ACTA STEREOL 1985; 4/2: 231—235
PROC ESS IV-1 GÖTEBORG 1985
ORIGINAL SCIENTIFIC PAPER

KARYOMETRY, WITH STEREOLOGICAL ESTIMATIONS, OF HUMAN GASTRIC DYSPLASIA

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ABSTRACT

Sections from human diagnostic biopsies of the gastric antrum have been studied with an image analyser. A stereological model for parallel prolate spheroids is applied to the measured data (nuclear profile major and minor axes), yielding mean nuclear parameters. It is possible, with high statistical significancies, to separate the normal and the inflammatory cases from all the dysplasias: all the stereologically estimated nuclear dimensions are larger in the latter, accompanied by a decrease in eccentricity. Dysplasia III can be separated from I and II. Nuclear profile area plots also allow a good classification; an additional peak (large nuclei) appears in dysplasia I, and decreases in II and III. This pattern resembles those observed in other putative pre-cancerous lesions.

Keywords: Dysplasia, image analysis, karyometry, spheroids, stereology, stomach.

INTRODUCTION

In morphological changes usually considered as precancerous lesions and/or conditions, such as epithelial dysplasias, relatively few DNA measurements have been made (review in Rigaut et al., 1985), compared with the abundant literature dealing with cancers. Increased DNA amounts have been found in dysplasias of uterine cervix and endometrium, bronchi, larynx, oral cavity, oesophagus and colon. This is also the case for the gastric antrum (Avtandilov, 1976; Sugar et al., 1982). As a well-documented correlation exists between nuclear size and DNA content (review in Rigaut et al. ,1982), a few karyometric studies have been made in pathology, but usually without any stereological estimation when using sections (review in Rigaut et al. ,1985). A recent work (Enchev and Tsanev, 1985) shows an increase in nuclear DNA and volume in chronic gastritis, gastric ulcers and adenomatous polyps.

METHODS

In the present study, we have studied fifteen human diagnostic biopsies of the gastric antrum (diagnoses: see Table 1), with an image analysis algorithm for the IBAS (Rigaut et al., 1982, as modified in Rigaut et al., 1985). Only the neck zones of the gastric pits were studied, as the most striking histological and cytological changes are observed by pathologists in such areas. The biopsy material was formalin-fixed for 24 hours and 5 µm-thick Feulgen-stained

sections were used for image analysis. For each section, a consecutive one was graded by the pathologist. The image analysis program includes an image enhancement (shading correction, histogram linearization of the grey values, modified median filtering and averaging filtering), followed by the binarization of the nuclear profiles, the estimation of their major and minor axis lengths (by moments of inertia) and area measurement. The only interactive step is usually the light-pen drawing of a contour around the zone to be studied (Fig. 1).

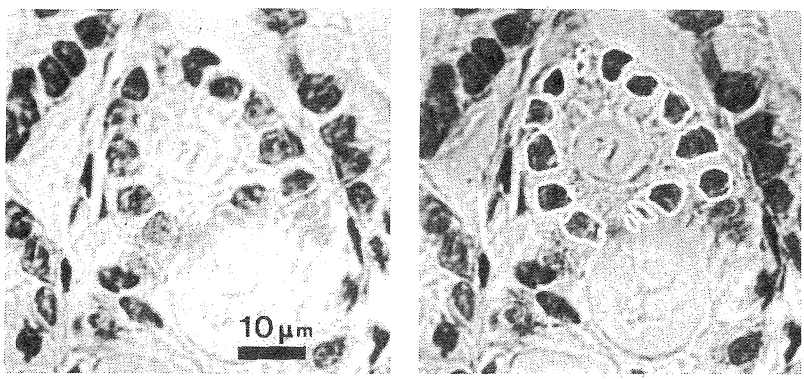

Fig. 1. Images on the IBAS TV monitor: (a) original image of a Feulgen-stained section crossing a gastric pit; (b) the white contours show the superimposition of the final result on (a).

For each biopsy (one per case), 100 nuclear profiles have been measured, in zones pre-determined (staged) by the pathologist. A stereological model for parallel prolate spheroids (Rigaut et al., 1982) yields mean nuclear parameter estimations for each zone (caliper diameter, axes, axial ratios, volume, surface area and volume/surface area ratio, numerical density). As no distribution unfolding is yet feasible for parallel spheroids of varying eccentricity, profile area plots were scanned for possible polyploidisms and the mean profile area (MPA) was computed.

RESULTS

It is possible, with high statistical significancies, to separate the normal and the inflammatory (superficial gastritis) cases from the dysplasias I, II and III, by using the U-test on the stereological results: all the nuclear dimensions are larger in dysplasias, accompanied by a decrease in nuclear eccentricity (Table 1). The differences in volume are striking (Fig. 2). The stereological parameters, however, do not allow significant separation of normal from inflammatory cases, although there appears to be a difference in volume which might become significant when more cases are studied (Fig. 2). Dysplasia III can be separated significantly from I and/or II by the eccentricity, and seems also to have a difference in volume (n.s.).

Table 1. Nuclear stereological estimations and mean profile areas.

NUCLEAR PARAMETERS:	HISTOPATHOLOGICAL STAGES: Normal	Inflammatory	Dysplasia I	Dysplasia II	Dysplasia III
Mean caliper diameter (μm)	2.4 (.3)	2.7 (.2)	3.9 (.5)	3.8 (.1)	3.4 (.3)
Mean axial ratio	0.44 (.09)	0.47 (.05)	0.61 (.03)	0.60 (.03)	0.50 (.05)
Mean volume (μm^3)	86. (18.)	124. (18.)	255. (60.)	243. (37.)	217. (38.)
Mean profile area (μm^2)	18.0 (6.8)	22.7 (10.8)	32.6 (11.1)	31.7 (11.8)	31.8 (11.5)
NUMBER OF BIOPSIES	4	3	2	3	3

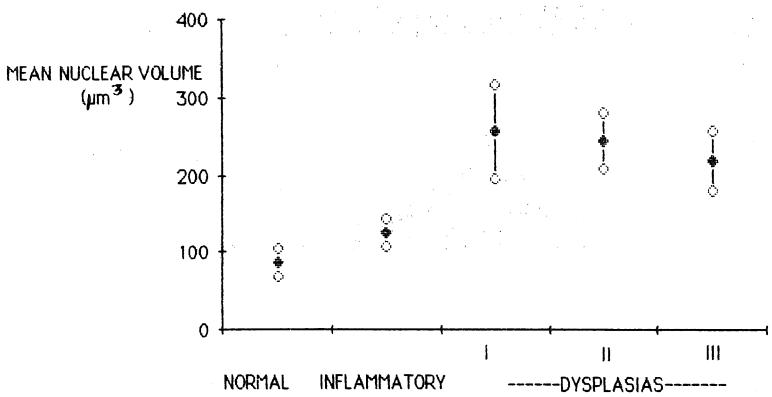

Fig. 2. Stereologically estimated nuclear volumes.

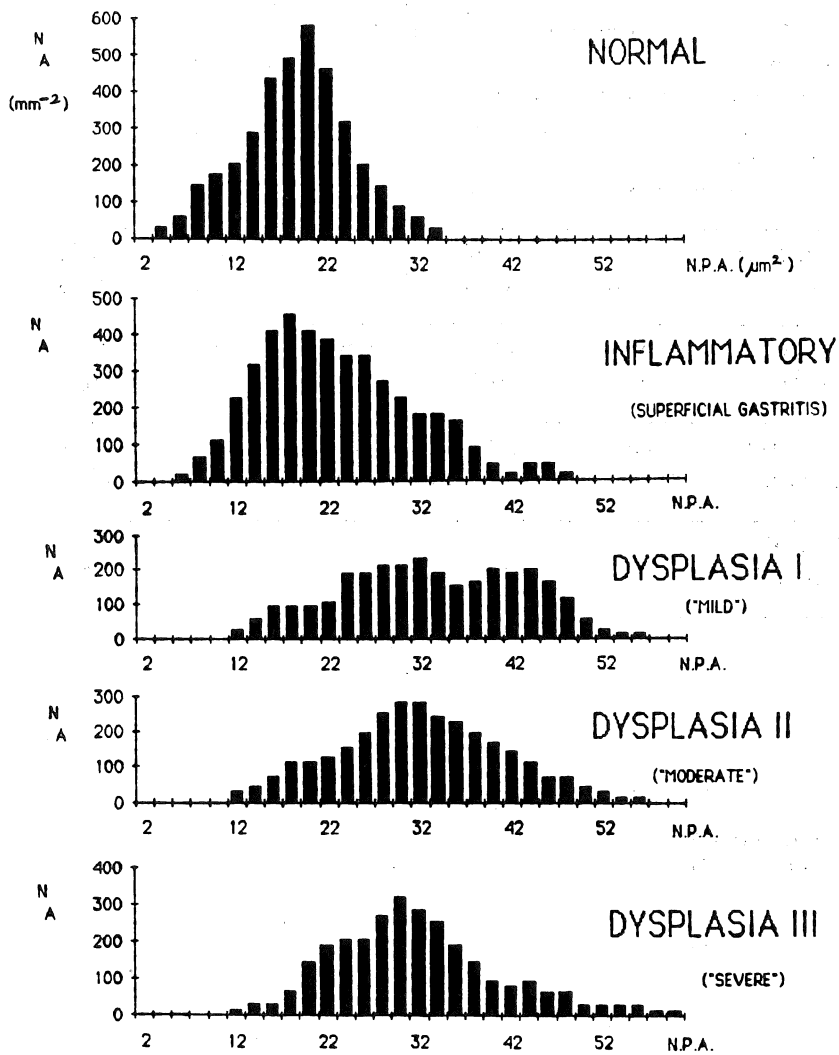

Fig. 3. Nuclear profile area (NPA) plots.

Some important conclusions can be drawn from the nuclear profile area plots (Fig. 3). The MPA allows significant separation of the normal or inflammatory cases from the dysplastic. Three MPA groups (Rigaut et al., 1985) can be used to classify the results ($i<20$, $20\leq i<30$, $30\leq i<40$). Normal and inflammatory cases all fall in groups 1 or 2, whereas only one dysplasic (II) case falls in group 2 and all the others in group 3. All normal and inflammatory cases show a major peak, in nuclear profile area plots (Fig. 3), at 20 μm^2, whereas the dysplasias never display it. Two major peaks exist in dysplasia I, the first one only being a major one in dysplasia II. In dysplasia III, contributions of very large nuclei tend to decrease and a peak appears around 25 μm^2. The Smirnov test shows that the distributions of nuclear profile areas are highly significantly different ($p<.0001$) between normal or inflammatory, and dysplastic cases. A difference ($p=.005$) is also noted here between normal and inflammatory.

DISCUSSION

The results show that morphometric nuclear parameters allow separation of dysplasias from other stages with a high significancy. Only the stereologically-estimated parameters allow this separation: the mean profile area values overlap, whereas dysplastic cases have a mean nuclear volume which never falls into the range of normal or gastritic cases. The estimated axial ratio allows a separation between dysplasias I + II and dysplasia III. On the other hand, only the nuclear profile distribution allows the separation of normal cases from inflammatory ones. Smaller nuclei seem to exist in dysplasia III.

Our results resemble strikingly those obtained in the nasal mucosa (Rigaut et al., 1982, 1985), where a progressive increase in nuclear dimensions is noted when passing from pseudostratified to metaplastic and dysplastic epithelia. Some dysplastic cases had small nuclei. It seems (Rigaut et al., 1982, 1985) that a pattern of polyploidization followed, in more severe cases, by a re-diploidization, would fit our observations, and two others in the literature (see Rigaut et al., 1985), of the existence of smaller nuclei in some severe dysplasias, as compared to a higher contribution of larger nuclei in metaplasias and moderate dysplasias. Karyometry should be a routine technique in cancer-oriented pathology (Rigaut et al., 1984; Voss et al., 1983).

REFERENCES

Avtandilov GG. Perspectives on the use of microspectrophotometry in the diagnosis of pretumorous processes and malignant tumors. Cancer 1976; 38: 254-258.

Enchev VG, Tsanev KG. Comparative cytomorphometric and cytospectrophotometric investigations of gastric lesions. Arch Geschwultsforsch 1985; 1: 37-46.

Rigaut JP, Boysen M, Reith A. Karyometry of pseudostratified, metaplastic and dysplastic nasal epithelium by morphometry and stereology. 2. Automated image analysis (IBAS) of the basal layer of nickel workers. Path Res Pract 1985; 180: 151-160.

Rigaut JP, Margulès S, Boysen M, Chalumeau MT, Reith A. Karyometry of pseudostratified, metaplastic and dysplastic epithelium by morphometry and stereology. 1. A general model for automated image analysis of epithelia. Path Res Pract 1982; 174: 342-356.

Rigaut JP, Reith A, El Kebir F.Z. Karyometry by automated image analysis. Application to precancerous lesions. Path Res Pract 1984; 179: 216-219.

Sugar J, Szentirmay Z, Decker A. The significance of cytophotometry in the characterization of malignant transformation. Acta Histochem 1982; 26-S: 59-66.

Voss K, Hufnagl P, Martin H, Roth K. Karyometric data by image analysis and their use in pathology. Acta Stereol 1983; 2: 312-318.