Virchows Archiv A Pathol Anat (1988) 413:249–255

Virchows Archiv A
Pathological Anatomy and Histopathology

Can cytomorphometry replace histomorphometry for grading of bladder tumours?

H.G. van der Poel[1], M.E. Boon[2], L.P. Kok[3], J. Tolboom[4], B. van der Meulen[4], and E.C.M. Ooms[1]

[1] Department of Pathology, Westeinde Ziekenhuis, The Hague, The Netherlands
[2] Leiden Cytology and Pathology Laboratory, Leiden, The Netherlands
[3] Institute for Theoretical Physics, University of Groningen, and
[4] Mathematical Institute, University of Groningen, The Netherlands

Summary. Cytomorphometry, using various cytopreparatory techniques on bladder washings and histomorphometry on the resected bladder tumours, was used in an attempt to answer the question: Can cytomorphometry replace histomorphometry for grading of bladder tumours? For the analysis of quantitative data, a probit model was used. Three out of the four cytomorphometric methods provided data supportive to the histomorphometry. Using one of the four cytomorphometric methods was sufficient to enhance grading accuracy and all were equally good. Two cases of high grade carcinoma in situ were properly identified by cytomorphometry (as judged on the follow-up data) but the concurrent resected papillary tumours were low grade. These findings indicate that cytomorphometry is a useful method in bladder tumour grading. In some cases it is preferable to histomorphometry.

Key words: Bladder cancer – Grading – Histomorphometry – Cytomorphometry

Introduction

The clinical relevance of the grading of urothelial bladder tumours depends on whether tumour grade correlates with prognosis. In our own material, we found that Grade I carcinomas (locally resected) had a 5 year survival rate of 87%, and Grade III carcinomas with extensive therapy a rate of 32%. Similar percentages were found by Jordan et al. (1987). High grade lesions account for almost all deaths from bladder cancer (Brawn 1982; Koss 1985).

Offprint requests to: H.G. van der Poel, Leiden Cytology and Pathology Laboratory, P.O. Box 16084, 2301 GB Leiden, The Netherlands

It is common practice that grading of the bladder carcinoma is done by well-trained pathologists, according to WHO standards. As the distinction between low-grade carcinomas (Grades I and II) and high-grade carcinomas (Grade III) is of paramount importance and a more refined gradation leads to practical complications, we focus sed our study on this distinction between low-grade and high-grade. If two pathologists are asked to make these assignments on the basis of a qualitative analysis according to the WHO system, the intra- and interobserver reliability is low (Ooms et al. 1983a, b; Ooms et al. 1985).

To achieve a higher degree of objectivity and reproducibility, three options are available. The first is a morphometric analysis of cytological material, the second a morphometric analysis of histological material, and the third is to base assignment on majority voting by two or more pathologists.

The third is not relevant from a practical point of view, and has not been shown to improve reproducibility (Ooms et al. 1983a). Morphometric analysis of cytological or histological material is an attractive option for diagnostic work. In this paper cytomorphometry using various cytopreparatory techniques and histomorphometry were applied in an attempt to answer our question: Can cytomorphometry replace histomorphometry for grading of bladder tumours?

Materials and methods

The material consisted of 50 consecutive cases, 39 with the diagnosis of bladder tumour and 11 with inflammatory changes. Each bladder tumour was graded histologically according to the WHO system by one pathologist. Eleven were graded as Grade I, 16 as Grade II, and 12 as Grade III. In all patients the follow up was complete until 2 years after the bladder washings. Prior cytology and histology specimen were available.

For the cytological preparations the bladder was rinsed with physiological saline solution (Flanagan 1978; Murphy et al. 1981; Zein et al. 1984). The bladder washings were per-

formed in the Westeinde Ziekenhuis in The Hague during 11 months (February–December 1986). The sample was centrifuged and the cell pellet resuspended in 50 ml fixation fluid prior to further processing. The fixative used was composed as follows: 70 cc polyethylene glycol (MW 300), 800 cc alcohol 96%, and 130 cc distilled water.

After centrifuging the sediment was divided into two parts. One was used to make the smears, the other to embed in paraffin. In this latter case the pellet was dehydrated in alcohol 96% twice, alcohol 100% twice, and toluene once. Paraffin was added to the dehydrated pellet and when it solidified the test tube was broken, and sections prepared from the block. Because not every sample contained enough material, all techniques could not be used in all cases. One smear was stained according to the Papanicolaou method (Pap-smear) and one according to the Feulgen method (Feulgen-smear). One section was stained according to the Papanicolaou method (Pap-section) and one according to the Feulgen method (Feulgen-section).

The cytomorphometrical measurements were made by means of a microscope connected with a computer MOP-video plan KONTRON. The nuclear area of 50 nuclei per slide was measured taking at most 15 min. The computer calculated the mean value of these 50 measurements and their standard deviation. All measurements were done by one person not knowing the histological grading. Interobserver discrepancies were negligible as other well-trained cytologists came to virtually the same values for nuclear area.

The slide to be measured was first screened to obtain a general impression and to circle the diagnostic cell groupings of the highest grade (for definition see further text). Only those nuclei that were clearly visible and not covered by other nuclei were taken for measurement.

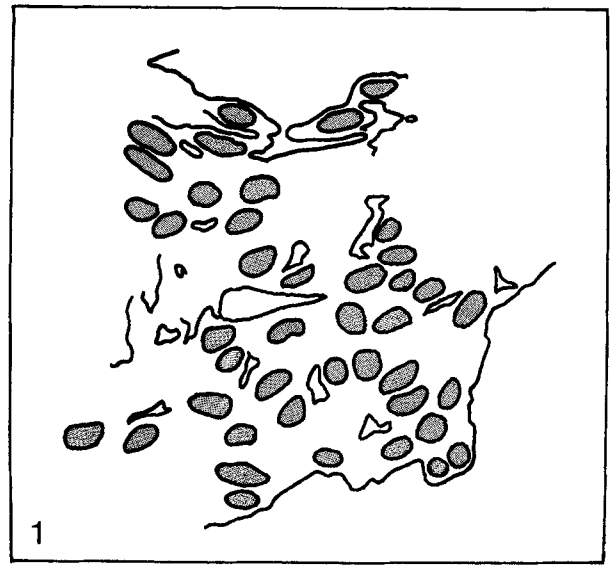
There were three types of diagnostic cell groupings.
Type 1: Large, dense papillary groups with smooth outlines.
Type 2: Smaller, less dense papillary groups with irregular outlines.
Type 3: Loose clusters of a few clearly malignant cells.

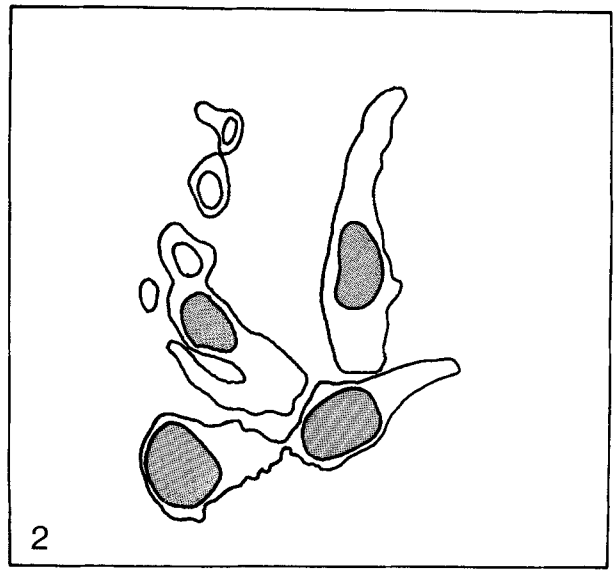
In addition, the washings contained sheets with ragged borders and lacking a papillary architecture.

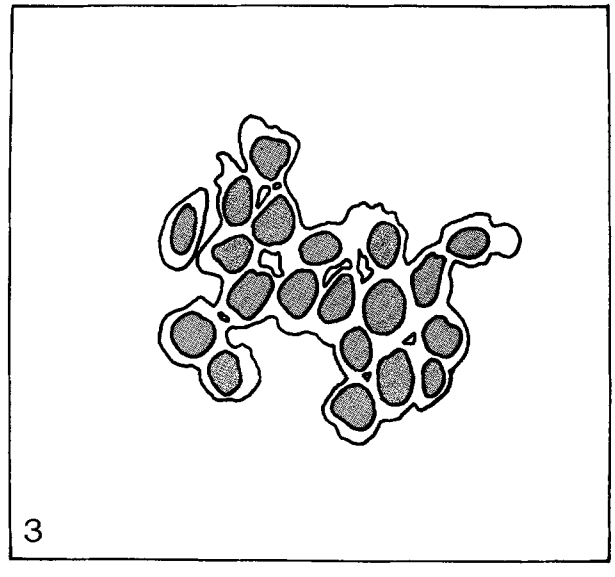
Grade I tumour cells are almost exclusively in type 1 cell groupings (Fig. 1). The nuclei are often pale in the Papanicolaou stain with nuclear clearing and there is a prominent nuclear envelope and clumping of some chromatin. The nuclei are predominantly oval or round with some abnormal shapes. The nuclei measured were these situated at the edges of the papillary groups. The nuclei in the center are not clearly visible, thus do not fit for measurement. Oval as well as round nuclei were measured.

Grade III tumour cells are mainly arranged as single cells and in addition in type 3 cell groupings. Cells selected for measurement have a very high N/C ratio (Bergkvist et al. 1965; Beyer-Boon 1977) and the chromatin pattern shows uneven distribution. Often there is more than one, irregularly shaped large nucleolus (Fig. 2).

Grade II tumour cells are arranged in predominantly type 2 cell groupings and sometimes as single cells or in type 1 group-







Figs. 1–3 are camera-lucida drawings of carcinoma cells selected for measurement (× 400)

Fig. 1. Grade I tumour cells

Fig. 2. Grade III tumour cells

Fig. 3. Grade II tumour cells

Table 1. The mean variables* for histomorphometry and cytomorphometry

	Grade I		Grade II		Grade III		Inflammation	
	n	mean SD	*n*	mean SD	*n*	mean SD	*n*	mean SD
Histomorphometry								
Large*	11	4.209±0.243	16	4.635±0.208	12	4.807±0.196	0	
Deep	11	3.767±0.214	16	4.184±0.348	12	4.332±0.318	0	
Superf	11	3.492±0.238	16	3.840±0.248	12	4.048±0.375	0	
Cytomorphometry								
Pap-smear	11	3.584±0.549	16	4.123±0.379	12	4.781±0.371	10	3.390±0.157
Feulgen-smear	9	3.441±0.380	12	3.985±0.356	9	4.440±0.463	1	3.045 –
Pap-section	11	3.424±0.260	15	3.758±0.220	11	4.020±0.229	10	3.428±0.142
Feulgen-section	10	3.118±0.363	16	3.646±0.282	11	3.948±0.284	9	3.056±0.198

* log mean values and standard deviation values of nuclear area. 50 nuclei per slide are measured

ings. It is apparent that the differences between the malignancy grades are graded with Grade II tumour cells in between Grade I and Grade II. The difference with Grade I is that the cellular and nuclear features are more malignant, for example, an unfavorable N/C ratio, moderate to highly hyperchromatic nuclei and irregularly shaped nucleoli (Fig. 3).

Cases with inflammation have mainly isolated cells and sheets. The cells are often cylindrical with an eccentric nucleus. In this group there are no papillary groups so nuclei in single cells are measured.

Prior to measurement the slide was screened and the diagnostic cell groupings of the highest malignancy grade were circled for measurement. Thus, in case of an admixture of type 1 cell groupings (with no or little atypia) and type 3 cell groupings (clearly malignant), the latter were circled for measurement. In case of type 2 and type 3 cell groupings, the scattered single clearly malignant cells in the neighborhood of the cell groupings were also measured.

Histological material was obtained by transurethral resection. Each biopsy was processed into 3 blocks. The Grade I tumours were all non-infiltrating in contrast with the Grade III tumours. In each case the most abnormal parts of the tumour tissue were selected for measurement. In these areas, three types of cells were measured: A, deep cells (Deep); cells near the stroma, B, superficial cells (Superf); cells near the bladder lumen, C, cells with large nuclei (Large).

The choice of superficial cells, deep cells, and large cells, was made on the basis that in routine diagnosis pathologists grade bladder tumours according to the cytomorphology of the cells. In each tumour ±240 cells were thus selected for morphometric study. Of each cell the contour of the nucleus was traced with the cursor and thus the area of the nucleus was measured with the graphic tablet. Cells with evident pyknotic nuclei or degenerating cells were not selected for this study.

Statistical methods. In our earlier cytomorphometric and histomorphometric publications (Boon et al. 1981; Ooms et al. 1982; Ooms et al. 1983), as in the present work, it was clearly shown that there is a strong linear dependency between standard deviation and mean value. For this reason, we choose as a tool for data reduction the calculation of the natural logarithm of the mean value in order to obtain a variance stabilizing transformation. We tested this procedure and found it acceptable. In this paper, we use log mean value of the measurements as variables.

The regression of grade (low versus high) on the histomorphometrical and cytomorphometrical variables to be considered was studied using a probit model (McCullagh and Nelder 1983). To test whether the cytomorphometric variables can be used in addition to histomorphometric variables in the grading of the bladder tumours and vice versa, the likelihood ratio test was applied (Serfling 1980).

The test was also used to investigate whether one cytopreparatory technique is sufficient. The statistical package GLIM (see McCullagh and Nelder 1983) was used to perform the analysis for the probit model.

Results

In Table 1 the values of the variables (log mean values and SD values) of the four groups are shown. For the bladder carcinomas, all histomorphometric and cytomorphometric values increase with increasing grade. The values for the group

Table 2. *t*-test results

	inflammation–I	Grade I–II	Grade II–III	Grade I+II–III
Histomorphometry				
Large		<	<	<
Deep		<	=	<
Superf		<	=	<
Cytomorphometry				
Pap-smear	=	<	<	<
Feulgen-smear		<	<	<
Pap-section	=	<	<	<
Feulgen-section	=	<	<	<

$p \leq 0.05$
" = " stands for not significantly different from each other
" < " stands for smaller and significantly different from each other

Table 3. Correlation matrix for the bladder carcinomas

Grade	1,0000 (0)							
Large	0,7265 (39)	1,0000 (0)						
Deep	0,5803 (39)	0,8461 (39)	1,0000 (0)					
Superf	0,6022 (39)	0,7398 (39)	0,7831 (39)	1,0000 (0)				
Pap smear	0,7430 (39)	0,6126 (39)	0,3874 (39)	0,5059 (39)	1,0000 (0)			
Feulgen smear	0,7156 (30)	0,6262 (30)	0,3429 (30)	0,5434 (30)	0,8942 (30)	1,0000 (0)		
Pap section	0,7123 (37)	0,6858 (37)	0,6191 (37)	0,6722 (37)	0,7582 (37)	0,7465 (28)	1,0000 (0)	
Feulgen section	0,7211 (37)	0,6312 (37)	0,4726 (37)	0,5372 (37)	0,8721 (37)	0,8763 (28)	0,8023 (35)	1,0000 (0)
	Grade	Large	Deep	Superf	Pap smear	Feulgen smear	Pap section	Feulgen section

The numbers between brackets indicate the number of cases used to calculate the correlation coefficient

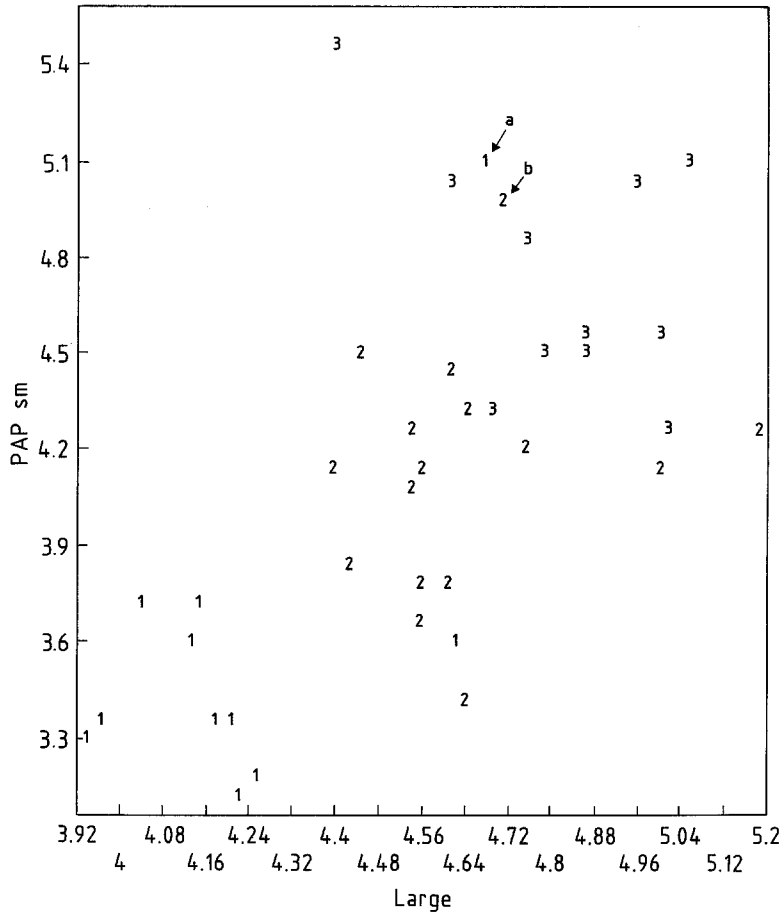


Fig. 4. Plot of Pap-smear with Large by grade. 39 cases plotted. 1: 1st grade, 2: 2nd grade, 3: 3th grade

of inflammatory cases are in the same range as for the Grade I carcinomas.

The significance of the differences between the four groups (see Table 1) was tested univariably, with the two sided *t*-test. Between the group of inflammatory changes and Grade I carcinomas, none of the variables proved to be significantly different. The results of the *t*-test for the bladder

Table 4. Significance of the additional information provided by cytomorphometry for histomorphometry and vice versa

Additional information		*n*	Significance
in:	with regard to:		
Pap-smear	Large/Deep/Superf	39	***
Large/Deep/Superf	Pap-smear		–
Pap-section	Large/Deep/Superf	30	*
Large/Deep/Superf	Pap-section		–
Feulgen-smear	Large/Deep/Superf	37	–
Large/Deep/Superf	Feulgen-smear		–
Feulgen-section	Large/Deep/Superf	37	**
Large/Deep/Superf	Feulgen-section		–
All cytomorphometry	Large/Deep/Superf	26	–
Large/Deep/Superf	All cytomorphometry		–

–: not significant
*: $0.05 < p\text{-value} \leq 0.01$
**: $0.01 < p\text{-value} \leq 0.001$
***: $p\text{-value} < 0.001$

carcinomas are shown in Table 2. For all variables, there were significant differences between Grade I and Grade II tumours, and between low grade (I + II) and high grade (III).

In Table 3 the correlation matrix for the variables of the bladder carcinomas is shown. All coefficients are positive and, with the exception of the coefficient between Deep and Feulgen-smear, significantly differing from zero ($\alpha = 5\%$, two sided test). Within the histomorphometry, as within the cytomorphometry, the correlation coefficients are high. Of the histomorphometric variables, Large correlates best with Grade, and of the cytomorphometric variables Pap-smear is best. In Fig. 4, these two are plotted for all cases to obtain an impression whether classification is feasible.

The results of the probit model for testing significance of additional information of cytomorphometry with regard to histomorphometry are given in Table 4. Three of the four cytomorphometric methods add to the value of the histomorphometric data, but the converse is not true. Note that for the calculation of the significance of the additional information of all four cytological methods combined we had only 26 cases available. This may be the reason that the p value was over 0.05. In addition we found that combining cytological methods did not improve the results. Using one out of the methods is sufficient and as the Pap-smear is the easiest we preferred this method. The probability for high grade, using histomorphometry, can be estimated using the formula:

$$\Phi\,(-17 + 4 \times \text{large} - 2 \times \text{deep} + 1 \times \text{superficial})$$

(see error curve of standard normal distribution). The probability for high grade using Pap-smear can be estimated using the formula:

$$\Phi\,(-10 + 2 \times \text{Pap-smear}).$$

Discussion

The importance of grading of bladder carcinoma is well recognized, however, the question is how to achieve reproducible results. Colpaert et al. (1987) showed that with precise criteria, with examination of the total section systematically field by field and with recording of a grading per field a better interobserver consistency can be obtained. The simple criterion of nuclear size (sometimes within a small subpopulation of cells) appeared to be a very important factor in the subjective grading of the histological sections. This is in accordance with our quantitative findings: of the histomorphometric parameters the value for the parameter Large Nuclei correlated best with Grade. It seems that architectural features of the tumour, such as arrangement of nuclei, coalescence of papillae and whorl formation occurring increasingly in the higher grades are closely correlated with nuclear size (Colpaert et al. 1987). Grading of bladder tumours based on morphometric nuclear parameters thus appears to be an acceptable practice, also for the subgroup of noninvasive transitional cell tumours (Montironi et al. 1985). As for the histological grading of cancer of the larynx, there is also a close correlation between tumour architecture and nuclear size variation, both less well correlating with degree of keratinization (Graem et al. 1980). It is likely that nuclear size as a discriminator is exclusively of value in tumours with a morphological continuum. Transitional cell carcinomas do represent a kind of tumour with increasing cell abnormalities in the higher grades (Bergkvist et al. 1965; Beyer-Boon 1977; Koss 1985; Mostofi et al. 1973). Helander et al. (1985) found a positive correlation between nuclear size as measured by flow cytometry and nuclear area as measured by morphometry. Tumour grade also correlates with the amount of DNA in the nucleus as measured with flow cytometry (Devonec et al. 1982; Farsund et al. 1984; Tribukait et al. 1979).

All three histomorphometric parameters used in the present study are closely correlated. Large (the best grade discriminator) correlates best with the cytomorphometric parameter Pap-smear (see Table 3). The value for this parameter depends on the presence of a subpopulation of cells with large nuclei in the sample. Highly malignant cells with

Table 5. Case history patients A and B

	Patient A	Patient B
1980	Histology Grade I tumour	–
1981	Histology Grade I tumour	Histology Grade I tumour
1982	–	Histology Grade I tumour
1983	Histology Grade I tumour	Histology Grade I tumour
1984	Histology Grade I tumour	Histology Grade II tumour
1985	Morphometry bladder washing Histology Grade I tumour	Morphometry bladder washing Histology Grade II tumour
1986	Histology Grade III CIS	Histology Grade III CIS
1987	–	Histology Grade III solid carcinoma

large nuclei are easily dislodged, thus are likely to appear in cytological material (Boon et al. 1986; Boon and Ooms 1988; Collste et al. 1980).

Patients A and B are interesting cases (see arrows in Fig. 5). Both were examples of patients with recurrent low-grade papillary carcinomas developing high grade carcinoma in situ during follow up (Boon and Ooms 1985). The follow up results of these two patients are summarized in Table 5. In both patients, the cytological bladder washings of 1985 reflected their true status, that is of a high grade carcinoma. In that year, easily visible concurrent low grade papillary tumours were removed for histologic diagnosis, giving the false impression that the prognosis was favorable. The Grade III carcinomas in situ, (exfoliating the highly malignant cells in the bladder washings) were not diagnosed initially due to the fact that they were not macroscopically identified during cystoscopy. A year later, in 1986, the foci of high grade carcinoma in situ were detected, probably partly due to the fact that at that moment there were no concurrent papillary carcinomas catching the attention of the cystoscopist. The bladder cystectomy specimen (1987) of patient B contained extensive high grade carcinoma in situ and many foci of solid carcinoma. Patient A is receiving (1987) radiation therapy with little success. In none of the other low-grade papillary carcinoma cases was this reversal to high grade cytology (due to carcinoma in situ) observed during a follow up of 1–2 years.

In the present study, bladder washings, not voided urine, were used. In particular in Grade I and Grade II carcinomas the harvest of tumour cell is much larger in washings, and there are more suitable for morphometry than voided urine (Badalament et al. 1987; Murphy et al. 1981; Ooms et al. 1982; Trott and Edwards 1973; Zein et al. 1984). As in our former studies (Boon et al. 1982; Ooms et al. 1982), the cell population measured was visually selected, in cases of high grade carcinomas this is particularly important. Many cases of Grade III carcinomas had many cell groupings of Grade I, with much smaller nuclei. When these less malignant cells are included in the measurements, the cases are classified morphometrically into the low-grade group.

The values for the four types of cytopreparatory techniques showed interesting patterns. The values for the Papanicolaou stained cells (both in the smears and the cell blocks) were larger than those for the Feulgen-stained cells. It is unlikely that staining itself changes the size of the nuclei (Boon and Drijver 1986). We postulate that the impression of the nuclear contour in the two different stainings is different, influencing the manual measurements. Automatic measurements might elucidate this point. The values for the smear preparations (both for the Papanicolaou and for the Feulgen-stain) were generally larger when compared with those for the cell blocks. In the Papanicolaou staining, this was the case in 21 of the 27 low grade carcinomas, whilst for all 12 high grade carcinomas the values in the smears were higher. For the nine Grade I carcinoma cases in which both smears and cell blocks were available the mean size difference was 8%, for the 13 Grade II carcinomas 14%, and for the 9 Grade III carcinomas 24%. Similar trends were observed for the Feulgen preparations. Physical factors such as nuclear rigidity are evidently important here (Boon and Drijver 1986). Another reason for this difference in nuclear area can be found in the Holmes' effect. Holmes describes a correlation between the mean nuclear area and the thickness of the section (Holmes 1927). The fact that not all nuclei are cut in the equatorial plane results in a smaller mean nuclear area of the sections, compared to the smears. This was also found by Helander et al. (1985). In the final evaluation, all four cytopreparatory techniques were equally good for morphometry. Since the Papanicolaou smear technique is the simplest of the four, we recommend this method for further studies.

Statistical evaluation showed that histomorphometry does not add to cytomorphometry, but cytomorphometry does add to histomorphometry. No doubt, this can be explained by the selective exfoliation of discriminating cells with large nuclei. Since it is easier to obtain material for cytology, this is a highly rewarding result.

References

Badalament RA, Hermansen DK, Kimmel M, Gay H, Herr HW, Fair WR, Whitmore WF, Melamed MR (1987) The

sensitivity of bladder wash flow cytometry, bladder wash cytology, and voided cytology in the detection of bladder carcinoma. Cancer 60:1423–1427

Bergkvist A, Ljungqvist A, Moberger G (1985) Classification of bladder tumors based on the cellular pattern. Acta Chir Scand 130:371

Beyer-Boon ME (1977) The Efficacy of Urinary Cytology. Thesis, Leiden

Boon ME, Blomjous CEM, Zwartendijk J, Heinhuis RJ, Ooms ECM (1986) Carcinoma in situ of the urinary bladder: Clinical presentation, cytologic pattern and stromal changes. Acta Cytol 30:360–366

Boon ME, Drijver JS (1986) Routine Cytological Staining Techniques: Theoretical Background and Practice. Macmillan Education Ltd, Hampshire and London

Boon ME, Kurver PHJ, Baak JPA, Ooms ECM (1981) Morphometric differences between urothelial cells in voided urine of patients with Grade I and Grade II bladder tumours. J Clin Pathol 34:612–615

Boon ME, Ooms ECM (1985) Cytomorphometric and histomorphometric evidence of the development of carcinoma in situ of the bladder in patients followed for low-grade papillary bladder carcinoma. Acta Stereol 4:255–259

Boon ME, Ooms ECM (1988) Carcinoma in situ of transitional cell epithelium: Clinical and pathological considerations. In: Fenoglio-Preiser CM, Wolff M, Rilke F (eds) Progress in Surgical Pathology, Vol VII. Field & Wood, Inc., Philadelphia, Pennsylvania, pp 153–167

Brawn PN (1982) The origin of invasive carcinoma of the bladder. Cancer 50:515–519

Collste LG, Devonec M, Darzynkiewicz Z, Traganos F, Sharpless TK, Whitmore WF, Melamed MR (1980) Bladder cancer diagnosis by flow cytometry, correlation between cell samples from biopsy and bladder irrigation fluid. Cancer 45:2389–2394

Colpaert C, Goovaerts G, Buyssens N (1987) Factors influencing the subjective grading of bladder cancer. Virchows Arch [A] 411:479–484

Devonec M, Darzynkiewicz Z, Kostyrka-Claps ML, Collste L, Whitmore WF, Melamed MR (1982) Flow cytometry of low stage bladder tumors: Correlation with cytologic and cystoscopic diagnosis. Cancer 49:109–118

Farsund T, Hoestmark JG, Laerum OD (1984) Relation between flow cytometric DNA distribution and pathology in human bladder cancer. Cancer 54:1771–1777

Flanagan MJ (1978) Evaluation of bladder washing cytology for bladder cancer surveillance. J Urol 119:42

Graem N, Helweg-Larsen K, Keiding N (1980) Precision of histological grading of malignancy. Acta Path Microbiol Scand Sect A 88:307–317

Helander K, Kirkhus B, Iversen OH, Johansson SL, Nilsson S, Vaage S, Fjordvang H (1985) Studies on urinary bladder carcinoma by morphometry, flow cytometry, and light microscopic malignancy grading with special reference to grade II tumours. Virchows Arch [A] 408:117–126

Holmes H (1927) Petrographic methods and calculations. Murby, London

Jordan AM, Weingarten J, Murphy WM (1987) Transitional cell neoplasms of the urinary bladder. Can biologic potential be predicted from histologic grading? Cancer 60:2766–2774

Koss LG (1985) Tumors of the urinary bladder. Atlas of Tumor Pathology, Fascicle No 11, Suppl 2nd series. Armed Forces Institute of Pathology, Washington, DC, pp 24–25

McCullagh P, Nelder JA (1983) Generalized Linear Models. Monographs on statistics and applied probability. Chapman and Hall, London, pp 64–92

Montironi R, Scarpelli M, Pisano E, Ansuini G, Marinelli F, Mariuzzi G (1985) Noninvasive papillary transitional-cell tumors: Karyometric and DNA-content analysis. Anal Quant Cytol 7:337

Mostofi FK, Sobin LH, Torloni H (1973) Histological typing of urinary bladder tumors. International Histological Classification of Tumours. No. 10, WHO, Geneva

Murphy WM, Crabtree WN, Jukkola AF, Soloway MS (1981) The diagnostic value of urine versus bladder washing in patients with bladder cancer. J Urol 126:320

Ooms ECM, Anderson WAD, Alons CL, Boon ME, Veldhuizen RW (1983a) Analysis of the performance of pathologists in the grading of bladder tumours. Hum Pathol 14:140

Ooms ECM, Blok APR, Veldhuizen RW (1985) The reproducibility of a quantitative system of bladder tumours. Histopathology 9:501–509

Ooms ECM, Kurver PJH, Boon ME (1982) Morphometrical analysis of urothelial cells in voided urine of patients with low grade and high grade bladder tumours. J Clin Pathol 35:1063–1065

Ooms ECM, Kurver PHJ, Veldhuizen RW, Alons CL, Boon ME (1983b) Morphometric grading of bladder tumors in comparison with histologic grading by pathologists. Hum Pathol 14:144

Serfling KY (1980) Approximation theorems of mathematical statistics. J Wiley and Sons, New York, pp 156–159

Tribukait B, Gustafson H, Esposti P (1979) Ploidy and proliferation in human bladder tumors as measured by flowcytofluorometric DNA-analysis and its relations to histopathology and cytology. Cancer 43:1742–1751

Trott PA, Edwards L (1973) Comparison of bladder washings and urine cytology in the diagnosis of bladder cancer. J Urol 110:664

Zein T, Wajsman Z, Englander LS, Gamarra M, Lopez C, Huben RP, Pontes JE (1984) Evaluation of bladder washings and urine cytology on the diagnosis of bladder cancer and its correlation with selected biopsies of the bladder mucosa. J Urol 132:670

Accepted March 3, 1988