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Quantitative analysis of nucleolar margination in diagnostic cytopathology

R. Montironi [1], M. Scarpelli [1], A. Braccischi [2], C. Magi Galluzzi [1], L. Diamanti [1], and R. Alberti [3]

[1] Institute of Morbid Anatomy and Histopathology, University of Ancona, Ancona, Italy
[2] Service of Morbid Anatomy and Histopathology, Civil Hospital, Ascoli Piceno, Italy
[3] Cytology Service of the Ancona Health Authority, Ancona, Italy

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Summary. The diagnostic value of nucleolar margination, defined as the percentage of nucleoli touching the nuclear membrane, was investigated in 359 cytological preparations of benign and malignant lesions of the thyroid, breast, prostate and central nervous system. Premalignant lesions of the uterine cervix and non-invasive papillary carcinomas of the bladder were also examined. It was observed that the percentages in benign lesions were, in general, lower than in the malignant and that the values increased progressively with increasing grade in the cervix and bladder. When the overlap index was calculated, this gave exact information on the usefulness of nucleolar margination in distinguishing benign from malignant lesions, particularly in the prostate and thyroid and, to a lesser extent, in the breast and central nervous system. As for lesions of different grades, the calculation of the index allowed the identification of two subgroups, one corresponding to low grades (mild cervical dysplasia or urothelial papillary carcinoma of grade 1), the other subgroup to high grades (severe cervical dysplasia and carcinoma in situ, or papillary carcinoma of grade 3). Moderate dysplasia cases and grade 2 papillary carcinomas do not appear as separate intermediate categories but rather show values falling into the range of either the higher or lower grades. The margination values obtained from the cytological preparations corresponded well to those in the histological slides obtained from the resected specimens. In conclusion, nucleolar margination appears to be a feature which is easy to evaluate in a reproducible way and useful in cytological diagnosis.

Key words: Nucleoli – Nucleolar margination – Cytopathology – Quantitative analysis

Offprint requests to: R. Montironi, Institute of Morbid Anatomy and Histopathology, University of Ancona, New Regional Hospital, I-60020 Torrette di Ancona, Italy

Introduction

Diagnostic cytopathology (DC) is directed towards several goals, in particular the distinction between benign and malignant lesions and the detection of malignant lesions or their precursors. During the last few years pathologists and clinicians have shown an increasing interest in the diagnostic value of clinical cytology because of its many procedural and non-procedural advantages, among which the speed with which cytological preparations and thus an immediate diagnosis can be made (Linsk and Franzen 1983). However, there are also some drawbacks to DC in terms of accuracy, shown to be inferior to other diagnostic procedures such as the frozen-section technique (Schricker and Hermanek 1974). For instance, in a recent paper by Thomas et al. (1990) a comparison of fine-needle aspiration (FNA) with the frozen section of palpable mammary lesions was made. The rate of false negatives for aspiration cytology was 6%, compared with 0% for frozen sections. The drawbacks in the use of DC can in part be related to the fact that DC is based primarily on the subjective evaluation of cell features and only to a minor degree on cell arrangement, whereas diagnostic histopathology can also rely on the architecture or pattern of growth (Linsk and Franzen 1983; Nguyen and Kline 1991). However, those in favour of DC have shown that refining the evaluation of the cellular, nuclear and nucleolar details can increase the accuracy (Kini 1987; Kline 1981; Koss 1979; Suen 1988).

While investigating the low accuracy of the pre-operative subjective discrimination between follicular adenoma and follicular carcinoma of the thyroid, we found that the problem is partly solved by using quantitative analyses of nuclear (including DNA) and nucleolar features. In particular, karyometric features had a discriminant power greater than those related to DNA (Montironi et al. 1989), whereas nucleolar frequency was more powerful than nuclear area and DNA (Montironi et al.

1990, 1991c). More recently, it was observed that the proportion of nucleoli touching the nuclear membrane was the most discriminant of the nucleolar features (Montironi et al. 1991b). Our observations on nucleoli appeared in agreement with the experimental findings of Wachtler et al. (1986), who investigated the nucleolus and its organizer regions (NORs) in interphases. They observed that in the course of induced cellular activation, NORs become transcriptionally active, move around within the nucleus and associate with one another, thus forming new or joining previously existing nucleoli. As for the nucleolar margination in particular, it was postulated that NORs change their position in order to find special sites where attachments to the nuclear membrane were possible and the development of one or a few large nucleoli more effective. This is probably because the accumulation of large amounts of preribosomal particles within a restricted area may create a steeper concentration gradient for diffusion into the cytoplasm than several small nucleoli.

The aim of the present research project was to evaluate the diagnostic cytology value – including reproducibility and correspondence to histology – of nucleolar margination, that is to say the percentage of nucleoli touching the nuclear membrane, in distinguishing benign from malignant lesions and in detecting malignancy or its precursors.

Materials and methods

The percentage of marginated nucleoli was investigated in 359 cytological preparations using a Leitz Dialux 20 EB microscope at a microscopical magnification of $\times 1000$ ($\times 100$ objective magnification, oil immersion; $\times 10$ ocular magnification) by one of us (AB) without knowing the final diagnosis of the single cases in order to avoid any form of bias in the evaluation. The analysis was performed on 100 nucleoli consecutively selected by adopting the vertical stratified method and starting from the top-left corner of the slide. From this point, parallel lines were scanned in the y direction, with each successive line farther towards the end of the slide. In order to visualize all the nucleoli present in each nucleus, the microscope was focused more than once so as to scan the whole nucleus in depth. The time needed for the analysis of each single case was approximately 8–10 min.

The material used in this study was divided as follows:

The preoperative cytology material included 229 cases of FNA performed by one of our team (RA) while working at the Cytology Service of the Ancona Health Authority. These included 125 cases from the thyroid gland, 63 from the female breast and 41 from the prostate gland. As for the thyroid (all were cold nodules), there were 15 nodular goitres (NG), 31 follicular adenomas (FA), 27 angioinvasive follicular carcinomas (FC), 14 papillary carcinomas (PC), 11 oxyphil neoplasms (ON) (of which 3 were benign, 2 intermediate and 6 carcinomas), 16 medullary carcinomas (MC) and 11 anaplastic carcinomas (AC). As for the breast, there were 31 benign lesions and 32 carcinomas. There were 24 cases of prostatic nodular hyperplasia and 17 prostatic adenocarcinomas. The subdivision of the cases was based on the diagnosis made on the corresponding histological slides retrieved from the files of the Institute of Morbid Anatomy and Histopathology of the University of Ancona. Besides the availability of histological slides, the other criterion for case selection was that the FNA samples had to be technically satisfactory, i.e. at least 2 slides per case containing a minimum of 8–10 tissue fragments of well-preserved epithelium. All smears had been wet-fixed in 95% ethyl alcohol and stained according to the Papanicolaou method.

Intraoperative cytology specimens included 35 consecutive cytological preparations consisting of brain tumours, biopsy specimens of which were sent to one of our team (MS) from the Neurosurgical Service of the Ancona Health Authority. The type of cytological preparation was of the "squash" type: a small fragment of tissue, usually about 1 mm^3, was placed on a slide; a second slide was placed on top of it, and enough pressure was applied to spread the tissue between the two slides. The slides were then pulled apart lengthwise and stained with toluidine blue. The cases were subdivided on the basis of the histological reports as follows: 23 astrocytomas (12 of low grade and 11 of high grade), 1 ependymoma, 3 oligodendrogliomas, 2 medulloblastomas, 2 meningiomas, 2 primary malignant lymphomas, and 2 pituitary adenomas.

Cytological screening for precancerous changes in the uterine cervix was assessed using 55 cervical smears obtained by employing a combination of aspiration and scraping at the outpatient gynaecology service. The cases included in this part were selected on the basis of the histological reports of the punch and cone biopsies so as to have cases representing the following categories: normal squamous epithelium (i.e. with no precancerous findings) (15 cases, NE), mild dysplasia (10 cases, MD), moderate dysplasia (10 cases, MoD), severe dysplasia (10 cases, SeD) and carcinoma in situ (10 cases, CIS). At least two Papanicolaou-stained slides were available in each case.

Urinary sediment screening for cancer detection provided 40 cytological preparations of voided (morning) urine collected in an equal amount of fixative (formalin) and sent to our institute during a previous investigation prior to a morphological quantitative study (Montironi et al. 1988). On the basis of the histological reports the cases were subdivided as follows: normal urothelium (10 cases, NU), non-invasive urothelial papillary carcinoma of grade 1 (10 cases UPC1), of grade 2 (10 cases, UPC2) and of grade 3 (10 cases, UPC3). Urinary samples were processed by cytocentrifugation. In each case, at least two Papanicolaou-stained slides were available.

Cytological, histological comparisons were made by evaluating the extent of agreement between the evaluation of the nucleolar margination in the cytological preparations and the corresponding histological slides. Sixty cases were used. These were selected at random from those already included in the first three parts and included 20 cases from the prostate set (10 of nodular hyperplasia and 10 of adenocarcinoma), 20 from the breast set (10 benign and 10 malignant) and 20 from the thyroid set (10 follicular adenomas and 10 follicular carcinomas). The nucleolar margination was evaluated in the histological slides without knowing the results obtained from the corresponding cytological preparations.

Reproducibility was assessed by investigating the extent of intra- and inter-observer reproducibility in the evaluation of the nucleolar margination, using the 41 cytological preparations from the prostate set. Two sessions were organized with two of our team (AB and CMG). In session 1, one slide per case was first given to one of the two and then to the other. The participants were not aware of each other's evaluation; nor was it possible to discuss the cytological features of the slides. One month later, a second session was held: the slides were presented in a different order from the first session, although the other circumstances were exactly the same as the first.

The overlap index (O_i) was calculated in order to quantify the degree of overlap between the two diagnostic categories. It is a non-parametric index originally developed by Hartz (1984). O_i is calculated as follows:

1. Combine the n_1 observations from sample 1 and the n_2 observations from sample 2, and then rank all the observations from the smallest to the largest.
2. For tied observations, first assign raw ranks to the tied values as if they were not tied. The corrected rank is the average of the raw ranks of the tied numbers.
3. Add ranks for one of the samples, e.g. sample 1. Generally it

is more convenient to add ranks for the smaller size sample. Call this sum of the ranks T_x.
4. Calculate $T_y = n_1\{(n_1+n_2+1)/2\}$. $T_y = T_x$ when the medians of the two samples are the same, i.e. when there is complete overlap.
5. Calculate $O_i = 1 - |\{[2(T_x - T_y)]/n_1 n_2\}|$. The value of O_i ranges from zero (if there is no overlap) to 1 (if the observations from the two samples have the same medians). The sum of the ranks was obtained using a Macintosh II computer whose statistical package contains the program for the Mann-Whitney U test.

Results

In the benign lesions included in the present study, nucleoli in general appear infrequent, single and small, whereas in the malignant cases nucleoli are always present, multiple and prominent. When dealing with lesions of different grades, for instance the pre-neoplastic lesions of the cervix and the urothelial non-invasive papillary carcinomas, the changes appear progressively greater with increasing grades.

When the nucleolar margination was evaluated, a similar pattern of changes was observed. The results are as follows.

In pre-operative cytology of the thyroid, the nuclear margination values in NG (mean ± standard deviation category values, 12.13 ± 3.81%) and in FA (15.45 ± 4.32%) are lower than in the carcinoma groups (FC, 23.11 ± 3.94%; PC, 24.64 ± 6.67%; MC, 24.24 ± 4.38; AC, 20.09 ± 2.91%). In NG and FA most of the values are similar, with a certain overlap (O_i 0.56). Overlap is almost complete between the carcinoma groups, the O_i values in general being greater than 0.90. The O_i values between NG and each of the carcinoma groups range from 0.00 to 0.05. Slightly higher O_i values are observed between FA and FC (0.19), PC (0.22), MC (0.13) and AC (0.38). The 11 cases of ON show margination-related percentages (25.72 ± 6.40%) within the ranges of the values of the carcinomas (Fig. 1).

As for the breast lesions, the percentages of marginated nucleoli in the benign lesions (17.96 ± 4.49%) overlap to a certain extent with those of the carcinomas (21.75 ± 4.12%), the O_i being 0.52. This value is due to the fact that, among the benign conditions included in this study, there are cases whose nucleolar margination values are in the range of carcinomas; their corresponding histology is that of the apocrine metaplasia, epitheliosis (papillomatosis) and fibroadenoma. Those benign cases whose values are below the lower limit of carcinomas morphologically are all non-proliferative mastopathies (Fig. 2).

Unlike the breast, the prostatic adenocarcinomas in general show percentages of margination (23.94 ± 4.86%) greater than those in nodular hyperplasia (15.29 ± 3.39%), the O_i being very low, i.e. 0.07 (Fig. 3).

Astrocytomas make up the majority of the cases of intraoperative cytological preparations in the tumours of the central nervous system (CNS). All but one of the low-grade astrocytomas have values equal to or lower than 32%, whereas high grade (malignant) astrocytomas show a wide range of percentages not always greater than 32% (low grade, 24.66 ± 10.95%; high grade, 38.36 ± 11.78%). The resulting O_i value is 0.39.

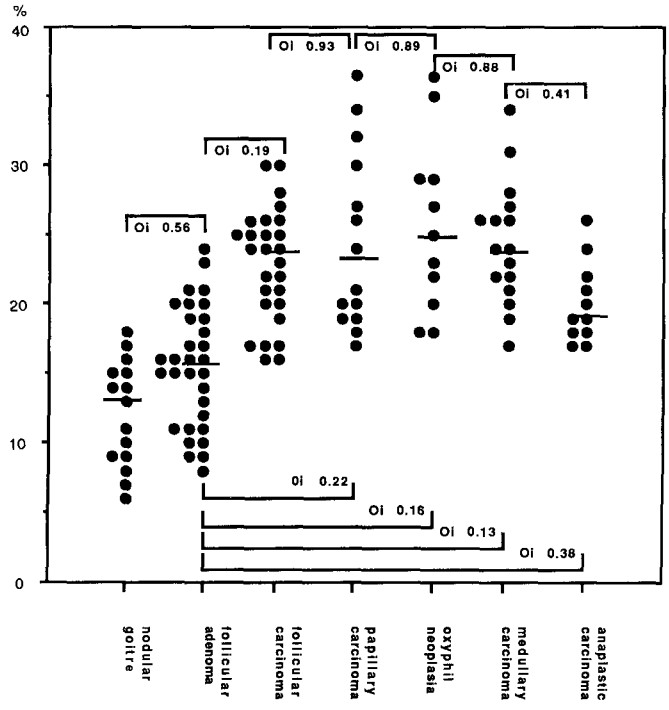

Fig. 1. Dot plot of thyroid fine-needle aspirations (FNAs). The nuclear margination values in the nodular goitres and in the follicular adenomas are lower than in the carcinoma groups. Overlap is almost complete between the carcinoma groups. The oxyphil neoplasias show margination-related percentages within the ranges of the carcinoma values. *Horizontal bars* indicate the median

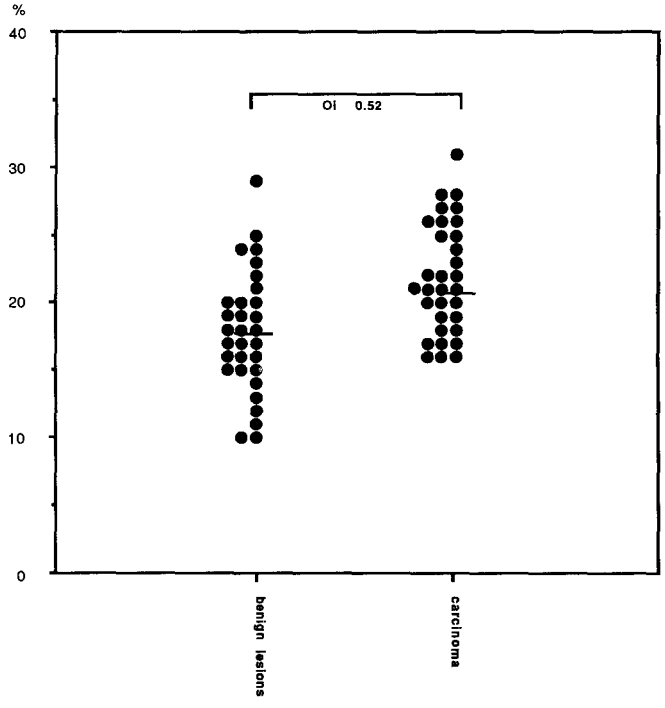

Fig. 2. Dot plot of breast FNAs. The percentages of marginated nucleoli in the benign lesions overlap to a certain extent with those of the carcinomas. Among the benign conditions, there are cases where the nucleolar margination values are in the range of carcinomas; their corresponding histology is that of the apocrine metaplasia, epitheliosis (papillomatosis) and fibroadenoma. *Horizontal bars* indicate the median

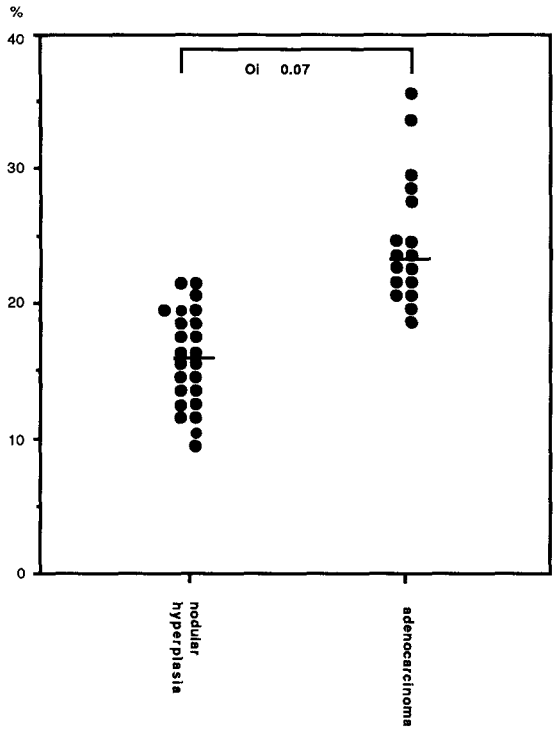

Fig. 3. Dot plot of prostate FNAs. The adenocarcinomas in general show margination percentages greater than those in nodular hyperplasia, the O_i being very low. *Horizontal bars* indicate the median

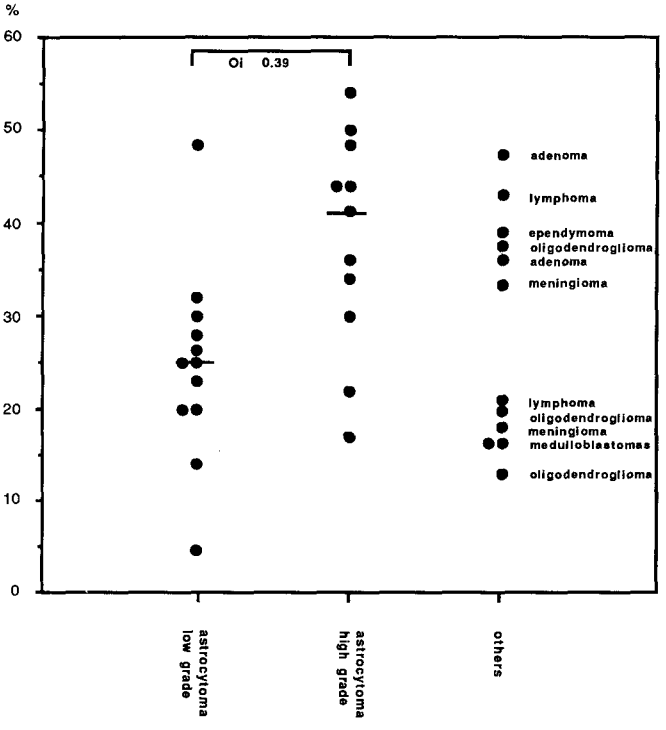

Fig. 4. Dot plot of the tumours of the central nervous system. All but one of the low-grade astrocytomas have values equal to or lower than 32%, whereas high-grade astrocytomas show a wide range of percentages not always greater than 32%. When the values of the non-astrocytoma cases are considered, this threshold is not valid because some of the biologically benign cases show values above and some of the malignants below 32%. *Horizontal bars* indicate the median

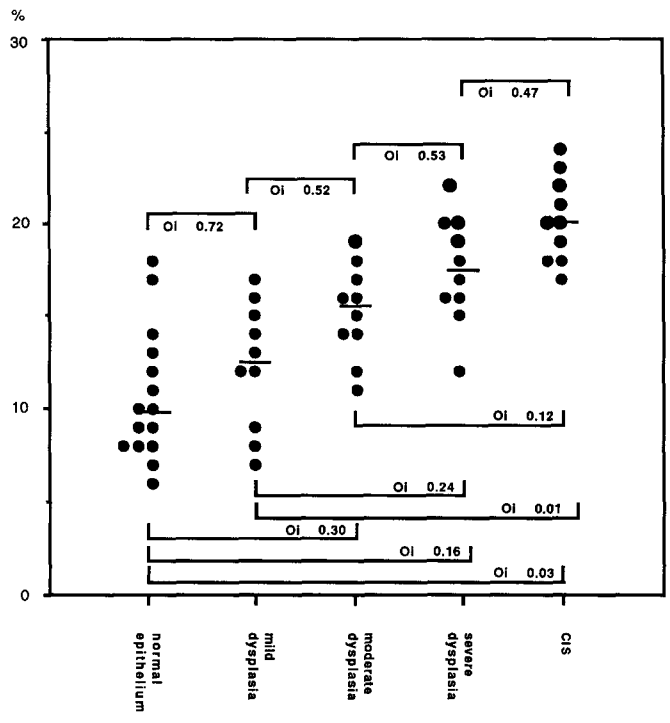

Fig. 5. Dot plot of the pre-cancerous lesions of the uterine cervix. The margination values are progressively greater, going from normal squamous epithelium and mild dysplasia to carcinoma in situ, with overlap between contiguous categories. Two subgroups can be identified, one with values lower than 15% including most of the normal squamous epithelium and mild dysplasia cases; the other subgroup with values equal to or greater than 15% includes all the cases of carcinoma in situ and all but one of the severe dysplasia cases; the moderate dysplasia cases show values both below and above 15%, the percentages being distributed over both subgroups. *Horizontal bars* indicate the median

As for the other tumours, the values are as follows: ependymoma, 39%; oligodendrogliomas, 13%, 19% and 38%; medulloblastomas, both cases had 17%; meningiomas, 18% and 34%; primary malignant lymphomas, 21% and 43%; pituitary adenomas, 36% and 48% (Fig. 4).

In the cytological screening for pre-cancerous changes in the uterine cervix, the margination values are progressively greater, going from NE ($10.66 \pm 3.53\%$) and MD ($12.30 \pm 3.40\%$) to MoD ($15.20 \pm 2.53\%$), SeD ($17.50 \pm 2.91\%$) and CIS ($20.20 \pm 2.30\%$), with overlap between contiguous categories. In particular, two subgroups can be identified, one with values lower than 15% including most of the NE and MD cases; the other subgroup with values equal to or greater than 15% includes all the CIS cases and all but one of the SeD cases; the MoD cases show values below and above 15%, the percentages being distributed over both subgroups. The O_i values fit in well with this artificial and simplified subgrouping. In fact, within each subgroup, the O_i is as high as 0.72 (NE vs MD) and 0.47 (SeD vs CIS). In contrast, the overlap values between categories from the subgroups are low (MD vs SeD, 0.24; MD vs CIS, 0.01). As for MoD, the values are 0.52 (MoD vs MD) and 0.53 (MoD

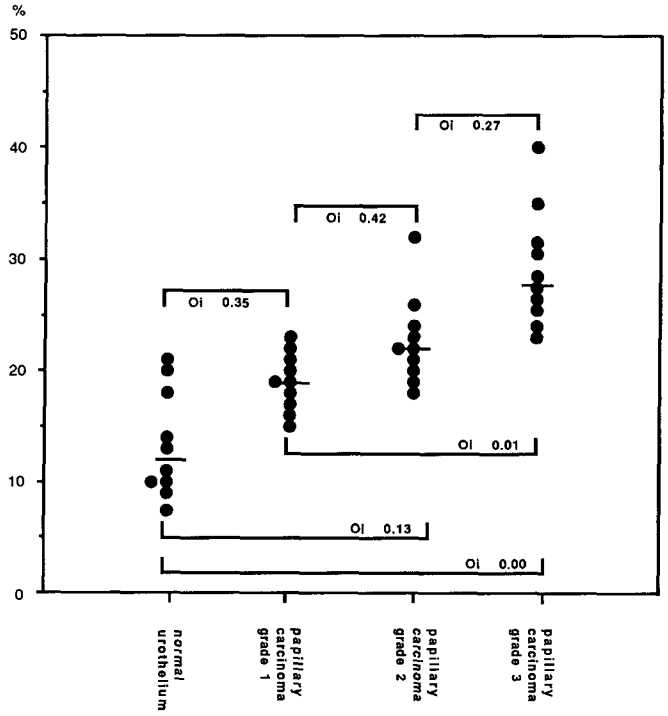

Fig. 6. Dot plot of the urinary sediment for cancer screening. The category margination values are progressively greater from normal urothelium and papillary carcinoma of grade 1 to papillary carcinoma of grade 3. A subgrouping similar to that of the cervix is feasible, one subgroup containing papillary carcinoma of grade 1 together with normal urothelium, the other papillary carcinoma of grade 3, whereas grade 2 cases fall into both. *Horizontal bars* indicate the median

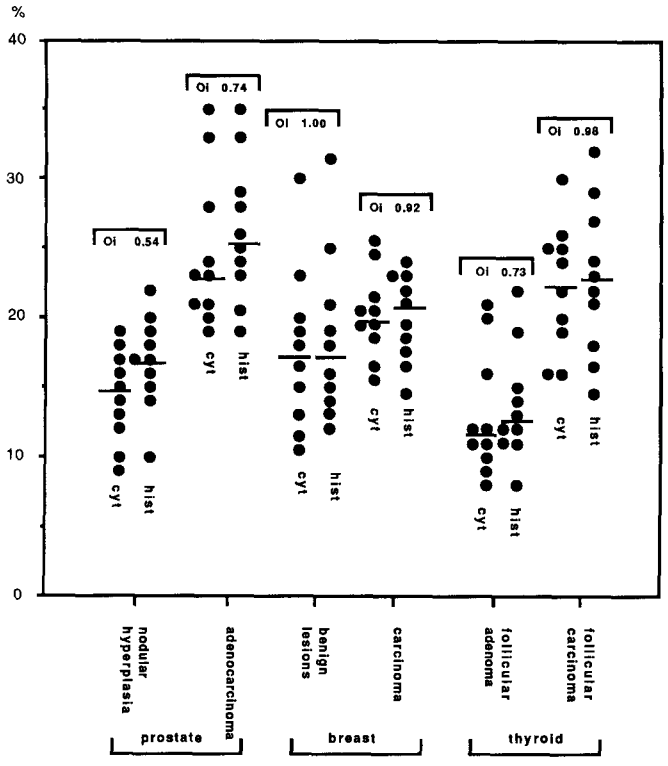

Fig. 7. Dot plot of nucleolar margination in the cytological/histological comparison. The O_i values are high for the breast and for the thyroid cases. In prostatic nodular hyperplasia the value is lower. *Horizontal bars* indicate the median. *cyt*, Cytology; *hist*, histology)

vs SeD). When considering the NE cases, some of the percentage values are lower and some similar to those in the MD, the O_i value being 0.72. In particular, among the NE cases, two show margination values in the range of the CIS cases, i.e. 17% and 18%, respectively. These two cases are associated with the presence of granulocytic infiltration (Fig. 5).

Nucleolar margination in the urinary sediment screening for cancer detection gave category margination values progressively greater from NU (13.40± 4.71%) to UPC 1 (19.00±2.58%), UPC 2 (22.70± 4.02%) and UPC 3 (28.90±5.30%), the O_i values between extreme categories being very low (UPC1 vs UPC3, 0.01) and higher between contiguous ones (NU vs UPC1, 0.35; UPC1 vs UPC2, 0.42; UPC2 vs UPC3, 0.27). A subgrouping similar to that of the cervix is feasible, one subgroup containing UPC1 together with NU, the other UPC3, whereas UPC2 cases fall into both (Fig. 6).

Nucleolar margination in the cytological/histological comparison was made to investigate the extent of agreement of the nucleolar margination evaluated in the cytological (cyt) preparations and in the corresponding histological (hist) sections. The O_i values are high for the breast (benign, cyt vs hist, 1.00; carcinomas, cyt vs hist, 0.92) and for the thyroid cases (adenoma, cyt vs hist, 0.73; carcinoma, cyt vs hist, 0.98). In prostatic nodular hyperplasia the value is lower (cyt vs hist, 0.54), whereas in the carcinoma group cyt vs hist the value is 0.74 (Fig. 7).

Reproducibility studies were performed to investigate the extent of intra- and inter-personal agreement in the evaluation of the nucleolar margination. This was separately calculated for benign (prostatic nodular hyperplasia) and malignant (prostatic adenocarcinoma) lesions. For the adenocarcinoma comparisons all but one of the O_i values are greater than 0.90 either in the intra-observer (AB, 0.87; CMG, 0.93) or inter-observer comparisons (AB1 vs CMG1, 0.97; AB1 vs CMG2, 0.94; AB2 vs CMG1, 0.91; AB2 vs CMG2, 0.95). For the prostatic nodular hyperplasia cases, some of the O_i values in the replicated evaluations are slightly lower, starting from 0.71 (intra-observer: AB, 0.92; CMG, 0.83; inter-observer: AB1 vs CMG1, 0.94; AB1 vs CMG2, 0.78; AB2 vs CMG1, 0.87; AB2 vs CMG2, 0.71) (the number 1 or 2 after the abbreviations indicates the first or the second session of the reproducibility study) (Fig. 8).

Discussion

Nucleolar alterations are some of the most constant features of the neoplastic state. When investigated in histological and cytological preparations, such alterations include an increase in the number or frequency, increase in size, and margination (Ghadially 1985).

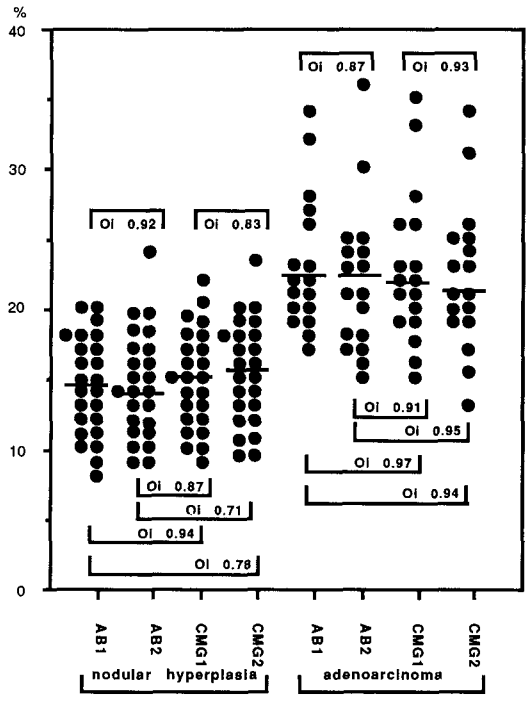

Fig. 8. Dot plot of the reproducibility in the evaluation of the nucleolar margination in the cytological preparations of the prostate. For the comparisons in the adenocarcinomas all but one of the O_i values are greater than 0.90 either in the intra-observer or inter-observer comparisons. For the prostatic nodular hyperplasia cases, some of the O_i values in the replicated evaluations are slightly lower, starting from 0.71. *Horizontal bars* indicate the median. (The abbreviations *AB* and *CMG* stand for the names of the observers who took part in the project; 1 or 2 after the abbreviations stands for the first or second session)

As for the nucleolar frequency, two subgroups of qualitative terms are used in cytopathological practice. The first includes terms such as "nucleoli absent, infrequent, inconsistent, always present"; the other includes terms such as "single or multiple" (Kini 1987; Kline 1981; Koss 1979). As for the nucleolar size, the qualitative terms suggested in the subjective cytological evaluation are "small, hypertrophic, prominent, macronucleoli" (Kini 1987; Kline 1981; Koss 1979; Kelemen et al. 1990). In the benign lesions of our study, nucleoli were in general infrequent, single and small, whereas in the carcinomas they were always present, multiple and prominent. However, although increase in frequency and prominence of the nucleolus were fairly constant features of the neoplastic state, it was by no means a hallmark of malignancy, because these characteristics were also present in some of the benign lesions, for instance in the breast, or of normal uterine cervix epithelium with inflammation (see below). These nucleolar alterations observed in our study are similar to those already observed either qualitatively or quantitatively (Ferrer-Roca et al. 1990; Helpap 1988; Huntington et al. 1989; Kamel et al. 1990; Lesty et al. 1990; Montironi et al. 1990; Murphy 1990; Shimazui et al. 1990; Sobrinho-Simoes et al. 1977; Suen 1988; van Diest et al. 1990).

In states of active protein synthesis, the nucleolus may come to lie against the nuclear membrane. This has been referred to as nucleolar margination (Ghadially 1985). Although close attachment of nucleoli to the nuclear membrane is only rarely found in normal cells of various types and species, margination is seen much more frequently in cells rapidly growing or engaged in the production of a protein-rich secretion (Ghadially 1985). For example, some 50% of the nucleoli move to the nuclear margin in the regenerating liver (Swift 1959). Nucleolar margination was observed during the growth phase of the keratoacanthoma (Ghadially 1985). However, this type of nucleolar arrangement has been more frequently seen in malignant tumours and has been quantified in studies mainly performed on histological sections calculating either location or eccentricity formulas. In general these studies take into consideration the prerequisite that the nucleus has to be round and has been investigated either as one of the factors contributing to the histopathological diagnosis or as a predictor of the clinical course of breast cancer, thyroid carcinoma, prostate adenocarcinoma, and some forms of non-Hodgkin's lymphoma (Ferrer-Roca et al. 1990; Helpap 1988; Lesty et al. 1990; Montironi et al. 1991 b, c; van Diest et al. 1990). In a study on benign, pre-neoplastic and neoplastic lesions of the prostate we investigated the diagnostic value of a nucleolar eccentricity index in comparison with other nucleolar features (Montironi et al. 1991 a). It was found that the proportion of nucleoli touching the nuclear membrane gives the same information as the eccentricity index. Therefore in the current study a simple method for determining the nucleolar location was preferred to others which require the use of complex calculations, such as the non-parametric approach suggested by Lesty et al. (1990), or of stereological tools, such as the "nucleator" described by Gundersen et al. (1988).

Little attention has been given to nucleolar margination in diagnostic cytopathology. For instance, Bahr and Bahr (1990), when describing the properties of the nucleolus, mentioned very briefly that the "nucleolus is eccentrically located, sometimes attached to the nuclear envelope". Helpap (1988) can be considered the first to investigate semi-quantitatively the central, intermediate and peripheral location of the nucleoli in cytological material from the prostate. He observed that the "carcinoma of grade II and III, which can be relatively easily diagnosed cytologically, shows distinct differences in the number and localization of nucleoli when compared with cases showing normal appearances, inflammation and typical hyperplasia". In a previous paper from our group (Montironi et al. 1991 b), margination was investigated in follicular neoplasms of the thyroid on FNA material. It was found that this change has a discriminatory power greater than that of the frequency and size. In agreement with our previous observations, the present study shows that there are differences in the proportion of marginated nucleoli between benign and malignant lesions in different organs, mainly in the prostate and in the thyroid and, to a lesser extent, in the breast and CNS. As for the thyroid gland, the carcinoma groups always showed values greater than 15%, whereas in most of the NG and FA cases, the values were below the threshold. This threshold is not applicable to the cases

of ON, whose values were always greater than 15%, irrespective of the diagnosis made on the histological slides; a similar lack of discrimination was previously observed for the nucleolar frequency (Montironi et al. 1990). This is in agreement with the concept that the ON of the thyroid include all potentially malignant cases, whose diagnosis depends on the presence of vascular invasion and/or capsular infiltration on the histological slides (Rosai and Carcangiu 1987). As for the breast, there was a partial overlap between the two diagnostic groups. This was due to the fact that there were cases among those reported histologically as benign whose values are in the carcinoma range (all but one were invasive, either ductal or lobular carcinomas, the exception being an intraductal carcinoma). When considering the corresponding morphology, these were represented by benign proliferative lesions such as apocrine metaplasia, epitheliosis and fibroadenoma. This finding is in some way in agreement with the observations made on histological sections of breast lesions by Derenzini et al. (1990), according to which silver-stained interphasic NORs, a feature closely related to nucleolar morphology, allow distinction between benign and malignant lesions only in a minority of cases.

As for the CNS tumours, the evaluation of the nucleolar margination can help in the separation of low- from high-grade astrocytomas. In fact, a threshold of 32% can be set in order to allocate most of the cases to the corresponding grade made on the histological sections. However, when the values of the non-astrocytoma cases were considered, this threshold was not valid because some of the biologically benign cases show values above and some of the malignant below 32%.

It is worth mentioning the behaviour of the nucleolar margination in lesions which form a continuous spectrum of changes and in which grades are identified, namely the pre-neoplastic lesions of the uterine cervix and the papillary carcinomas of the bladder. In these two, the values were progressively greater moving from the low grade towards the high grade, with overlap between contiguous grades, further supporting the idea that the grading systems draw artificial lines in lesions with progressively greater changes (Mariuzzi et al. 1991). From the practical point of view, two broad subgroups which are quite well separated can be identified, one containing low grades, i.e. MD in the cervix or UPC1, and the other containing SeD/CIS or UPC3; intermediate grades form a non-homogeneous category, not distinct from the contiguous ones. This is in agreement with studies in which a similar conclusion was drawn by using computer-aided methods of analysis (Mariuzzi et al. 1991; Montironi et al. 1988; Wied et al. 1984). Moreover, this subgrouping is in agreement with the Bethesda classification according to which a two-grade system is the simplest and most useful approach to the cytological grading of the cervical lesions (National Cancer Institute Workshop 1989). Two cases of NE of the cervix showed margination percentages in the range of the CIS cases and were characterized by the presence of acute inflammation. Their cytological changes were compatible with "benign proliferative reactions of the uterine cervix", a group which can be confused with neoplastic lesions (Patten 1983).

Good correspondence exists between margination in cytological and histological preparations in benign and malignant lesions of the breast and thyroid, whereas the O_i values in the prostate are lower, especially for the nodular hyperplasia. This mild discrepancy could have been caused by the fact that the cytological preparations were not obtained from exactly the same areas from which the sections were cut. An additional cause may have been the selection criterion adopted in the evaluation of the margination in histological sections, that is, the most cellular area. In the nodular hyperplasia of the prostate gland, this corresponds to the secretory cell layer in non-atrophic glands, that is, the only type of epithelial cells in which nucleoli appear clearly. The same criterion may have influenced the O_i obtained in the follicular adenomas of the thyroid, in which the follicles in the sub-capsular zone are smaller and with more "active" nuclei than towards the centre of the nodule (Montironi et al. 1989).

In conclusion, nucleolar margination is easy to evaluate in routinely prepared cytological material and shows a level of reproducibility similar to that observed by Huntington et al. (1989) in the evaluation of the nucleolar diameter in ocular melanoma. Taken alone, nucleolar margination does not discriminate benign from malignant cases in all situations. However, it can be considered together with other qualitative and quantitative features to obtain the best discrimination. As for the follicular lesions of the thyroid previously investigated, margination was the best discriminator of nucleolar features and allowed accurate identification of the category diagnosed in the corresponding histological material (Montironi et al. 1991 b).

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