in this symposium. Automated instruments for cell counting are now widely accepted in routine laboratory practice. Emerging information like MCV, MCH and MCHC can now be used more adaequately, due to a higher accuracy and precision, to develop better and simpler diagnostic programmes and for training medical students in this field. The role of other new parameters like platelet crit and distribution width and the analysis of other cell distribution curves has to be further investigated. Image analysis and flow cytometry systems can both give reliable results in the normal white cell differentiation count. Through these machines the significance of the differential count for daily practice has come into discussion.
The instruments have far more possibilities in quantitative cytometry than the differential count alone. DNA measurements both by image analysis and in flowsystems, cell content and even cell function analysis, antigen and receptor detection and antibody coating of cells will be summarily discussed. Some other new approaches to cell characterization by automated measurements are also shortly mentioned.

INTRODUCTION

In 1979 a symposium was held in Paris on "Automation: what to measure and why" (Ref. 1). The subject was then the measurements of haematological parameters, but the main question could naturally be well extended to today's congress. Although the first question should be, as put by Wintrobe (2), "whether to measure or not", there is an ever increasing amount of data available in clinical practice nowadays, which can not be ignored.Unfortunately, not always is this information used correctly and sometimes even not used at all. In a short clinical survey in an university hospital, it was shown that the newer data as RDW, PCV and PDW generated by a Coulter S Plus were acted upon in less than 20% of the cases, when abnormal and also that such common information as abnormal differentials was not used consistently by the clinicians (ref. 3). The selection procedure for what to measure and what to automate poses therefore a difficult problem (Ref. 4). It is known that a number of laboratory tests has been chance discoveries and not developed because there was a need for it. In reverse, the increased accuracy of automated measurements has brought indiscussion the utility of some long used laboratory tests, such as the differential count (Ref. 5). Thus, better possibilities for quantitation and automation in clinical and laboratory medicine should not only be used for the already established measurements, but also for exploration of new data and their significance for the patient. It must be kept in mind also, that improvement of the quantitative measurements has an important role in education and training in medicine. For instance, the better accuracy and precision in the determination of values like MCV, MCH,MCHC and platelet parameters enables better diagnostic programmes in primary health care. Finally, it is hoped that the experimentation going on in quantitative and automated cytometry will lead to new approaches, as discussed below.

In today's symposium, time does not permit to go into all details of already established procedures or the developments in haematological cytometry. Some aspects will be shortly mentioned in this review and some of these aspects will be discussed in more detail by the other speakers (Ref.6,7,and 8).

PHILOSOPHY OF QUANTITATIVE CYTOMETRY IN HAEMATOLOGY

Quantitative cytometry in haematology started with cell counting and haemoglobinometry. The information obtained for the diagnosis, the natural course and the treatment of diseases was purely empericial. In the same way the differential count became a widely used laboratory tool. In these first haematological measurements the mere quantitation of cell numbers (and amount of haemoglobin) had to bring the correlation with disease states. Starting with the differentiation of the white cell count, the characterization of individual cells or specific cell population plays an ever increasing role. This characterization can now be done in different ways:

- analysis of physical properties, like mechanical, electronic, optical and thermal (Ref. 9) or radiation properties (as in nuclear magnetic resonance, Ref. 10) of the cell;
- the characterization of the morphology (Ref. 11,12), alone or in combination with
- analysis of the cell contents like DNA, RNA, proteins,polysaccharides, lipids and enzymes by specific staining or labeling:
- analysis of the cell surface properties, like antigenic markers or receptors (Ref. 13). The use of xenogeneic antibodies and erythrocyte rozette tests, later on followed by the development of monoclonal antibodies via the hybridoma technique, has facilitated another way of differentiating blood cells.
- analysis of functions. Sometimes, the latter properties can be expressed by one of the other measurements, but this is mostly an indication of the presence of that function rather than a quantitation of the functional activity.
- Although the distribution of cell properties in a certain class had already been introduced in haematology as early as 1933 with the Price-Jones curve as a measurable characteristic (Ref. 14), the analysis of such distributions possible now with the new instruments, could add a new dimension to quantitate cytometry (Ref. 9,15). Kamentsky (9) advocated the use of second and higher order moments to characterize the distributions, since they are available by the use of microprocessors.

Most of the measurements mentioned above can be performed with the two main types of instruments, that have now been developed: flowcytometers and image analysis machines. Both have their own advantages and disadvantages. Some of these are shown in table 1.

TABLE 1. Advantages and disadvantages of image analysis and flow cytometry systems.

Image analysis system (pattern recognition)
- cell by cell measurement
- specific individual cell labeling
- combination with morphology
- relatively slow (improvement!)
- usually small numbers measured
Flow systems
- fast
- large numbers measured statistically more reliable
- needs highly specific labeling
- quantity of label not measured.

Bernard (16) made a difference between the quantities and the specificities of cells to be measured. Perhaps a pattern image analysis (IAS) is better suited for measuring and characterizing properties of the individual cells, while flow systems (FCS) have the advantage of better statistics through larger numbers. However, speeding up of measurements by improving selection criteria for cells to be measured or hard ware adaptation of measuring procedures in IAS can vastly increase the number of measured cells.On the other hand,specific labeling of cells (enzymes,proteins,antigens) have proven that flowcytometers can also measure specific properties and functions of cells (see below,Ref.17,18,19).

Replacement of the manual methods by automated quantitative cytometry on grounds of increased precision or economy does not need to be the sole

purpose for the introduction of new instruments or techniques. In our own experiments with a Texture Analysis System (T.A.S., Leitz) the first goal was to objectivate the criteria on which blood cells are recognized and classified (Ref. 20,21). Analysis of the criteria was made possible by the interactive statistical system for pattern analysis, developed by Gelsema (22). Results indicated that 1) with changing values of parameters objective criteria could be defined to classify mature and immature white blood cells; 2) abnormal cell populations could be recognized in peripheral blood of lymphoma patients based on criteria for deviating monocytes and lymphocytes (Ref. 23). With interactive systems like the T.A.S. other applications are also possible (see below). As mentioned, with certain flowcytometers also new techniques can be developed, even concerned with function of the cells, for instance the antibody dependent cell-mediated cytotoxicity (ADCC) (Ref. 19,24).

Finally, one of the new approaches is to study cells in their own or in an artificial environment, as has been done with erythrocyte rheology. This has led to information on red cell deformability under varying shear stress in different diseases by the use of an ektacytometer, to be discussed later in this paper (Ref. 25).

INSTRUMENTS AND TECHNIQUES USED IN QUANTITATIVE HAEMOCYTOMETRY

Cell counters

There is an ever increasing number of these instruments now commercially available. They are based on either the aperture-impedance principle,or light-or laser scattering techniques.

Principles and results of an aperture-impedance system and a light scattering system have been recently reviewed by respectively Jones (26) and Groner and Epstein (27).
The use and reliability of these instruments in daily practice has been extensively investigated and is now widely accepted.
Quaility control of automated cell counting will be reviewed by Bull (this issue).

Most important is also the accuracy and precision of the secondary parameters like MCV and other red cell indices and the platelet data, obtained with the new instruments. The increased reliability of the first parameters can and must lead to their better use and acceptance in clinical medicine, but also as mentioned, will have an impact on primary health care and the training of students.

The gain obtained by analysis of cell size distribution curves is still under debate. The problems in analysis and interpretation have recently been reviewed by England (28) and recommendations for the analysis have been issued by ICSH (Ref. 29).

In general, red cell size distribution have been longer accepted as having practical significance. White cell size analysis could probably replace in part or in total the classical white cell differential count and thereby at least reduce the number of differential counts in the laboratory.
Rowan and Fraser (15) implicated that perhaps platelet size analysis could be as interesting or even more as platelet counts, but infer also from the literature that no conclusive evidence for the clinical value of this analysis has been published so far.

White cell differential counting systems

Several machines have been introduced on the market for the differential count and some have already been withdrawn again.

There is no doubt that these instruments can perform the task of normal differentiation adaequately. There is also no doubt that the instruments based on pattern recognition contain much more capabilities than the normal differential count alone. They can extract a wealth of information from the blood smear both in the white cell series as well as in the red cells and platelets. However, only part of this information may be generated in the results and especially the immature and abnormal cell classification is not officially accepted. In today's paper on morfometry of white cells Dr. Bins will go into some of the problems related to the characterization and classification of immature and abnormal cells (Ref. 7). Equally doubtless is the fact that instruments based on flow cytometry principles are

also capable of extracting more information than the normal differentiation from their blood samples. In the last years a constant flow of publications has been produced to confirm this. The proceedings of the Technicon symposia have given excellent overviews of the possibilities with their instruments. In Dr. Wessels paper (Réf.8)comparisons will be made between light scatter systems and a laser scatter apparatus in practical use in a haematological laboratory.

Other developments (Table 2)

Table 2. Techniques used in quantitative haemocytometry.

Red cell image analysis

Red cell deformability (ektacytometer)

Image analysis in development

- objectivation of morphology
- teaching of critera
- cytochemistry (DNA, enzymes)
- immunological methods

Flowcytometry in development

- cell populations subsets (specific labeling)
- antigen distribution
- DNA etc. quantitation
- chromosome analysis
- kinetics of cells
- cell function analysis

Red cell parameters can be derived from the modern general purpose cell counters. But red cell morphology contains similar information, as will be shown in the paper of Dr. Bacus in this symposium (Ref. 6,11).Using a high speed image processor, six red blood measurements can be simultaneously extracted: size, haemoglobin content, central pallor and three shape factors.

Combinations of measurements allow to define five subpopulations of red cells and to characterize these subpopulations. In anemias the characteristics are markedly altered. The question to be put of course whether these measurements can be competitive with the information obtained with other instruments and especially whether they can bring new information, leading to better, simplified diagnostic problems.

A totally different approach to red cell characteristics has been proposed by Bessis and Mohandas (25,30,31). The automated ektacytometer used by these investigators possesses a concentric cylinder viscometer in which varying shear stress can be induced. Cells in suspension are measured by the diffraction pattern caused by a laser beam. Red cell deformability and indices can be studied, providing a new approach to study an important rheologic property of the erythrocytes and also the diameter and volume of these cells in different circumstances. The advantage is that cells can be studied under conditions more resembling their natural environment. Especially in haemolytic anemias this can prove to be useful in establishing the severity of haemolysis.

Image analysis instruments can be (and are) used for recognition and classification of blood cells on their morfology. The paper of Dr. Bins in this symposium (Ref. 7) will show that they can be used also to teach us how objective our own criteria are for classifying cells and which objective criteria are most suited to identify each cell class. The possibilities for the distinction of immature and abnormal cells can increase with such an approach (Ref. 21,23,32).

One of the problems related to automated measurements lies in the reproducibility of the preparation of blood films, summarized by Koepke (Ref.33). An ICSH reference method on the routine use of pure dyes for staining the smears has only partly solved the problem (Ref. 34). The reproducibility of this staining method and the conditions necessary for proper use of the stain can be investigated nicely with the TAS (Ref. 7).

It has been mentioned that the morphological analysis of cells can harbour more information than displayed so far. Special stainings can strengthen

this. Cytochemical properties of the individual cell can be measured both by image analysis and flowcytometry systems. Bins (7,35) has developed a method to measure the DNA content of cells by using densitometry on a Feulgen stain. The advantages of the method are that tissue sections can be used also and that the DNA measurement is related to other morphological properties of the cells, thereby distinguishing on a cell to cell basis the cell population that interests the investigator.

As already mentioned some of the flow cytometry instruments can be used for more applications than cell counting alone. Improvement of these possibilities came with the use of multiparameter measurements (for instance, two scatter and a fluorescence signal) and the increasing availability of the recognition of the existence and production of specific cell markers.

Lymfocyte subsets can be quantitated in this way (Ref. 8) but also the presence of antibody covered cell populations or antibodies against erythrocytes, granulocytes or platelets (Ref. 36). Wessels' paper in this symposium will also discuss the possibilities to quantitate DNA kinetics of homogenous or mixed cell populations by the multiparameter technique. Kinetics of the myeloid, erythroid, lymfocytoid and monocytoid series can be examined to a fair degree of reliability. The same techniques can be used for karyotyping of cells and to measure the uptake of some cytostatic drugs in individual bone marrow cells. Finaly, this paper will show that a start has been made with the analysis of cell function on a flowcytometer (Ref. 19,24). De Mulder has shown that the ADCC capacity of monocytes is increased in patients with Hodgkin's disease, but also in patients with other tumours. The results prove that with a careful choice of methods it is possible with automated instruments to record quantitatively certain cell functions.

CONCLUSIONS

Automated instruments for cell evaluation have clearly won their place in routine laboratory measurements as far as cell counting is concerned. This is mainly a question of cost-performance considerations. Part of the data generated by the new machines are well known parameters, but since they are measured now with far higher reliability, they should be reintroduced in clinical practice. They can be used to improve diagnostic procedures and should be well incorporated in student education and medical training programmes. Other data like cell distributions curves have still to be evaluated further for their clinical utility, although for the white cell differentiation the distribution analysis of granulocytes, lymphocytes and monocytes instead of the classical differential count seems already a good proposition. Other ways of measurements like red cell indices by image analysis, cell deformability, immature blood cell recogniton and abnormal cell detection are also worthwhile studying for their usefulness in routine diagnosis. It has been shown that the commercial instruments have greater capabilities and possibilities than for their original purpose alone. These extras can be used to quantitate better and more hameatological procedures; on the other hand they help us to estalish new and other approaches, as for instance, cell function measurements in a flow system. Finally, they are of great value in setting up selection and control procedures as has been done in monoclonal antibody production, DNA measurements and cell sorting.

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