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DNA IMAGE CYTOMETRY ON BIOPSIES CAN HELP THE DETECTION OF LOCALIZED GLEASON 3+3 PROSTATE CANCERS

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ABSTRACT

Purpose: In spite of classifications based on digital rectal examination, prostate specific antigen (PSA), transrectal echography and histological analysis, 20% to 40% of operated prostate adenocarcinomas are not yet organ confined. New diagnostic features to predict extracapsular invasion before treatment are needed to avoid surgical extraction within positive margins.

Materials and Methods: A retrospective study was performed for 74 prostate adenocarcinomas with Gleason 3+3. A total of 54 organ confined tumors (T1T2) at digital rectal examination were compared with 20 nonorgan confined tumors (T3T4). Image cytometric DNA analysis was performed on prostate initial biopsies. DNA ploidy results were compared in both groups for values of PSA greater than 15, less than 15 and less than 10 ng/ml.

Results: For a PSA rate less than 15 ng/ml, 83.8% of T1T2 were diploid vs 33% T3T4 ($p = 0.042$). For a PSA rate less than 10 ng/ml, 96% of T1T2 were diploid vs 33% T3T4 ($p = 0.025$).

Conclusions: DNA ploidy appears to be an interesting feature at diagnosis. When in doubt about the localized character of a tumor, DNA ploidy it makes it possible to predict a nonorgan confined tumor. Gleason 3+3 prostate cancers that are organ confined at digital rectal examination are more often diploid than T3T4 tumors. The prognostic interest in DNA ploidy is more reserved because of its correlation with PSA level.

KEY WORDS: ploidies, image cytometry, prostatic neoplasms

Prostate carcinoma is the most frequent cancer in men older than 50 years and its incidence increases with age. This cancer represents 15% of all the cancers and is the second greatest cause of cancer mortality. Today the treatment of prostate cancer depends on the stage of the disease and the life expectancy of the patient at the time of cancer discovery. The management of localized tumors is controversial with the 2 possibilities of watchful waiting or curative treatment. Because of the slow progression of prostate adenocarcinoma and the risk of postoperative consequences, surgical treatment is only discussed for patients with a life expectancy longer than 10 years. At diagnosis 62% of patients have a localized tumor and can benefit from curative treatment.[1] The prediction of pathological stage is of great importance in detecting clinically organ confined disease principally because of the therapeutic and prognostic implications.

In spite of the present classifications based on the data of rectal examination, serum prostate specific antigen (PSA), transrectal echography and histological analysis of prostate biopsies (mainly Gleason score), 20% to 40% of prostate cancers are not localized to the prostate gland after examination of the radical prostatectomy specimen.[2] The careful examination of the surgical specimen often shows extracapsular spread with involvement of surgical margins. This finding implicates the presence of residual malignant tissue which can lead, sooner or later, to an increase in serum PSA. This increase corresponds to biological recurrence which frequently precedes local or metastatic recurrence.[2–4]

Thus, the sensitivity of the usual features to determine tumor extension seems to be inadequate. It does not allow precise differentiation between tumors strictly localized to the prostate gland and those having already crossed over the capsule. Indeed, in multivariate analysis margin status is an independent predictor of cancer progression.[5] The involvement of seminal vesicles also represents a 72% risk of progression at 5 years.[1,2,4] Thus, new diagnostic and prognostic features are needed to distinguish patients in whom cancer cannot be controlled with surgical treatment. It is necessary to predict the risk of extracapsular spread of the lesion to adopt the best treatment and to avoid prostatectomy with positive margins.

The determination of the DNA ploidy pattern is now well-known as a prognostic factor in many tumors, particularly in prostate cancer.[6–9] Few studies have presented the importance of DNA ploidy in predicting pathological stage and particularly extracapsular spread.[10,11] We assessed whether the DNA profile can help predict the clinical stage of prostate cancer on prostate biopsies showing a Gleason 3+3 grade adenocarcinoma.

PATIENTS AND METHODS

This retrospective study was performed on 100 patients presenting with Gleason 3+3 prostate adenocarcinoma diagnosed on prostate biopsy between 1999 and 2002 at the department of urology of the CHU of Reims. After patient selection histological diagnosis and Gleason score were confirmed by 2 pathologists. Patients were separated into groups of organ confined (OC) at digital rectal examination, not organ confined (NOC) at rectal examination and tumors presenting unclear localized character at digital rectal examination. Our goal was to determine if DNA ploidy can predict the clinical stage of prostate cancer, thus the third group of patients was excluded from the DNA ploidy study.

For every patient an average of 10 biopsies was performed according to the current recommendations. The biopsies were

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formalin fixed and paraffin embedded, and 3μ serial sections were performed for histological analysis after standard hematoxylin and eosin staining. An additional 5μ section of a representative biopsy chosen by a pathologist was used for DNA ploidy determination after Feulgen staining. Briefly, the slides were dewaxed, rehydrated and submitted to a 1-hour 5N HCl hydrolysis at room temperature followed by staining with thionine.

DNA image cytometry was performed by interactive measurement using the CAS 200 image analyzer (Becton Dickinson, Leiden, The Netherlands) without knowledge of the clinical status of the patient. About 200 nuclei were measured after an external calibration with rat hepatocytes, followed by an internal calibration performed on normal glands of the biopsy. Histograms were classified as diploid, aneuploid, polyploid (2c+4c) and multiploid (at least 2 aneuploid peaks).

The chi-square and Fisher exact tests were used for the statistical analysis with StatView software (SAS Institute, Cary, North Carolina). If an expected count was less than 5 the chi-square test was not valid. In these cases we used the Fisher exact test. We examined whether there was a statistical difference in DNA ploidy status between patients presenting with localized and those with nonlocalized prostate adenocarcinoma at any PSA level, and then at PSA less than 15 and less than 10 ng/ml.

RESULTS

A total of 74 men 55 to 97 years old (average 71.3, median 73) with prostate adenocarcinoma Gleason 3+3 were included in the study. There were 5 (7%) patients with clinical stage T1 (normal digital rectal examination), 49 (66%) clinical stage T2, 17 (23%) stage T3 and 3 (4%) stage T4. Therefore, 54 patients were grouped together in the group OC (stages 1 and 2) and 20 in the group NOC (stages 3 and 4). In the OC group 43 (79.6%) were diploid, 2 (3.7%) were polyploid, 1 (1.8%) was aneuploid and 8 (14.8%) were multiploid. In the NOC group diploidy was observed in 8 cases (40%), polyploidy in 3 cases (15%), aneuploidy in 1 case (5%) and multiploidy in 8 cases (40%).

According to ploidy results tumors were separated into diploid and nondiploid. Aneuploid, polyploid and multiploid tumors were grouped together in the nondiploid group. We deliberately chose to include tetraploid (polyploid) tumors in the aneuploid group because the statistical significance was higher in comparison when we grouped tetraploidy and diploidy together. The determination of the serum level of PSA was possible in all 74 patients. Of the patients 36.5% (27) had a PSA less than 10 ng/ml and 50% (37) less than 15 ng/ml. The distribution of ploidy status in OC and NOC according to the rate of PSA is presented in the table.

Whatever the serum PSA rate 80% of the OC group were diploid and 20% were nondiploid. However, in the 20 NOC group, 40% were diploid and 60% were nondiploid (chi-square test 10.7, $p = 0.0018$). The sensitivity of ploidy status to predict the clinical stage of a tumor was 60% (95% CI 48.8%–71.1%), the specificity, positive predictive value (PPV) and negative predictive value (NPV) were 79.6% (95% CI 70.4%–88.8%), 52% (95% CI 40.6%–63.4%) and 84% (95% CI 75.6%–92.3%), respectively.

Distribution of ploidy status and clinical stage according to serum PSA

	All PSA Levels	PSA Less Than 10 Ng/Ml	PSA Less Than 15 Ng/Ml
No. pts	74	27	37
No. tumor type (%):			
Diploid localized	43 (58.1)	23 (85.2)	31 (83.8)
Nondiploid localized	11 (14.8)	1 (3.7)	1 (2.7)
Diploid nonlocalized	8 (10.8)	1 (3.7)	2 (5.4)
Nondiploid nonlocalized	12 (16.2)	2 (16.2)	3 (8.1)

In the group of patients with a serum PSA less than 10 ng/ml (27), 96% of the OC group were diploid and 4% were nondiploid, whereas 33% of the NOC group were diploid and 67% nondiploid (chi-square test 10.54, $p = 0.025$). Thus, the sensitivity of a diploid DNA profile to predict the localized character of a tumor was 66% (95% CI 48.1%–83.8%), the specificity, PPV and NPV were 96% (95% CI 88.7%–100%), 66% (95% CI 48.1%–83.8%) and 96% (95% CI 88.7%–100%), respectively.

In patients with serum PSA less than 15 ng/ml (37), 91.2% of the OC group were diploid and 8.8% were nondiploid, whereas 33% of the NOC group were diploid and 67% nondiploid (chi-square test 7.89, $p = 0.042$). In these cases diploidy could predict the localized character of a tumor with a sensitivity of 66% (95% CI 50.7%–81.3%), a specificity, PPV and NPV of 91.2% (95% CI 82%–100%), 40% (95% CI 24.2%–55.8%) and 96.8% (95% CI 91.1%–100%), respectively.

DISCUSSION

DNA ploidy determination of prostate carcinomas in needle biopsy and/or prostatectomy is a significant prognostic factor as demonstrated in the literature.[8,10,12,13] However, the diagnostic and therapeutic interest of DNA ploidy determination to help the clinician determine clinical stage and adapt the therapeutic treatment of a patient has been less studied. We deliberately chose to work on biopsies to determine the diagnostic and therapeutic interest of DNA ploidy measured by image analysis to select the patients who could benefit from curative surgical treatment.

DNA ploidy determination by image analysis is definitely adapted to biopsies in comparison to flow cytometry because it can be used on small specimens.[14,15] Moreover, image analysis by preserving the histological architecture of the specimen allows us to quantify DNA only in tumor cells after calibration on adjacent normal cells. Despite that some authors consider that the biopsy may be not representative of tumor heterogeneity,[4,15] it has been demonstrated that DNA ploidy results on biopsies are correlated with the results of ploidy determination on the prostatectomy specimen in 92% of lesions.[16]

We analyzed only 1 biopsy containing tumor because this study was conducted to use the DNA ploidy measurement in routine clinical guidance. Such analysis is relatively time-consuming and could not be realized routinely on several tumor biopsies. Some authors have reported that DNA ploidy is the reflection of a high Gleason score.[3,17] While deciding to study only Gleason 3+3 grade tumors we had a homogenous group and we avoided the bias to a majority of nondiploid tumors by including high Gleason scores.

The extension of the carcinoma occurs with invasion beyond the capsule, involving the basis of seminal vesicles and lymph nodes and, finally, the external iliac nodes. Local tumor aggressiveness is determined by the breakdown of the capsule, which can be detected at rectal examination and determines the clinical stage. Pretreatment prognostic factors like serum PSA, Gleason score and clinical stage are used in clinical practice.[18] In prostate cancer, like in the majority of cancers, DNA ploidy appears as a marker of tumor aggressiveness at local and locoregional levels.[15]

In our series we observed a majority of diploidy in the group of localized tumors and, on the other hand, nonlocalized tumors were significantly more often nondiploid. This result is observed whatever the PSA level, but the specificities PPV and NPV significantly increase with PSA less than 10 to 15 ng/ml. These results are of great interest in clinical practice because prostatectomy is decided for patients with low serum PSA and localized nonmetastatic tumors. However, sometimes the rectal examination is unclear in the determination of the localized character of the tumor. The knowledge of DNA ploidy profile is helpful in this case. A

diploid tumor is organ confined at 96% (NPV 96%) in the group with PSA less than 10 ng/ml.

CONCLUSIONS

Prostate biopsy specimens provide reliable data concerning tumor ploidy status,[11] which can be determined by image cytometry on an additional paraffin section. Feulgen staining is an inexpensive and easy procedure. Thus, a routine DNA ploidy determination, especially on clinical localized carcinoma, should become an important factor in the therapeutic and surgical treatment of the patient.

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