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HISTOPATHOLOGICAL SUBGROUPING OF WHO II UROTHELIAL NEOPLASMS BY CYTOPHOTOMETRIC MEASUREMENTS OF NUCLEAR ATYPIA

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The grade of nuclear atypia was objectively assessed in 21 cases of non-invasive WHO II transitional cell bladder neoplasm. Measurements were performed by stage scanning absorbance cytophotometry, registering nuclear optical density, nuclear area, and the variability of these two factors, in 5 μm thick Feulgen-stained paraffin sections. The material was subgrouped into a 2– and a 2+ group according to the degree of histopathological atypia. Cytophotometrically determined atypia showed close correlation to the subjectively judged atypia, and there was no overlap between 2– and 2+. A difference in tumour ploidy level between different WHO II tumours is the most likely explanation of the grouping recorded.

Key words: Urothelial neoplasms; cytophotometry; subgrouping.

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The histopathological grading of transitional cell tumours of the human urinary bladder is of considerable clinical importance, but owing to its subjectivity it does not show perfect reproducibility (3, 10). DNA cytophotometry has proved to be a useful, objective, tool in this context and several studies have shown that aneuploidy of the tumour stem cell is a prognostically negative sign (4, 7–9, 11–17).

Tumours of higher grade more commonly have aneuploid cell populations. *Tribukait and Gustafsson* (16) noted that tumours in the WHO II group are diploid in 60%, and aneuploid in 40% of cases, whereas WHO I and WHO III are almost without exception diploid and aneuploid respectively. The ploidy dividing line that passes through the WHO II group is very interesting, because the WHO II group is inhomogeneous both regarding tumour histology and clinical behaviour.

The two main features of nuclear atypia, namely nuclear area and stainability, can be measured by means of scanning absorbance cytophotometry in Feulgen-stained tissue sections (1). Cytophotometric measurements of this kind in selected cases of urothelial bladder carcinomas WHO I, II, and III showed that objective differentiation is indeed possible (2). This kind of measurement, provides a reliable measure of atypia that can be used as a reference for subjective histopathological grading. It also allows more accurate evaluation of the prognostic influence of nuclear atypia.

The aim of the present investigation is to measure the atypia in WHO II tumours and to find objective criteria for a refined classification.

MATERIAL AND METHODS

Biopsy material from all new cases of urothelial bladder neoplasm, discovered during 1974 and 1975 at the University Hospital, Linköping, was re-examined. This gives a follow-up period of at least 6 years. Only

single, papillary, non-invasive tumours were included. The urinary cytology was of equal or lower grade than in the biopsy specimen in every case. 21 cases fulfilled these criteria. 3 patients died from intercurrent disease during the follow-up period, and 2 were lost to follow-up after 3 and 5 years respectively. Recurrences, progress of disease, and mode of treatment were read from the clinical records.

The biopsy specimens had been fixed in neutral buffered formalin and prepared by routine techniques. Using a Leitz 1212 microtome constantly set at 5 μm section thickness, two consecutive sections were cut from each paraffin block: one was Feulgen-stained as described by *Duijndam and Van Duijn* (5, 6) and one was stained in haematoxylin and eosin (H&E). The final histopathological grading was made on these newly-cut sections to assure optimal comparability when evaluating the correlation between measured atypia and subjectively judged atypia. These specimens were histologically subclassified into two groups; 2– with a lower and 2+ with a higher degree of atypia.

Cytophotometric measurements were made with a computerized stage-scanning cytophotometer, Leitz MPV 2, in combination with the HISTOSCAN program. This measuring system is described elsewhere (1). In each specimen 120 epithelial-cell nuclei were systematically measured within a representative field selected from the H&E section. The staining in each section was checked by measuring 30 lymphocyte nuclei.

RESULTS

The results are presented in three-dimensional diagrams of nuclear area, nuclear mean optical density, and cluster size (cluster size represents the total variability of both mean optical density and area). In Fig. 1 for comparison, the previously

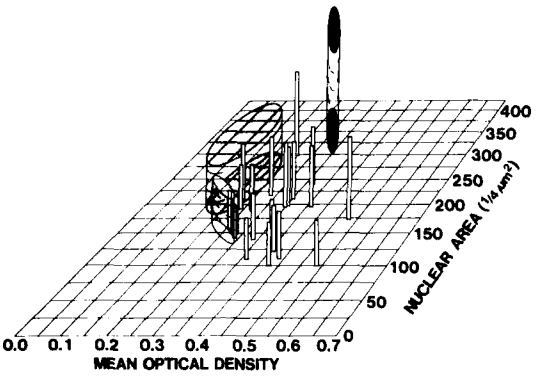

Fig. 1. Nuclear mean optical density, nuclear area, and cluster size for all measured specimens. The height of each column represents the relative cluster size. Previously determined values for WHO I, II, and III are indicated thus,
○ WHO I, ○ WHO II, ● WHO III.

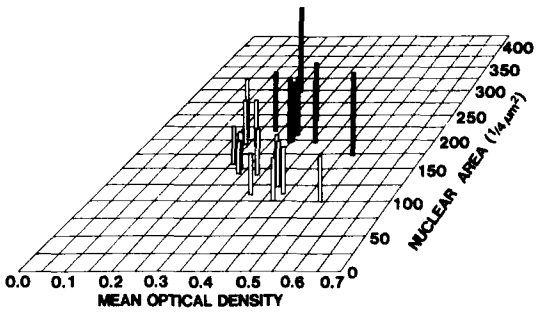

Fig. 2. Correlation between subjective histopathological grading and cytophotometrically measured atypia. White columns, 2–, black columns, 2+.

TABLE 1. *Nuclear Optical Density, Nuclear Area, and Cluster Size for WHO Specimens Displaying a Lower (2–) or Higher (2+) Grade of Histological Atypia. (S.D.)*

Histology	Optical density	Area (1/4 μm²)	Cluster size	No
2–	0.35 (0.1)	138 (35)	63 (8)	13
2+	0.36 (0.1)	210 (40)	93 (15)	8

determined cytophotometric values for WHO I, II, and III (2) are indicated, and it can be seen that the newly measured WHO II lesions take an intermediate position between the WHO I and WHO III specimens. The WHO II group is inhomogeneous however: most of the specimens have values close to those of the formerly measured WHO I lesions, whereas others have values close to those of the WHO III group. Furthermore, the WHO-I-like lesions seem to be separated from the WHO-III-like lesions by a rift in the diagram which gives the distribution a bimodal appearance.

The correlation between subjective, histopathological grading and cytophotometry is evaluated in

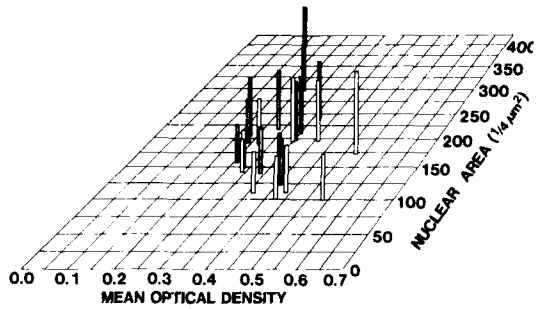

Fig. 3. Correlation between cytophotometric results and recurrence rate. □ 0–1 recurrence, □ 2–4 recurrences, ■ > 4 recurrences, during the 6-year follow-up period.

Fig. 2, where the histological classification of the specimens into 2– and 2+ subgroups is indicated. All specimens judged as 2+, *i.e.* displaying higher grade of atypia, are included among those with cytophotometric values close to those of the WHO III group. The 2+ specimens are all above the rift in the diagram, and the 2– specimens are all below it.

The differences between subgroups 2– and 2+ are studied in detail in Table 1, which shows that the 2+ specimens have larger nuclear area and cluster size. The two subgroups do not differ with respect to mean optical density or variability of optical density. The greater cluster size recorded in the 2+ specimens thus exclusively reflects the area factor.

In Fig. 3 the measurements are correlated to the results of follow-up. Of the 8 specimens in the 2+ group, 2 were associated with more than 4 recurrences, 3 with 2–4 recurrences, and 3 cases with 0–1 recurrence. The corresponding figures for the 13 histologically less atypical specimens, 2–, were 2, 4 and 7. Thus there is a slightly higher tendency towards recurrences in the 2+ specimens, but the series is too small to give statistically valid figures. In 3 cases invasive carcinoma subsequently developed. These cases all were in the 2+ group and included the 2 specimens with the highest recurrence rate, but also specimens from the intermediate recurrence group.

The treatment was the same in all cases and consisted of local electrocoagulation.

DISCUSSION

The great clinical importance of histopathological grading of urothelial bladder neoplasms makes objective registration of nuclear atypia desirable. In a previous study we showed that nuclear atypia could be objectively recorded by scanning absorbance cytophotometric determinations of nuclear area and nuclear stainability in Feulgen-stained, 5 μm thick sections cut from routinely paraffin-embedded material, and that the three WHO grades could indeed be differentiated (2).

Clinical experience shows that the WHO II group is inhomogeneous, and recent flow cytometric studies of nuclear DNA content suggest that there is a dividing line between high-grade aneuploid, and low-grade diploid tumours within the WHO II group (16).

By microscopy, *Tribukait and Esposti* (14) and *Tribukait et al.* (15) divided the WHO II group into subgroups IIa and IIb, and found a higher incidence of aneuploidy in the IIb specimens. No direct correlation between tumour ploidy level and nuclear atypia has been shown, but there are probably at least two kinds of WHO II tumours, those with diploid stem cell and low atypia, and those with aneuploid stem cell and high atypia.

Against this backgroud it is highly interesting that the WHO II group can be subdivided by objective assessment of nuclear atypia into two apparently non-overlapping groups. As is shown in Fig. 2, this subgrouping correlates with the careful histopathological subgrouping into 2– and 2+ performed. Of the two factors measured, nuclear area and optical density, the former had a strong discriminative power, whereas the optical density was the same in both 2– and 2+.

The nuclei in the 2+ group are larger but have the same optical density as those in 2–, which indicates that they must have a higher DNA content. The results are therefore what were to be expected if the 2+ tumours had been hyperdiploid, *i.e.* aneuploid. The fact that the relation between 2– and 2+ in this study (13 cases/8 cases) is exactly the same as the relation between diploid and aneuploid tumours (40/26) in the study of *Tribukait and Gustafsson* (16) is perfectly consistent with this concept. The presence of a dividing line inside the WHO II group both regarding tumour ploidy and objectively determined nuclear atypia clearly indicates that a refinement of tumour classification is desirable and also possible. The type of measurement used here, made under direct visual inspection of every measured nucleus, has the benefit of transferring a sense of objectivity to the operator, which is useful in routine diagnostic work.

Owing to the limited number of cases studied (21 cases), the correlation between measured atypia and outcome of the disease will inevitably be unreliable. However, all 3 patients who subsequently developed invasive carcinoma belonged to subgroup 2+. No clear-cut correlation to rate of recurrence was noted, but there is a tendency in Fig. 3 towards a higher rate in the 2+ specimen.

In conclusion, our results show that the WHO II group of transitional cell neoplasms is inhomogeneous regarding nuclear atypia. This inhomogeneity most likely reflects the difference in tumour ploidy level discussed above.

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