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ORIGINAL PAPER

Diagnostic Yield of Touch Imprint Cytology of Prostate Core Needle Biopsies

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Abstract Touch imprint cytology may provide additional information to core needle biopsy interpretation according to previous reports. The aim of this study was to investigate the diagnostic yield of this method in the diagnosis of prostate carcinoma. For this purpose, 452 transrectal prostate needle biopsies were evaluated from 56 patients. All patients were clinically suspicious of having prostate carcinoma. Two touch imprints were prepared from each fresh biopsy cylinder. Results of the standard histology and of the touch imprint evaluation were compared. Histologically negative biopsy cylinders were further evaluated for prostate carcinoma by fine step serial sectioning. The standard histological examination showed adenocarcinoma in 27 patients. Touch imprint cytology revealed atypical cells suspicious of carcinoma in 38 patients. This group included all 27 patients with positive standard histology and further 11 patients with initially negative core biopsy. Following serial sectioning, in three out of these 11 samples, histological evidence of a carcinoma could be proven. Fine step serial sectioning of all 29 core biopsies negative for carcinoma by standard histological examination, 26 patients remained negative. All three core biopsies initially negative by standard histology but positive after serial sectioning had cytology findings suspicious of carcinoma. We conclude, that in problematic cases the additional use of touch imprint cytology and serial sectioning of prostate core needle biopsies significantly improve the diagnostic accuracy.

Keywords Biopsy · Diagnosis · Imprint cytology · Prostate · Cancer

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Introduction

The introduction of systematic sextant biopsy scheme recommended by Hodge et al. significantly improved early prostate cancer detection rates [1]. However, subsequent studies revealed a 20–40% false-negative rate of systematic sextant biopsies and, consequently, repeat biopsy has been suggested for all men providing a negative biopsy result [2]. In order to minimize sampling errors, various attempts such as more laterally directed sampling, higher number of cores taken at first biopsy, or the saturation biopsy technique have been recommended, resulting in a 20–35% cancer detection improvement of these extended biopsy schemes when compared with the standard sextant scheme.

Although these techniques substantially decreased the need for repeat biopsies, considerable uncertainty about the presence or absence of prostate cancer persists in cases with elevated PSA levels and repeated histological negativity. This particular case may occur due to the poor specificity of PSA determination, but also due to missed cancer at biopsy resulting from sampling error, or missed cancer at the level of pathological work-up, therefore, further endeavours to enhance the accuracy of prostate biopsy appear justified.

Jacobs et al. demonstrated that touch imprint cytology of core needle biopsies of non palpable breast cancers is a

highly informative and enables to decrease the number of biopsies required for safe diagnosis [3]. Concordantly, Gentry et al. could show that touch print cytology of pelvic lymph nodes in patients with prostate cancer is a simple, rapid, reliable, and highly sensitive method for the detection of lymph node metastases [4]. Lo et al., and more recently, Chieco et al. already emphasized touch imprint cell preparation from fresh needle biopsy of the prostate as a useful adjunct to histopathological evaluation [5, 6].

In order to improve the diagnostic yield and potentially decrease the number of necessary biopsies for establishing the correct diagnosis, we investigated in our prospective study the impact of touch imprint cytology from core needle biopsy material in patients undergoing transrectal ultrasound guided biopsy of the prostate because of an elevated PSA and/or abnormal digital rectal examination.

Material and Methods

Patients and Samples

In 2002, between March and August 452 transrectal needle biopsies from 56 patients were collected in the Department of Urology, Medical University of Graz. The patients age ranged from 45–87 years with a median age of 68 years. The median of the total prostate specific antigen level (PSA) was 14,3 ng/ml, the range was 2,0–205,2 ng/ml. 27 patients had an atypical digital rectal examination.

The biopsies were taken from the same urologist by ultrasound-guided needle biopsy, using a 18 gauge needle. The median number of the core needle biopsies per patient was eight with a range between 2–12.

From one patient only two biopsies were taken because of patient's early break off.

The results of the histology and the cytology were subdivided in positive and negative for carcinoma.

Preparation and Evaluation of Touch Imprints

After receiving the fresh (unfixed) biopsy cylinder two touch imprints were immediately prepared on two glass slides. The biopsy cylinder was gently rolled over the surface of the glass slides.

One slide was air dried and stained with Diff-Quik (DADE BEHRING Inc., Switzerland), the other was fixed with a spray-fixative (Merckofix, Merck, Germany) and stained according to the Papanicolaou method.

The classification of cytologic features in the touch imprints followed the criteria described by Willems [8], Epstein [9] and Leistenschneider [10]. The following classical features were registered as signs of malignancy: nuclear pleomorphism, moulding of nuclei, presence of prominent nucleoli, granular chromatin pattern with “salt and pepper” appearance and increased nuclear-cytoplasmic ratio. Furthermore, loss of polarity of the nuclei at the edge of cohesive clusters with acinar arrangement was also considered.

According to these features the samples were categorized as either positive (highly suspicious) or negative (= not suspicious) for carcinoma blind and independent from the histological evaluation.

Processing of the Biopsy Cylinders

After the preparation of the touch imprints biopsy cylinders were fixed in buffered 4% formaldehyde (pH 6.9) and were embedded individually in paraffin. Each biopsy cylinder was cut in four step sections and stained with hematoxylin and eosin.

In addition, all biopsy cylinders with an inconclusive or negative result for prostate cancer were serial sectioned in up to 60 fine step sections. All sections were stained with hematoxylin and eosin and evaluated by two pathologists independently (S.M. and F.M.). Diagnoses of carcinoma were confirmed according to the World Health Organisation criteria on tumours [7] following prostatectomy.

Clinical and pathological follow up reached for all patients until the end of the year 2007.

Results

Standard histological examination was positive for carcinoma in 27 out of the 56 patient sample evaluated (Table 1). Touch imprint cytology from the same core biopsies enabled the safe identification of cancer related morphological changes (Fig. 1.) Such cytological abnormalities could be detected in all cases positive for carcinoma by standard histology. In additional 11 patients, carcinoma was suspected by cytology but was not found in the standard histological preparations. Following serial sectioning, three out of these 11 samples turned out to present cancer (27.3%). No carcinoma was found after serial sectioning in any of the patients who were initially negative by both cytology and histology. This corresponds to 10% (3/29) false negative rate for standard histology alone, and 0% false negative rate when using the combination of standard histology and imprint cytology. Selected for extensive serial sectioning were those biopsies, which were negative on initial histology, but positive cytology. The results of serial sectioning of biopsies of 29 patients that were negative in initially routine histology are also shown in Table 1.

Table 1 Correlation of standard histological serial sectioning and touch imprint cytological findings in 56 cases of prostate core needle biopsies

Type of histology	No. of cases	Result of histology	Total	Touch imprint cytology	
				Suspicious	Non-suspicious
Standard histology	56	malignant	27	27	0
		non-malignant	29	11	18
Serial sectioning	29[a]	malignant	3	3	0
		non-malignant	26	0	26
Follow-up biopsies[1]	13[b]	malignant	3	2	1
		non-malignant	10	4	6

[1] based on serial sectioning
[a] all non-malignant cases diagnosed by standard cytology
[b] all follow-up cases available for the original patient cohort

Surprisingly, only those three cases which were suspicious for carcinoma on the basis of the initial touch imprint cytological examination showed evidence of prostate cancer while none of the biopsies from the remaining 26 cases showed cacinomatous change in these sections. The concordance of 100% when using serial sectioning and cytology means that all false-negativity obtained by standard histology could be identified and that the sensitivity of the methods reach 100% in this cohort.

All patients evaluated by both histology and touch imprint cytology were monitored 25–46 months after their first biopsy. During this time period, 13 out of 26 patients initially negative for carcinoma underwent a repeat biopsy. In 3/13 patients, a carcinoma could be diagnosed. In two of these three patients, touch imprint cytology proved to be positive already during the first sampling (66.7%). Among the remaining ten patients being rebiopsied and still histologically negative for carcinoma, four cases were recorded as cytologically positive during first-time biopsy (60%).

The Gleason score distribution of the patients with prostate cancer was as in the usual range. In more detail, Gleason score five was stated in six patients, Gleason score six in 11 patients, Gleason score seven in eight patients, Gleason score eight in three patients and Gleason score nine in two patients. All three cases with positive imprint cytology and manifest adenocarcinoma after serial sectioning presented with Gleason score six.

Discussion

Transrectal fine-needle aspiration biopsy and core needle biopsy technique are reliable and standard procedures in the diagnosis of prostate carcinoma [8, 11]. The incidence of false-negative sextant prostate biopsies is approx. 23%, as it was demonstrated in patients who were undergoing radical prostatectomy [12]. This relatively high incidence of false-negative biopsies can be reduced by modification of the diagnostic treatment modalities [12]. One clinical possibility is a systematic five region biopsy, which can significantly increase the chance of finding the carcinomatous areas [13]. However, the number of tissue cores it to be obtained for optimal diagnostic yield and to reduce the incidence of false-negative biopsies is still debated [8, 11–14]. For this reason any further method that potentially reduces false-negativity from the side of the pathologists is highly desired.

In our study we tried to establish and evaluate the utility of combining histology and touch imprint cytology in order to improve diagnostic accuracy without causing further stress to the patients. This approach relies on the use of established cytomorphological parameters of malignancy [3, 4, 8]. According to our observation, touch imprint cytology was able to identify all carcinomas that were diagnosed by standard core biopsy histology. Moreover, furthrer cases were excluded, the carcinomatous nature of which could only be cleared due to serial sectioning of the biopsy core. We were able to improve the sensitivity of routine core biopsies by the additional evaluation of touch imprints of the same material. This means, for those patients who were negative by standard histology, but showed a positive result by touch imprint cytology, a serial sectioning of the biopsy core should be recommended. Standard histology of biopsy cores, without the application of any of the additional methods, bears the potential for false negativity in 10% of the cases.

There are several reasonable explanations for the high sensitivity of imprint cytology compared to that of histology of prostatic needle biopsy:

1) For cytology, cells are collected from the entire surface of the tissue core. Rolling over the glass slide, the sampling surface is increased “pi”-times compared to a

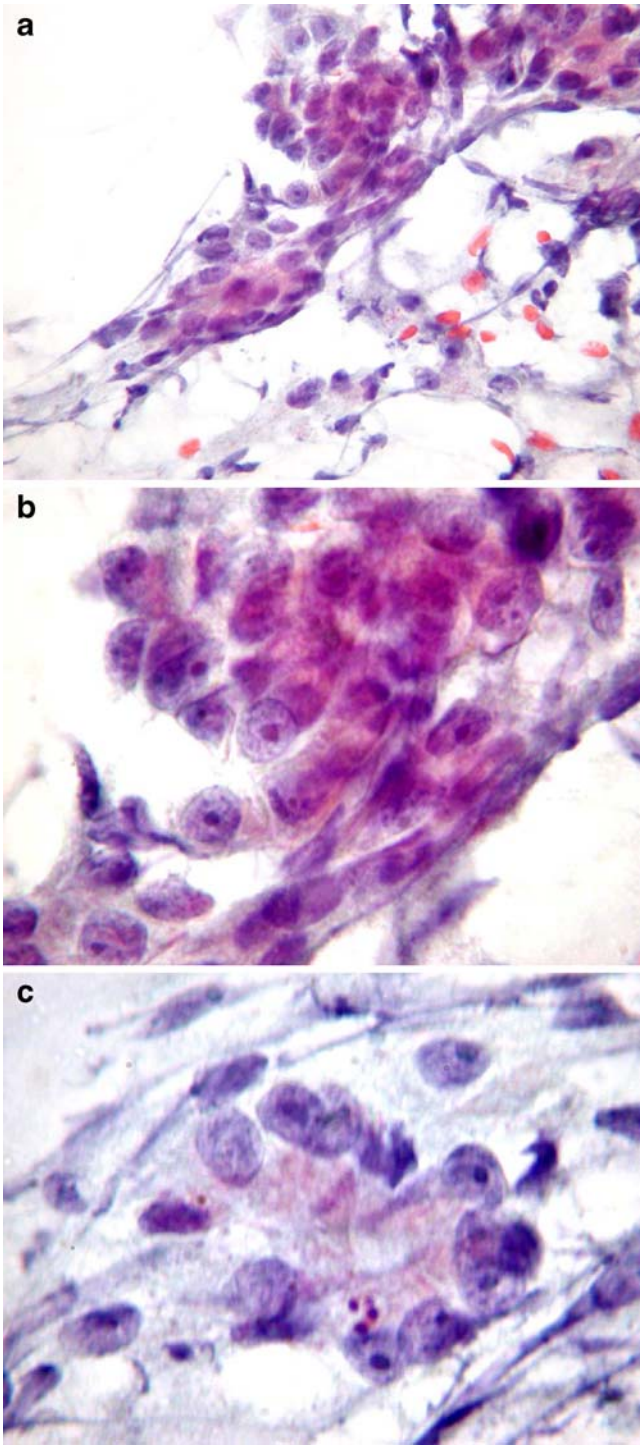

Fig. 1 Cytologic features of prostate carcinoma. **a** Prostate carcinoma cells with loss of polarity and increased nuclear-cytoplasmic ratio (Papanicolaou staining, 400× magnification), **b** Same case with higher magnification, note the vesicular and large nuclei with prominent nucleoli (Papanicolaou staining, 1000× magnification), **c** Highly atypical cells with acinar arrangement, without basal cell component (Papanicolaou staining, 1000× magnification)

longitudinal section through the middle of the biopsy core, which is the best possible case in routine histology.

2) According to the general experience, biopsy cylinders often cannot be perfectly cut along their axis. The problem engraves obviously when more than one cylinder is embedded in a block which can lead to problems of detecting small foci of prostate cancer [15].
3) A significant part of the core needle biopsy cylinder is wasted during sectioning of the paraffin embedded biopsy cylinder. Similar problems of work-up and the utility of step serial sectioning have been discussed with respect to the sentinel lymph nodes in breast cancer [16, 17]. Lane et al. [18] demonstrated the necessity of cutting at least three levels of the prostate biopsy cylinder. They showed that sampling the cylinder at only one level missed an average of 23.4% of the total biopsy length and sampling the tissue at three levels improved this to 7% [18].
4) The possibility of an aspiration effect during core biopsy sampling should also be considered. Tumor cell groups are generally characterized by reduced cohesiveness which makes them easier to aspirate even by minimal forces. Therefore, the tissue fluid covering the sample surface may be selectively enriched in detached tumor cell groups giving a unique source for cytological analysis.

Touch imprint cytology, similar to aspiration cytology has also its limitations due to numerous conditions, including e.g. granulomatous prostatitis or cell clusters from the seminal vesicles [8]. Moreover, uncertain atypia or atypical cells of prostate intraepithelial neoplasia (PIN) are not reliably distinguishable from invasive carcinoma based on the cytology alone. In the presented series of 56 patients, however, we could not demonstrate such events.

Should imprint cytology be performed for every core needle biopsy? Clinically suspicious cases with an elevated PSA level and atypical digital rectal examination, especially if previous routine biopsies had an inconclusive result for malignancy, may clearly benefit from it. The diagnostic improvement by imprint cytology may also reduce the number of core biopsies in high-risk cases (e.g. patients with coagulopathies). Touch imprints with suspicious cells offer also an excellent basis for further molecular analysis. Individual cells identified in stained preparations can be nicely evaluated for genetic alterations, e.g. by FISH or PCR after microdissection, that enables further proof of malignancy in selected samples.

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