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Automated image analysis for bladder cancer

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Abstract The genetic and epigenetic changes that occur during cancer development result in apparent morphological changes. Light microscopic image analysis provides objective assessment of cellular and nuclear morphology. The complexity of changes reflects the basic nature of dedifferentitation: a multi-hit process. Image analysis methods proved valuable for the assessment of malignancy in bladder cancer. Clinically applicable systems have been developed to diagnose urothelial cell cancer and predict prognosis. What is the place of these systems in daily practice?

Key words Image analysis · Bladder cancer · Morphometry · Karyometry · DNA ploidy

Introduction

The biology of tumour development is characterized by many morphological cellular and nuclear changes. These changes can be detected using light microscopy. Study of these changes in benign and malignant tissue showed typical differences that are widely used for the grading of cancer. The causes of the morphological changes are ill-understood. Both genetic and epigenetic factors influence cell morphology. This explains the correlation between morphological changes and cell behaviour as is found in malignant dedifferentiation.

The low reproducibility of both cytological [37] and histological [33] grading systems for bladder cancer illustrates the need for objectivation of the morphological changes. Here we will review the literature on the causes of the morphological cellular and nuclear changes. Moreover, the development and use of image analysis systems for quantitation of these changes is discussed.

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Nuclear matrix and nuclear morphology

In 1974, Berezny and Coffey [3] observed disruption of nuclear structure of isolated rat liver nuclei by mild digestion with proteases, whereas other chemical treatments had no influence on nuclear morphology. Removing chromatin, DNA, RNA and phospholipids from the nucleus, they found a spherical residual particle consisting of more than 98% polypeptides and accounting for less than 15% of the mass of an entire cell nucleus. The major function of this nuclear matrix is organization of the DNA into loop domains. By topoisomerase II activity in the matrix it modulates DNA topology. Moreover, it plays a role in RNA synthesis by association of the transcribed genes to the matrix and binding RNA processing intermediates [19, 20, 34, 35]. The mechanism by which the nuclear matrix structures nuclear shape is called tensegrity. This is defined as a dynamic structure based on a framework composed of compression elements and tension cables [35].

The nuclear matrix has been shown to be cell type specific [19, 20]. Moreover extracellular factors determine the composition and morphology of the nuclear matrix [21, 34, 35]. Recent studies have shown differences in nuclear matrix protein composition in transitional cell cancer compared with normal bladder mucosa, illustrating the role of the nuclear matrix in tumour development [30].

The nuclear matrix determines nuclear morphology and regulates gene expression. Mulder et al. [31] described the genetic changes in colorectal cancer and correlated these changes with nuclear morphology. The nuclear shape and chromatin pattern features established in light microscopic images were predominantly associated with tumour progression in colon carcinoma and were not influenced by the individual molecular genetic alterations such as allelic deletions in chromo-

somes 5q, 18p, 17p, and mutations in the *ras* gene. Nuclear chromatin texture, however, was inversely correlated with fractional allelic loss, a measure of overall genetic changes in the cell. Moreover, for urothelium it was shown that extracellular components influence nuclear matrix organization and in this way may regulate the epithelium [21]. These findings suggest that, in normal as well as cancerous tissue, genetic and epigenetic changes determine nuclear morphology. Hence, nuclear morphology could be applied to monitor the overall genetic and epigenetic changes in tumour development.

Image analysis techniques

Image analysis systems are composed of a light or fluorescence microscope and a charge-coupled device (CCD) camera for image acquisition. Image processing and analysis are performed on a (personal) computer with a framegrabber board. The microscope can be equipped with an automated cross table for computer-directed selection of areas of interest. Moreover, autofocus methods can be applied. Several of these systems have been tested and are commercially available. Only a few systems, however, provide built-in routines for daily use. Standardization and data recording and presentation determine the value of the system and should be thoroughly tested before the method can be of value for daily practice.

Using different staining techniques, objects of interest can be differentiated. The Feulgen-Schiff staining method is used for analysis of nuclear morphology and chromatin analysis [18]. Since DNA is stained in this method it is also well suited for analysis of nuclear DNA content. Furthermore, in situ hybridization methods and immunostaining can be quantified by image analysis techniques [10]. In this review nuclear morphological analysis by image analysis in transitional cell carcinoma of the bladder will be discussed on the basis of recent literature. Earlier studies have been reviewed elsewhere [45].

Cytology

The criteria for routine cytological grading have recently been reviewed [32]. Both cells in urine and bladder washings can be graded, although superior results from bladder washings have been reported. Automated grading using image analysis has been extensively studied. Between 1975 and 1989 several publications appeared from the Montefiori Medical Center in New York on computer image analysis of voided urine cytology [25, 38]. A system was constructed for the automatic grading of urinary cytology based on the cell-to-cell reference of a panel of observers. Sensitivity of the system was 84% for the detection of urothelial cell carcinoma, as compared with 63% for visual cytology of the same cases [38]. A special algorithm reduced the number of cells necessary for analysis.

In another study the analysis of 32 nuclear features in bladder wash material from patients with superficial bladder cancer was tested and nuclear shape and the 2c deviation index [7] were found to correlate with tumour grading [44, 46]. This quantitative cytology system (Quanticyt) was tested in a study population of 1412 patients, bladder wash samples being divided into low, intermediate and high risk on the basis of the karyometric analysis. The system is now commercially available.

Analogous to the system described by Koss et al. [25, 38] the Quanticyt system automatically analyses nuclei in light microscopic images. In contrast to the system of Koss et al., the Quanticyt system used the histological findings and follow-up data (tumour recurrence and progression) as reference, instead of cytology grading. Moreover, bladder wash samples were applied instead of voided urine. When compared with expert cytology the sensitivity of automated cytology systems was equally good (unpublished data).

Recently Veltri et al. [49] showed that a combination of cellular and nuclear features predicted tumour recurrences more accurately than routine cytology.

Several disadvantages of automated cytology still exist. Although sensitivity for low-grade lesions is superior for automated systems compared with visual cytology, many papillary lesions will still not be detected. Hence, cystoscopy is still required to confirm the presence of small papillary lesions in patients suspected of having bladder tumours. Future studies should be directed to the issue of predicting progression rather than recurrence. Low-grade papillary lesions may be left unresected for a period of time without increasing the risk of progression. Another issue consists of the application of automated microscopy in daily practice. Although a report is generated it still has to be incorporated in clinical use and, ultimately, influence clinical decision making. Prospective evaluation comparing different tumour markers in cytology is required to determine whether markers will influence cystoscopy frequency and timing.

The application of image analysis methods to the assessment of antibody staining techniques specific to bladder tumours has been studied [8]. The role of image analysis can only be as important as the sensitivity of the antibody used. In the context of this article it should be stated that image analysis methods will be important in the accurate assessment of the distribution and location of staining when tumour markers are applied.

DNA ploidy analysis

Earlier flow cytometry studies found increased aneuploidy in malignant cell populations [43]. Bass et al. [2, 4, 8, 23] developed a fluorescence method for ploidy analysis in bladder cytology. Shackney et al. [36] found a correlation of ploidy and S-phase fraction changes with tumour behaviour and therapy resistance in bladder cancer. When compared with flow cytometry, image

cytometry in histological sections proved to be more sensitive for small populations of aneuploid cells [13]. Although of additional prognostic value to grade and stage [41] in histological material, flow cytometry analysis was never used extensively in daily practice. The need for tissue disaggregation makes the method too elaborative for daily use. Moreover, when other features were compared with DNA flow cytometry, the predictive value dropped [5] and was found to be of no clinical use in pTa-pT1 tumours [42].

In cytological material image cytometry and flow cytometry proved equivalent for the detection of aneuploidy [11]. DNA ploidy analysis in addition to routine cytology improved sensitivity up to 84% [1]. DeVere White and Deitch [17] reviewed the use of flow cytometry in bladder cancer and concluded that the method is particularly useful for monitoring the condition of the mucosa and predicting tumour recurrence.

Hence, the use of DNA ploidy assessment is probably only of value for bladder cancer in cytological material. The simultaneous morphometric evaluation when image cytometry is applied is an advantage of this method. Currently, several systems are available for the determination of DNA ploidy in urine or bladder wash material.

Histology

Several multivariate studies have been published on histological automated tumour grading. Blomjous et al. [6] proposed an interactive nuclear selection system for the histological grading of bladder cancer: a mean value over 95 mm^2 of the largest selected nuclei was correlated with an increased progression rate. In Ta-T1 low-grade cancers, however, Lipponen et al. [29] found the mean nuclear size of the ten largest selected nuclei only of predictive value in combination with the mitotic/volume index, indicating that proliferation rather than nuclear size was of predictive value [27, 28]. Sowter et al. [39, 40] proposed a grading system based on both subjective histological grading and nuclear size analysis, whereas Borland et al. [9] found nuclear shape useful for predicting progression after cystectomy.

De Meester et al. [16] developed a system for the automated grading of histological bladder tumour material including assessment of tissue architectural features. The system, however, was sensitive to the area selected and was not tested against clinical outcome. Lipponen et al. [28] suggested D_{max}, the longest nuclear axis, as a prognostic clinical marker in pTa-pT1 papillary bladder cancer. A comparable multivariate analysis on histological material was performed by Colombel et al. [14, 15]. Again a combination of several nuclear features resulted in prognostic information regarding tumour recurrence and progression of superficial as well as invasive tumours [14]. They advocated the use of their system mainly in superficial cancers.

A different approach to histological grading was applied by van Velthoven et al. [48]. Instead of using histological sections, pronase-digested, resuspended material was used. The chromatin pattern of each Feulgen-stained nucleus was quantified. In combination with ploidy analysis this yielded a 91% correct prediction of tumour recurrence. Similar evidence of the value of chromatin pattern characterization for the grading of tumours was provided by Choi et al. [12]. Before widespread clinical application is feasible, however, study is needed to determine the influence of digestion techniques on chromatin patterns.

In summary, early studies focused on nuclear size as a prognostic marker. When more sophisticated systems came available the complexity of morphological features increased. Multivariate analysis showed that a combination of nuclear shape and chromatin features probably adds to clinical findings. Although all these studies support the value of quantitative analysis methods for interpretation of microscopic images, none compared the method with a range of other markers. Hence, before imaging methods become of clinical value, studies comparing different prognosticators are required. However, these methods can already support pathological diagnosis. In particular reproducibility increases using quantitative methods. Moreover, cell-to-cell analysis of, for example, DNA ploidy may aid in more accurate (sub)grading of, for example, of grade 2 tumours.

Follow-up of bladder cancer patients

Several clinical factors predict the chance of tumour recurrence. Multiplicity, tumour grade and stage, and earlier tumour recurrence rate are correlated with tumour recurrences despite intravesical instillation treatment [24, 26]. Hall et al. [22] proposed a follow-up plan based on multiplicity and recurrence at 3 months after resection – two indicators of increased recurrence rate. Hence, available clinical data can aid in the planning of follow-up schemes. Cystoscopy remains the gold standard in the detection of tumour recurrences. A reliable marker on cytological material could reduce cystoscopy frequency in patients with a low risk for tumour recurrence and thus reduce costs and patient inconvenience. Moreover, predicting tumour recurrences more reliably can aid in the stratification for (intravesical) treatment modalities.

In a low-risk group of patients as defined by Kurth et al. [26], still 7% of tumours progressed to invasive disease within 3 years of diagnosis and this group comprised 52% of their patient population, whereas the high-risk group comprised only 6.7% of the patients, of whom 42% progressed. Hence the absolute number of progressive tumours in the low-risk group is still higher than the absolute number in the high-risk group. These data indicate that changing treatment and follow-up schemes based on relative risk alone underestimates the potential risk of the less aggressive but more frequent cancers and may put an equal number of patients at risk of late detection of progression as is found in time by

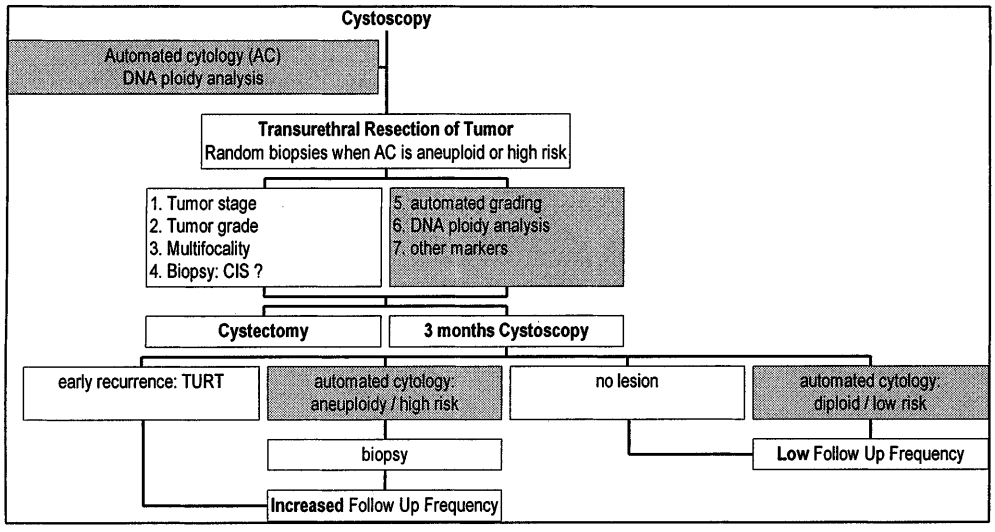

Fig. 1 The place of automated image analysis in the follow-up of patients with bladder cancer. *TURT* transurethral resection of tumour

meticulous follow-up of the high-risk patient group. Two factors are important to overcome this dilemma: more accurate prognostic markers and a reliable tool for the monitoring of bladder mucosal chances during patient follow-up, in particular in the less progressive cancers.

Follow-up schemes and automated image analysis

Since the majority of patients who experience tumour recurrences do so within 2 years after transurethral resection of a tumour, cystoscopy is traditionally recommended to be performed every 3 or 4 months for the first 1–2 years. The chance of developing tumour progression due to later detection and thus resection of tumour recurrences is not known. Moreover, tumour characteristics seem to change over time, making prediction of tumour behaviour in an early phase of disease more difficult [47].

A clear report is mandatory for clinical integration of automated imaging systems. A high frequency of follow-up cystoscopies may not be necessary in large groups of "low-risk" patients. The presence of small lesions of low malignancy grade may be efficiently treated expectantly or with laser coagulation. In these cases information on malignancy grade of the bladder mucosa and the lesions present can be provided by automated image analysis supported cytology. Figure 1 shows a possible treatment protocol in which image analysis is applied for decision-making in (1) treatment after transurethral tumour resection and (2) follow-up schemes.

Automated image analysis is easily accessible for daily practice. Urologists acquainted with markers such as DNA ploidy and morphology can apply the method to support decision-making. Only with effective collaboration of urologists and pathologists will new methods find their way into daily practice to optimize clinical decision making.

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