

APPLICABILITY OF MORPHOMETRICAL METHODS IN CLINICAL CYTOLOGY

Frej Stenbäck

Department of Pathology
University of Oulu
Kajaanintie 52 D, SF 90220 Oulu22, Finland

ABSTRACT

The results from a study of the applicability and significance of different morphometrical parameters in estimates of malignancy in exfoliated cells and fine needle biopsies are presented. The most useful were the nucleo/cytoplasmic ratio and cellular and nuclear area. Similar patterns were seen in cell shape and short and long axis estimates. Form factors for cell irregularity, cellular angle and cellular diameter provided no useful information. Morphometrical analysis was able to determine degree of malignant potential of cells and to identify cells not classifiable by conventional methods as well as cell of origin.

INTRODUCTION

The needs for accurate, reliable and reproducible analytical methods in cytology are perhaps greater than in any other field of morphological pathology due to the large number of specimens regularly studied, the limited number of criteria applicable to diagnosis of each lesion and the minimal structural differences between lesions of widely varying biological potential. Also the criteria used are basically morphometrical though applied in a diffuse and inconsequent manner.

Morphometrical and stereological approaches to cytology are done from two different directions. The basic approach relates to biological considerations of cell structure in relation to anatomy and function. The clinical approach deals with quantitative estimates of morphological abnormalities of prognostical and therapeutical significance and applicable to daily routine practice. The former requires special technics, ultrastructural analysis etc., the latter deals with standard specimens submitted for cytological diagnosis. As this presentation involves application

of morphometry in clinical cytology basic aspects will be briefly discussed.

Early studies started in the 1950's reported quantitative characteristics of exfoliated cells usable for automatic prescreening of large populations for cancer (Mellors et al. 1951, 1952, Tolles et al. 1961). Later studies have employed highly sophisticated methods: absorption cytophotometry and rapid flow cytometry of DNA (Böhm and Sandritter 1975, Atkin 1979, Fosså et al. 1982). These methods are sensitive and accurate but require expensive equipment and extensive expertise in this specialized field. Due to the high cost, difficulties in studying large number of specimens and problems in specimen selection and preparation these methods have been in limited use.

Stereological studies in cytology require in addition to those for homogenous tissues additional precautions due to the nature of the sample studied. We are dealing with mixed cell populations instead of homogenous tissues, the cytological preparation technics cause additional artefacts if not properly observed and comparisons to previous observations are hampered by the lack of information on this type of studies. However, studies are not affected by histological preparation technics i.e. penetration of fixatives and plane of sectioning.

In this study the morphometric findings from a study of various cytological specimens will be presented and the results discussed in relation to their methodological, clinical and biological aspects.

MATERIAL AND METHODS

For this study samples from clinical examination for suspected malignant disease were studied. These included fine needle aspirates (FNA) of lung, mammary, prostatic and thyroid neoplasms as well as exfoliated cells (CEF) in samples from ascites, pericardial and pleural fluid, 20 specimens altogether. The specimens were fixed in ether-alcohol and filtered by the Millipore technique. The filters were stained by the Papanicolau method.

The cells and nuclei, their size and shape were measured with a Videoplan digitizer system (Carl Zeiss, Inc., New York, New York) interfaced with a laboratory computer. The cells were outlined over the electromagnetic tablet using a stylus projected over the microscopic image through a camera lucida attachment. All measurements were made using a 40 x

or 400 x magnification. The figures given are the average of 10 measurements and the standard deviation.

RESULTS

For morphometrical evaluations a number of parameters were evaluated for usefulness in cytological analysis. For size analysis the cellular and nuclear area were selected. As shown in Fig. 1a, cell and nuclear size varied considerably in, as well as between, different types of neoplasms in fine needle aspirates. Typical for well differentiated adenocarcinomas was a large cell size, cellular pleomorphism is indicated by the farly large standard deviation.

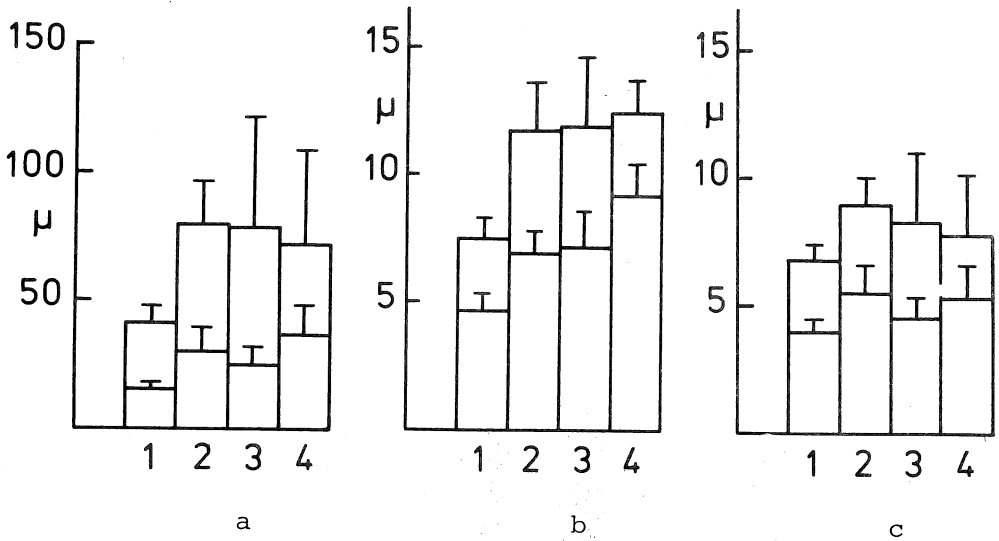


Fig. 1. Size estimates of fine needle aspirates. 1 = pulmonary squamous cell carcinoma, 2 = mammary adenocarcinoma, 3 = prostatic adenocarcinoma and 4 = thyroid papillary carcinoma. a = size, b = major axis, c = minor axis. Upper line denotes cell dimension, lower line nuclear dimension.

The other parametres shown for size were the major axis, and minor axis. As shown in Figs 1b and 1c they follow the same pattern as cell and nuclear size, with nuclear major axis almost approaching cellular major axis in some neoplastic cells.

In exfoliated cells (Fig. 2), the variation in cell size was obvious with a distinct increase in malignant cells (Fig. 2a). In these cells the cellular pleomorphism in malignant ascites cells significantly increased when compared to benign ascites cells as seen by the standard deviation. The differences in

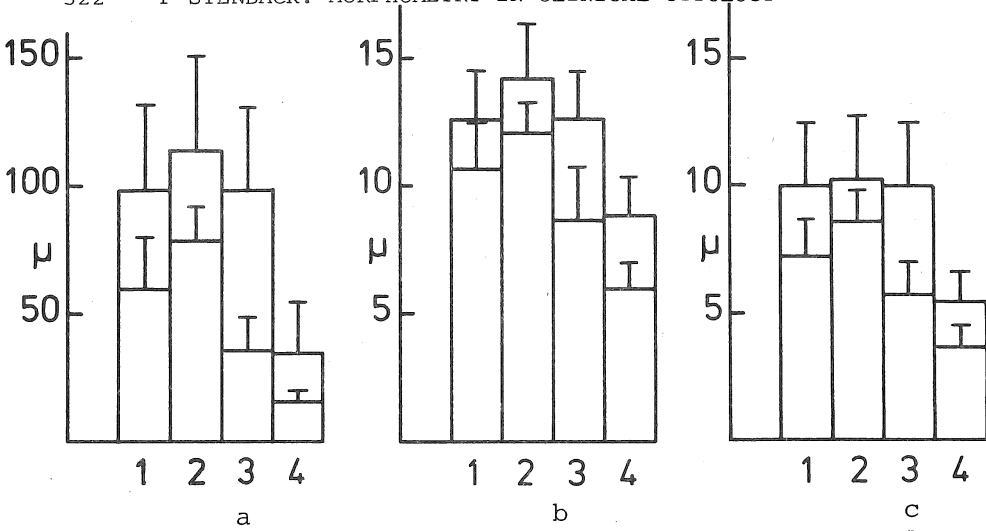


Fig. 2. Size estimates for exfoliated cells. 1 = normal ascites cells, 2 = malignant ascites cells, 3 = malignant pericardial fluid cells and 4 = pleural fluid cells from patient with mammary adenocarcinoma. a = size, b = major axis, c = minor axis. Upper line denotes cell dimension, lower line nuclear dimension.

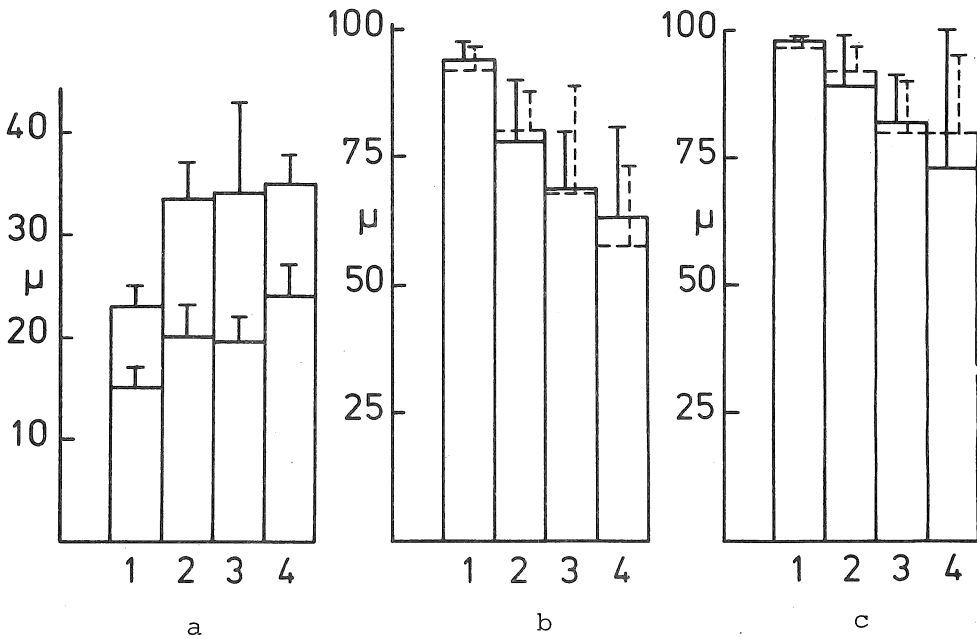


Fig. 3. Shape estimates for fine needle aspirates. 1 = pulmonary squamous cell carcinoma, 2 = mammary adenocarcinoma, 3 = prostatic adenocarcinoma and 4 = thyroid papillary carcinoma. a = perimeter, b = axis ratio, c = shape ratio. Solid line denotes cellular estimates, dotted line nuclear estimates.

nuclear size were less significant. The increase in N/C ratio was also seen in malignant exfoliated cell though not as distinctly as in fine needle aspirates.

For shape dimension estimates the major and minor axis ratio was studied. The perimeter indicates the size of the cell in addition to shape. The shape ratio indicates the ratio area/perimeter. The perimeter as seen in Fig. 3a showed the same type of changes as previous described with a larger perimeter in malignant cells, indicating cellular polymorphism. Axis ratio was associated with cellular origin with rounded cells in squamous tumors and columnar cells having considerable differences between short and long axis (Fig. 3b). Shape ratio (Fig. 3c) followed the same pattern.

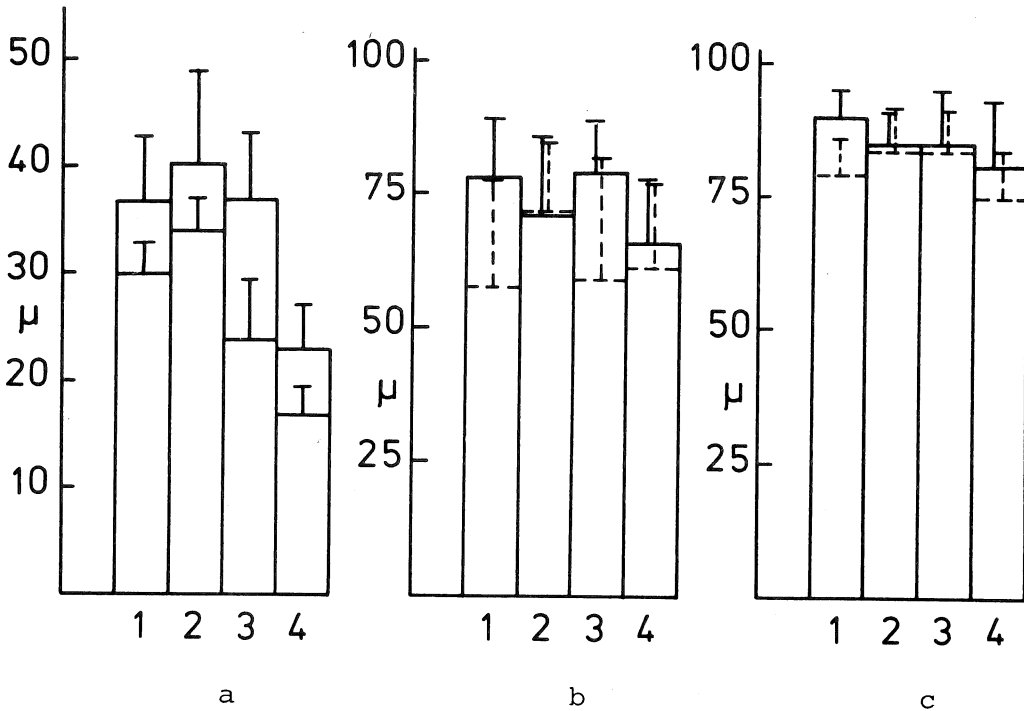


Fig. 4. Shape estimates for exfoliated cells. 1 = normal ascites cells, 2 = malignant ascites cells, 3 = malignant pericardial fluid cells and 4 = pleural fluid cells from patient with mammary adenocarcinoma. a = perimeter, b = axis ratio, c = shape ratio. Solid line denotes cellular estimates, dotted line nuclear estimates.

Shape estimates in exfoliated cells (Fig. 4) shows the difficulties in estimating morphological changes in a simple manner. The perimeter of normal ascites cells (Fig. 4a) was high indicating the need

to separate cell size and cell polymorphism. The axis ratio as shown in Fig. 4b indicated that the columnar shape of cell and nuclei not always were similar. The shape ratio area/perimeter as shown in Fig. 4c separated malignant cells from benign, but was similar in malignant cells of different origin. In malignant cells the nuclear and cellular shape pattern was virtually identical.

Also studied were projected lengths of cellular diameters, center of gravity, cellular angle and form factors to determine irregularity of surface. Additional meaningful information was not derived from these measurements.

DISCUSSION

Until now, morphometric methods have been applied to human pathology only to a very limited degree. The main reasons are probably the lack of generally accepted morphological criteria and lack of suitable morphometric models for analyzing the often complex alterations taking place in pathologic conditions. The aim of the present study was to develop a method for quantitative analysis of cellular changes by an objective comparison between the different types of neoplasms in specimens submitted for cytological analysis.

Previous observations have indicated the need for objective assessment of the diagnosis of precursors of malignancy and carcinoma. Quantitative microscopy makes this possibly (Weibel 1969), and there are an increasing number of applications of this technique in pathology. This method may reveal differences and changes which escape subjective observation. It can be used to identify malignant populations in sections and smears as well as to identify and classify cells in regards to origin and behavior. The main advantage of the application of quantitative techniques over subjective impressions is however the ability to obtain consistent reproducible results.

Previous studies on cytological specimens embrace three main groups (Mayhew and White 1980), freefloating cells in fluid milieux i.e. blood leukocytes, alveolar macrophages, cells and serous exudates, cells found in tissues but having retained a distinct individuality i.e. histiocytes, lymph node lymphocytes and connective tissue fibroblasts as well as cell from a variety of tissues which have been dissociated for tissue culture. To these groups should be added a fourth involving cytological

preparations from epithelial surfaces taken by imprint smear etc.

Isolated cells offer certain advantages for studying cellular biology. They are often available in large numbers and reasonable pure populations showing little morphological variations. Stereological data for populations of isolated cells can be correlated with parallel cytochemical, biochemical and physiological data (Fosså et al. 1982). Also isolated cells are easy to sample in a representative fashion as they are independent of another, structural anisotropy is absent.

Methodological aspects have to be taken in consideration when planning morphometrical studies as summarized by Mayhew and White (1980). This affects the sampling procedure necessary to ensure a random representative sample (Weibel 1969) as well as the parameters studied. The main problems in histological studies, pellet section planes and nuclear biased versus unbiased sampling technics (Stenbäck and Arranto 1981, 1983) do not relate to exfoliated cells in smears.

Efforts to determine morphometric criteria for estimation of malignancy have mainly centered around the nuclear size. This approach has been useful when applied to cytological diagnosis of gastric smears (Boon et al. 1981) as well as intestinal cells (Sato et al. 1981). Different opinions have also been presented (Nödskov-Pedersen 1971) failing to obtain prognostic data from nuclear size estimates of cervical malignancies. Cell size determinations have been used to a lesser extent and mostly in connection with estimates of the nucleo/cytoplasmic ratio. The mean N/C ration and the standard deviation of the nuclear area were essential in discriminating between benign and malignant lesions in gastric cytologic (Boon et al. 1981) and cervical smears (Johnston 1952).

Other cells size estimates have been used to a lesser extent. Nuclear short axis and long axis were not significantly different in studies on endometrial cancer and its precursors (Baak et al. 1981). Epithelial volume density were suggested as indicators for malignancy by Ortner (1983). Nuclear and nucleolar perimeter also failed to yield significant results in Sato's et al. (1981) studies on large bowel carcinoma and its precursors.

Many parameters have been used to characterize the extent and irregularity of cell and nuclear

membranes, including indices of nuclear indentation and cell surface amplification (Mayhew 1980). The cell surface amplification factor provides a useful index of how much more surface a cell has than a sphere of equivalent volume. The nuclear contour index has achieved recognition of the success in discriminating between cutaneous T cell lymphomas, mycosis fungoides and Sezary's syndrome (Van Der Loo et al. 1981).

Shape estimates have been used to lesser extent. The form factor nuclear area/nuclear perimeter showed high variability in studies on large bowel tumours (Sato et al. 1981) indicating that the cells tend to become increasingly circular as they acquire carcinomatous character. Nuclear shape factors estimates were less useful than glandular shape determinations in Baak et al. (1981) studies on endometrial neoplasia and its precursors.

When comparing morphometrical results to those obtained by rapid flow cytometry and nuclear DNA-Feulgen absorption cytophotometry the results are generally in agreement (Fosså et al. 1982, Van Der Loo 1981), though not in all studies (Nödskov & Petersen 1971). Extensive efforts have been made to improve cellular DNA measuring instruments (Wied et al. 1983) still their use is fairly limited.

Already in 1951 Mellors and Silver suggested that automatic mass screening for cervical cancer might be feasible if an instrument could be devised that could distinguish reliably between normal cytological smears and those smears in which there was the suspicion of cancer. The effect of such a prescreening instrument would be to remove from human attention the large percentage of normal smears, thus allowing the trained observer to concern himself with only those smears exhibiting some degree of cellular abnormality. As indicated in this study morphometrical analysis using suitable parameters may be developed for clinical use. Their main application, however, is probably to aid in determining the significance of individual cell changes, not as a replacement for the human eye.

REFERENCES

- Atkin NB and Kay R: Prognostic significance of modal DNA value and other factors in malignant tumours, based on 1465 cases. *Br J Cancer* 40: 210-231, 1979.
- Baak JPA, Kurver PHJ, Diegenbach PC, Delemarre JFM, Brekelmans ECM and Nieuwlaat JE: Discrimination of hyperplasia and carcinoma of the endometrium by quantitative microscopy feasibility study. *Histopathology* 5: 61-68, 1981.
- Boon ME, Kurver PJ, Baak JPA and Thompson HT: The application of morphometry in gastric cytological diagnosis. *Virchows Arch (Pathol Anat)* 393: 159-164, 1981.
- Böhm N and Sandritter W: DNA in tumours: a cytophotometric study. *Curr Topics Pathol* 60: 151-181, 1975.
- Fosså SD, Marton PF, Knudsen OS, Kaalhus O, Børmer O and Vaage S: Nuclear feulgen DNA content and nuclear size in human breast carcinoma. *Hum Pathol* 13: 626-630, 1982.
- Johnston DG: Cytoplasmic nuclear ratios in cytological diagnosis of cancer. *Cancer* 5: 945-949, 1952.
- Mayhew TM and White F: Ultrastructural morphometry of isolated cells: methods, models and applications. *Pathol Res Pract* 166: 239-259, 1980.
- Mellors RC, Glassman A and Papanicolaou GN: Microfluorometric scanning method for detection of cancer cells in smears of exfoliated cells. *Cancer* 5: 458-468, 1952.
- Mellors RC and Silver R: Microfluorometric scanner for differential detection of cells; application to exfoliative cytology. *Science* 114: 356-360, 1951.
- Nødskov-Pedersen S: Degree of malignancy of cancer involving the cervix uteri, judged on the basis of clinical stage, histology size of nuclei and content of DNA. *Acta Pathol Microbiol Scand Sect A* 79: 617-628, 1971.
- Ortner A: Epithelial compartment volume density: a proposal of a prognosis score in endometrial adenocarcinoma. *Gynecol Oncol* 15: 190-200, 1983.

- Sato E, Goto M and Nakamura T: Nuclear ultra-structure in carcinoma, adenoma, Peutz-Jeghers polyp and dysplasia of the large bowel: a morphometric analysis. *Gann* 72: 245-253, 1981.
- Stenbäck F and Arranto A: Volume density relationship of tumor epithelium, glandular lumen and stroma in development and classification of uterine adenocarcinoma. *Stereol Iugosl 3 I*: 397-401, 1981.
- Stenbäck F and Arranto A. Development and progression of squamous cell carcinoma of the uterine cervix. Quantitative morphometrical aspect in ultrastructural studies. In: Collan Y et al (editors): *Morphometry and stereology in pathology*. Kuopio University Press, Kuopio 1983. Published as a special issue of *Acta Stereol 2*, 1983.
- Tolles WE, Horvath WJ and Bostrom RC: A study of the quantitative characteristics of exfoliated cells from the female genital tract. I. Measurement methods and results. *Cancer* 14: 437-454, 1961.
- Van Der Loo EM, Van Vloten VA, Cornelisse CJ, Scheffer E and Meijer CJLM: The relevance of morphometry in the differential diagnosis of cutaneous T cell lymphomas. *Br J Dermatol* 104: 257-269, 1981.
- Weibel EW: Stereological principles for morphometry in electron microscopic cytology. In: *Int Rev Cytol* (ed. GH Bourne and JF Danielli) 26: 235-302, Academic Press Inc., London, 1969.
- Wied GL, Bartels PH, Dytch HE and Bibbo MC: Rapid DNA in clinical diagnosis. *Acta Cytol* 27: 33-37, 1983.