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The Combination of DNA Ploidy Status and PTEN/6q15 Deletions Provides Strong and Independent Prognostic Information in Prostate Cancer

Dissertation

zur Erlangung des Grades eines Doktors der Medizin
an der Medizinischen Fakultät der Universität Hamburg.

vorgelegt von:

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Hamburg 2017

Angenommen von der Medizinischen Fakultät der Universität Hamburg am: 25.10.2017

Veröffentlicht mit Genehmigung der Medizinischen Fakultät der Universität Hamburg

Prüfungsausschuss, der/die Vorsitzende: Prof. Dr. Guido Sauter

Prüfungsausschuss, zweite/r Gutachter/in: PD Dr. Gunhild von Amsberg

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The Combination of DNA Ploidy Status and PTEN/6q15 Deletions Provides Strong and Independent Prognostic Information in Prostate Cancer

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Abstract

Purpose: Aberrant DNA content has been discussed as a potential prognostic feature in prostate cancer.

Experimental Design: We analyzed the clinical significance of DNA ploidy in combination with prognostic relevant deletions of PTEN and 6q15 in 3,845 prostate cancers.

Result: The DNA status was diploid in 67.8%, tetraploid in 25.6%, and aneuploid in 6.8% of tumors, and deletions of PTEN and 6q15 occurred in 17.8% and 20.3% of tumors. Abnormal DNA content and deletions were linked to high Gleason score, advanced tumor stage, and positive nodal stage ($P < 0.0001$ each). The risk of PSA recurrence increased from diploid to tetraploid and from tetraploid to aneuploid DNA status ($P < 0.0001$ each). However, 40% of patients with Gleason score $\geq$4+4 and 55% of patients with PSA recurrence had diploid cancers. This fraction decreased to 21% (Gleason $\geq$4+4) and 29% (PSA recurrence) if PTEN and/or 6q deletion data were added to ploidy data to identify cancers with an aberrant DNA status. The significance of combining both deletions and ploidy was further demonstrated in a combined recurrence analysis. Presence of deletions increased the risk of PSA recurrence in diploid ($P < 0.0001$), tetraploid ($P < 0.0001$), and aneuploid cancers ($P = 0.0049$), and the combination of ploidy data and deletions provided clinically relevant information beyond the CAPRA-S nomogram. Multivariate modeling including preoperatively and postoperatively available parameters identified the "combined DNA status" as a strong independent predictor of poor patient outcome.

Conclusions: The combinatorial DNA content analysis involving general (ploidy) and specific events (deletions) has the potential for clinical utility in prostate cancer. *Clin Cancer Res; 22(11); 2802–11. ©2016 AACR.*

Introduction

Prostate cancer is the most prevalent cancer in men in Western societies (1). While most cancers have a rather indolent clinical course, prostate cancer still represents the third most common cause of cancer-related death in men. Despite recent advances, the only established pretherapeutic prognostic parameters currently include Gleason grade and tumor extent on biopsies, preoperative prostate-specific antigen (PSA), and clinical stage. Because these data are statistically powerful but not sufficient for optimal individual treatment decisions, there is a tremendous need for better prognostic parameters enabling a more reliable prediction of the aggressiveness of individual prostate cancers.

Measurement of the cellular DNA content by either flow cytometry or static image cytometry has been extensively discussed as a potential prognostic tool in many cancer types in the 1980s and early 1990s of the last century (2–6). At that time, ploidy measurement represented the best possible method to globally assess tumor DNA content. It was postulated that a non-diploid DNA status would indicate genomic instability, which in principle should be linked to an aggressive cancer phenotype. Various studies have demonstrated that tetraploidy and aneuploidy are associated with unfavorable disease outcome in many cancer types, such as cancers of the colon, breast, oral cavity, urinary bladder and lungs (2–6). However, cytometry has not become a routine tool in any of these cancers, mostly because the prognostic information was clinically not relevant or because better predictors were identified. Moreover, current methods, for example fluorescence in situ hybridization (FISH) or—more recently—next-generation sequencing (NGS), enable detection of much more subtle genomic alterations and have thus recently gained more attention by researchers.

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-15-0635

Translational Relevance

Distinguishing between the indolent and aggressive forms of prostate cancer as early as possible is a major goal in current prostate cancer research. DNA cytometry has been discussed as a potential diagnostic test for more than a decade but has never entered clinical practice. Here, we demonstrate that the predictive power of measuring gross alterations of DNA content can be significantly improved by additional FISH-based measurement of deletions of small chromosomal fragments that will not become detectable by DNA flow cytometry. The gain of prognostic information beyond established clinicopathological parameters of this combinatory approach was particularly strong in the subgroup of Gleason 3+4 cancers, which is the most disputed subset with respect to a possible inclusion in active surveillance concepts.

Studies evaluating DNA ploidy in prostate cancer have come to varying conclusions. Some studies on 45–850 (mean 139) cancers have suggested a relevant prognostic impact of ploidy (7–20), while other studies on 33–447 (mean 122) cancers have failed to find significant associations (7, 20–24). Deletions affecting various chromosomal loci are a hallmark of prostate cancer. In particular, deletion of PTEN and 6q15 has been linked to adverse tumor phenotype and early biochemical recurrence in several studies, including our own work (25–28). In this study, we took advantage of our large Hamburg prostate cancer cohort to evaluate the potential impact of DNA cytometry data in a large consecutive series of cancers. Moreover, we evaluated the combined impact of measuring both a generally disturbed DNA content (ploidy status) and presence of subtle genomic alterations—such as small chromosomal deletions with known prognostic relevance, including PTEN and 6q15 (25, 28). The data demonstrate that quantitative DNA measurement in combination with deletion measurement is a powerful predictor of prognosis in prostate cancer.

Materials and Methods

Patients

Radical prostatectomy specimens were available from 3,845 patients for which both PTEN and 6q15 deletion status had been successfully determined in earlier studies (25, 28). All patients underwent surgery between 1992 and 2012 at the Department of Urology and the Martini Clinics at the University Medical Center Hamburg-Eppendorf. Follow-up data were available from a total of 3,845 patients with a median follow-up of 45.1 months (range, 0–240.4 months). The clinical and pathological parameters of the prostate cancers are given in Table 1. PSA values were measured following surgery and PSA recurrence was defined as a postoperative PSA of 0.2 ng/mL and increasing at subsequent measurements. In addition, patients were grouped into 4 subsets defined by clinically and biologically important features: Group 1 "organ confined tumor growth" included 444 patients with organ-confined tumors and no evidence of local or systemic dissemination [no histological sign of extraprostatic extension and no biochemical relapse (BCR) in long-term follow up ($\geq$3 years after surgery)], group 2 "local invasive tumor growth" included 172 patients with histological proof of extraprostatic tumor growth (pT3a or pT3b), but no BCR in long-term follow-up, or pT3 tumors with BCR but permanent response to salvage radiation, group 3 "occult systemic dissemination" included 307 cancers with BCR after two local therapies (radical prostatectomy and secondary—adjuvant or salvage—radiation), but without development of overt distant metastases in long-term follow-up, and group 4 "metastatic tumor growth" included 119 patients with development of distant (bone and/or visceral) or regional (lymph node) metastases. The remaining 2,803 cancers had not sufficient clinical information to allow for unequivocal allocation to these four groups. All prostate specimens were analyzed according to a standard procedure, including a complete embedding of the entire prostate for histological analysis (29).

Flow cytometry

Cell nuclei were extracted from formalin-fixed paraffin-embedded tissues and stained for flow cytometry analysis using a modified standard protocol (16, 21, 30). Two punches (diameter 0.6 mm) per tissue block were taken with a hollow needle from 3,845 prostate cancers included in this study. Special emphasis was placed on taking the punches from the same tumor area as used for PTEN/6q15 deletion analysis before. For preparation of cell suspensions, punches were re-embedded in paraffin, and cut into 50-μm sections using a microtome. The sections were placed in a nylon bag with a mesh of 50 μm, dewaxed in xylene, and rehydrated in a descending series of ethanol (100%, 96%, 70%, water) prior to digestion in 5 mg/mL pepsin (Serva #31820.02), and resolved in 0.07 mol/L HCl in a 37°C water bath for 30 minutes. Five milliliter cold phosphate buffered saline was added to stop the reaction. The suspension was centrifuged at 2,500 rpm to release cell nuclei from the nylon bags, resuspended to a volume of 450 μL and transferred to 5 mL FACS tubes. RNAse A (Sigma #R4875) was added to a final concentration of 0.05 mg/mL (adjusted to pH 7.4) before incubation at 37°C for 30 minutes. Nuclei were then stained by adding 100 μL propidium iodide solution (1 mg/mL) (Sigma, #P4864) for 5 minutes at 4°C in the dark. The DNA content was measured in at least 1,000 stained nuclei using a FACS Canto II using the blue laser (488 nm) with the filter configuration longpass 556 and bandpass 585/42.

Interpretation of the FACS results

DNA flow histograms were interpreted following standard criteria as described elsewhere (16, 17, 22). In brief, the first analyzable peak, indicating the fraction of cells with the lowest DNA content, was considered DNA diploid and was given a DNA index of 1.0. DNA indices for the following peaks were calculated relative to the 1.0 peak. Samples were considered DNA aneuploid when one or more unequivocal peaks between DNA index 1.0 and 1.8 or >2.2 was present. Tetraploidy was defined as the presence of one unequivocal peak between DNA index 1.8 and 2.2 with more than 10% of all cells. Otherwise, the sample was considered DNA diploid.

CAPRA (cancer of the prostate risk assessment) post-surgical (CAPRA-S) score

The CAPRA-S score was calculated for the entire set of 3,845 prostate cancers as described before (31).

Statistical analysis

Statistical calculations were performed using JMP 11 software (SAS Institute Inc.). Contingency tables and the χ^2 test were performed to search for associations between molecular parameters and tumor phenotype. Analysis of variance (ANOVA) analysis was performed to compare the preoperative PSA levels with molecular and cytometry data. Survival curves were plotted

Table 1. Associations between tumor phenotype and clinical endpoints and molecular features of prostate cancer (including PTEN deletion, 6q15 deletion, DNA ploidy, and the 3-level DNA score)

			PTEN deletion			6q15 deletion			ploidy			3-Level DNA score			
		n	Normal	Deletion	*P*	Normal	Deletion	*P*	Diploid	Non-diploid	*P*	Negative	Intermediate	High	*P*
pT stage	pT2	2381	11.3	88.7	<**0.0001**	81.8	18.2	**0.0001**	74.5	25.5	<**0.0001**	55.0	34.1	10.8	<**0.0001**
	pT3a	911	24.8	75.2		77.7	22.3		58.8	41.2		34.8	42.8	22.4	
	pT3b	529	34.2	65.8		73.7	26.3		54.1	45.9		27.8	41.2	31.0	
	pT4	24	37.5	62.5		70.8	29.2		41.7	58.3		16.7	37.5	45.8	
cT stage	T1c	2830	14.6	85.4	<**0.0001**	80.4	19.6	**0.0423**	70.7	29.3	<**0.0001**	50.1	35.9	14.0	<**0.0001**
	T2a	548	25.4	74.6		79.6	20.4		62.8	37.2		38.7	40.9	20.4	
	T2b	238	30.3	69.7		71.0	29.0		56.7	43.3		31.5	37.8	30.7	
	T2c	60	35.0	65.0		80.0	20.0		40.0	60.0		23.3	43.3	33.3	
Gleason	≤3+3	780	91.8	8.2	<**0.0001**	88.6	11.4	<**0.0001**	77.4	22.6	<**0.0001**	63.6	29.1	7.3	<**0.0001**
	3+4	2047	83.9	16.1		81.6	18.4		70.9	29.1		49.3	37.7	13.0	
	3+4 TG5	152	80.9	19.1		78.3	21.7		66.4	33.6		43.4	39.5	17.1	
	4+3	392	70.2	29.8		63.5	36.5		58.9	41.1		25.0	46.9	28.1	
	4+3 TG5	256	69.9	30.1		68.8	31.3		50.8	49.2		24.6	41.4	34.0	
	≥4+4	218	68.8	31.2		72.5	27.5		39.9	60.1		21.1	37.2	41.7	
Nodal stage	pN0	2203	81.5	18.5	<**0.0001**	77.0	23.0	0.2172	66.2	33.8	<**0.0001**	43.0	38.9	18.1	<**0.0001**
	pN>0	245	61.2	38.8		73.5	26.5		47.8	52.2		22.9	40.4	36.7	
Resection margin	R0	3019	83.6	16.4	**0.0004**	80.1	19.9	0.3559	69.8	30.2	<**0.0001**	48.6	36.2	15.2	<**0.0001**
	R1	761	78.1	21.9		78.6	21.4		60.2	39.8		38.1	40.5	21.4	
Clinical endpoints	Group 1	444	90.1	9.9	<**0.0001**	86.7	13.3	<**0.0001**	72.3	27.7	<**0.0001**	57.2	32.9	9.9	<**0.0001**
	Group 2	172	79.1	20.9		82.6	17.4		61.0	39.0		40.1	40.7	19.2	
	Group 3	307	68.4	31.6		71.7	28.3		58.0	42.0		30.6	41.7	27.7	
	Group 4	119	60.5	39.5		71.4	28.6		40.3	59.7		19.3	38.7	42.0	
Pre-surgical PSA	PSA (ng/mL)	3845	10.5	10.0	0.5358	9.9	10.5	0.4737	9.7	10.8	0.0869	9.4	10.5	10.8	0.134

NOTE: The 3-level DNA score is defined as follows: Negative: DNA diploid and no deletion; Intermediate: DNA diploid with deletion or DNA tetraploid without deletion; High: DNA tetraploid with deletion or DNA aneuploidy. Numbers in bold indicate significant values.

according to Kaplan–Meier. The log-rank test was applied to detect significant differences between groups. Cox proportional hazards regression analysis was performed to test the statistical independence and significance between pathological, molecular, and clinical variables. Gleason grade (in biopsies and radical prostatectomies), clinical (cT) and pathological tumor stage (pT), pathological nodal stage (pN), resection margin stage, and preoperative PSA levels were selected for multivariate analysis because they represent the clinically most relevant variables. Logistic regression was used to quantify the area under the receiver-operator curve (ROC).

Results

DNA cytometry

Between 1,043 and 85,049 cell nuclei were measured per tumor (mean 7,282 ± 2,317). The average coefficient of variation was 8.03 ±2.05 for the first peak. DNA cytometry revealed a diploid DNA content in 2,605 of 3,845 (67.8%), a tetraploid DNA content in 984 (25.6%), and an aneuploid DNA content in 256 (6.7%) of interpretable cancers. Aberrant ploidy was significantly associated with pT category, Gleason grade, clinical T stage, R status, pN category, and clinical endpoints ($P < 0.0001$ each; Table 1). Of note, among 218 patients with a Gleason score of ≥4+4 = 8; 60.1% had an abnormal DNA content, but there were still 39.9% of these highly aggressive cancers with a normal diploid DNA content. Abnormal DNA content was also tightly linked to early PSA recurrence ($P < 0.0001$; Fig. 1A).

PTEN/6q15 deletions

Deletions of PTEN and 6q15 were earlier shown to be linked to unfavorable tumor phenotype and PSA recurrence in our patients (25, 28). The respective data for the 3,845 cancers with interpretable cytometry profiles are shown in Table 1. As expected, significant associations were seen with phenotypical parameters and PSA recurrence ($P < 0.0001$) for both parameters.

Combination of ploidy and deletion data

The combination of crude DNA alterations (ploidy) and subtle specific changes (deletions) leads to substantial improvements of associations with phenotype and prognosis. We create a "3-level DNA score" for the combination according to the following criteria: negative, DNA diploid and no deletion; intermediate, DNA diploid with deletion (of at least one of PTEN and 6q) or DNA tetraploid without deletion; high, DNA tetraploid with deletion or DNA aneuploidy. As for deletions and ploidy alone, the combined approach revealed a tight link with unfavorable phenotype (Table 1). It is remarkable that the fraction of cancers with Gleason score ≥4+4 with a diploid DNA content and no deletion decreased to 21.1% in this combinatorial approach. The relevant effect of deletions over ploidy alone is best demonstrated in the PSA recurrence data (Fig. 1H–J). Here, the presence of deletions resulted in a massive deterioration of outcome in the subgroups of diploid, tetraploid, and aneuploidy cancers. The combination of ploidy and deletions was also tightly linked to our alternative clinical endpoints ($P < 0.0001$; Fig. 2A).

Impact on PSA recurrence and clinical endpoints in subgroups

Separate analyses in subgroups of cancers with Gleason score ≤3+3, 3+4 (with and without tertiary Gleason 5), 4+3 (with and without tertiary Gleason 5), and Gleason ≥4+4 revealed that the assessment of DNA alterations was particularly relevant in Gleason 3+4 cancers (Fig. 1C), while the effect was less relevant and failed to reach statistical significance in the other subgroups. Also the analyses of our alternative clinical

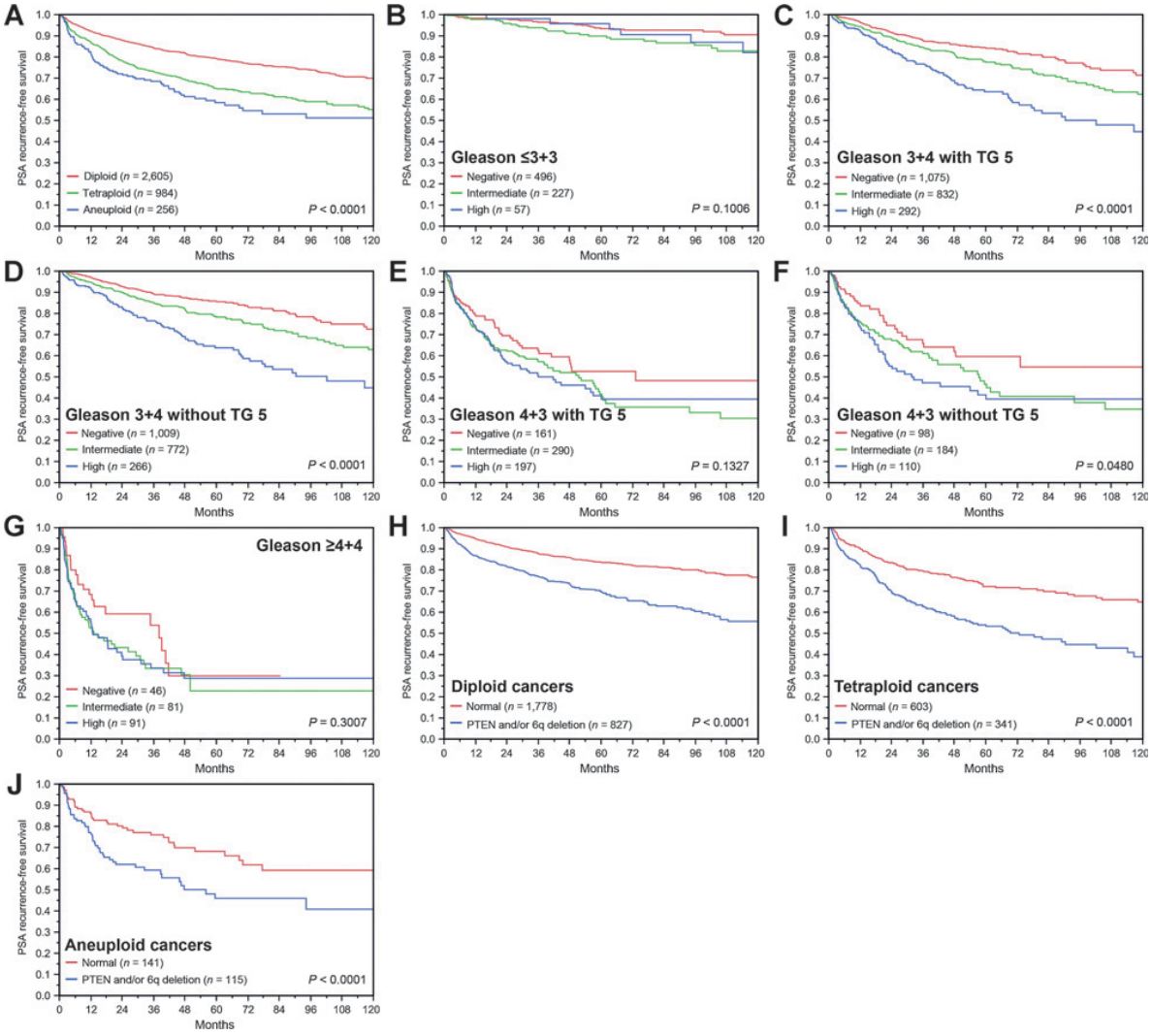

Figure 1.
PSA recurrence-free interval of patients with diploid, tetraploid, and aneuploidy cancers in all cancers (A), subset of Gleason ≤3+3 cancers (B), Gleason 3+4 with tertiary Gleason grade (TG) 5 cancers (C), Gleason 3+4 without TG 5 cancers (D), Gleason 4+3 with TG 5 cancers (E), Gleason 4+3 without TG 5 cancers (F), Gleason ≥4+4 cancers (G). Prognostic impact of deletions of PTEN and/or 6q15 in subsets of diploid cancers (H), tetraploid cancers (I), aneuploid cancers (J).

endpoints show that the effect was only relevant in the Gleason 3+4 subset, too (Fig. 2B).

Multivariate analyses

Four multivariate analyses were performed evaluating the clinical relevance of our "3-level DNA score" in different scenarios (Table 2). Scenario 1 was utilizing all postoperatively available parameters, including pathological tumor stage, pathological lymph node status (pN), surgical margin status, preoperative PSA value, and pathological Gleason grade obtained after the morphological evaluation of the entire resected prostate. Scenario 2 was utilizing all postoperatively available parameters with exception of the nodal status. The rationale for this approach was that the indication and extent of lymph node dissection is not standardized in the surgical therapy of prostate cancer and that excluding pN in multivariate analysis can markedly increase case numbers. Two additional scenarios had the purpose to model the preoperative situation. Scenario 3 included the "3-level DNA score," preoperative PSA, clinical tumor stage (*c*T stage), and Gleason grade obtained on the prostatectomy specimen. Because postoperative determination of a tumor's Gleason score is more accurate than the preoperatively determined Gleason grade (subjected to sampling errors and consequently under-grading in more than one third of cases), another multivariate analysis was added. In scenario 4, the preoperative Gleason grade obtained on the original biopsy was combined with preoperative PSA, *c*T stage, and the "3-level DNA score." In all these analyses, the "3-level DNA score"

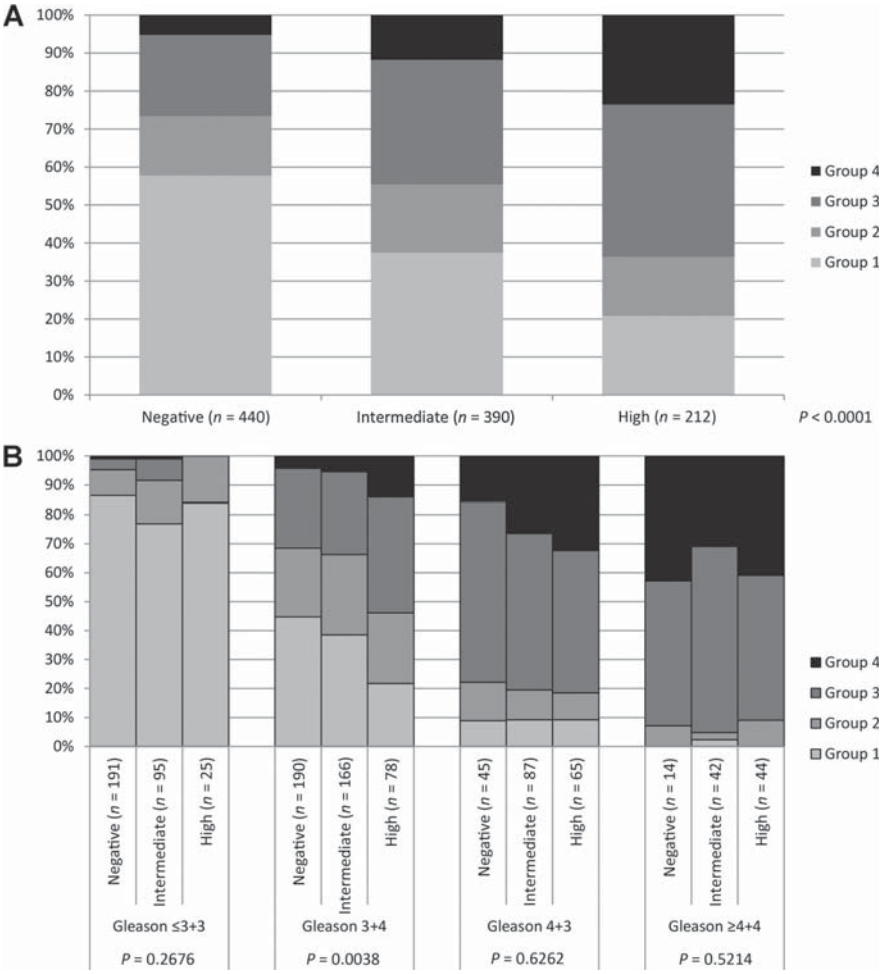

Figure 2.
Association between clinical endpoints and combination of DNA ploidy with PTEN/6q15 deletions (negative: DNA diploid and no deletion; intermediate: DNA diploid with deletion or DNA tetraploid without deletion; high: DNA tetraploid with deletion or DNA aneuploidy) (A) and in Gleason subgroups (B).

emerged as an independent predictor of prognosis with high statistical significance. ROC analyses were then performed to estimate whether the "3-level DNA score" can improve the predictive power in the 4 different clinical scenarios in subsets of 2,392 to 3,780 cancers for which data on these parameters and ploidy/deletion were available. These analyses revealed a gain of the predictive accuracy by 0.6% in the strongest scenario 1, 1.3% in scenario 2, 1.6% in scenario 3, and 2.6% in scenario 4 containing the preoperative prognostic parameters (Gleason at biopsy, clinical stage, preoperative PSA; Table 3).

Comparison with a clinical nomogram (CAPRA-S)

High scores of the established clinical CAPRA-S nomogram were strongly linked to early biochemical recurrence in our cohort ($P < 0.0001$; Supplementary Fig. S1a). In order to estimate if our "3-level DNA score" can improve the predictive power beyond the nomogram, Kaplan–Meier plots of the 3-level DNA score were prepared in subsets of cancers with an identical CAPRA-S score. This analysis revealed that the assessment of DNA alterations provided additional prognostic information beyond the nomogram. The "3-level DNA score" added significant prognostic information in subsets with CAPRA-S scores 0–1 ($P = 0.0007$; Supplementary Fig. S1b), 2–3 ($P = 0.0016$; Supplementary Fig. S1c), 4–5 ($P = 0.0278$; Supplementary Fig. S1d), and 6–8 ($P = 0.0010$; Supplementary Fig. S1e). For the highest score (9–12), there was at least nonsignificant trend toward a poor prognosis for cancers with an intermediate or high 3-level DNA score ($P = 0.4273$; Supplementary Fig. S1f).

Discussion

DNA flow cytometry revealed 6.8% aneuploid and 25.6% tetraploid prostate cancers in this study. All earlier studies analyzing series of at least 30 cases are summarized in Fig. 3. These data show that our rate of non-diploid cancers is in the range of earlier studies analyzing consecutive patient cohorts (9, 11–14, 16–19, 22). Markedly higher rates—ranging from 39.7% to 74.8%—were mainly found in studies enriched for advanced (7, 10, 14, 16, 17, 23, 32) or high grade cancers (22, 33). Some of the studies resulting in particularly low frequencies of non-diploid cancers had preselected for T1a or clinically localized cancers (7, 8, 13, 16, 20, 34).

Table 2. Multivariate analyses including established clinicopathological prognostic parameters and the combined ploidy/deletion score ("3-level DNA score") in different clinical scenarios

Scenario			*P*	HR	95% CI
1	Preoperative PSA level	>20 vs. 10–20	0.0019	1.4	1.1–1.8
		10–20 vs. 4–10	0.0048	1.3	1.1–1.6
		4–10 vs. <4	0.0819	1.3	1.0–1.9
	pT stadium	pT4 vs. pT3b	0.2294	1.4	0.8–2.2
		pT3b vs. pT3a	0.0009	1.4	1.1–1.7
		pT3a vs. pT2	<0.0001	1.9	1.5–2.3
	Gleason grade prostatectomy	≥4+4 vs. 4+3	0.0403	1.3	1.0–1.6
		4+3 vs. 3+4	<0.0001	1.9	1.6–2.3
		3+4 vs. ≤3+3	0.0004	1.8	1.3–2.6
	N status	N1 vs. N0	0.0005	1.5	1.2–1.9
	R status	R1 vs. R0	0.1463	1.1	1.0–1.4
	3-level DNA score	High vs. intermediate	0.0382	1.2	1.0–1.5
		Intermediate vs. low	0.1753	1.1	0.9–1.4
2	Preoperative PSA level	>20 vs. 10–20	0.0019	1.4	1.1–1.7
		10–20 vs. 4–10	<0.0001	1.4	1.2–1.7
		4–10 vs. <4	0.1111	1.2	1.0–1.6
	pT stadium	pT4 vs. pT3b	0.2800	1.3	0.8–2.1
		pT3b vs. pT3a	<0.0001	1.6	1.3–1.9
		pT3a vs. pT2	<0.0001	1.8	1.5–2.2
	Gleason grade prostatectomy	≥4+4 vs. 4+3	0.0030	1.4	1.1–1.8
		4+3 vs. 3+4	<0.0001	2.0	1.7–2.4
		3+4 vs. ≤3+3	<0.0001	2.1	1.6–2.7
	R-Status	R1 vs. R0	0.0016	1.3	1.1–1.5
	3-Level DNA score	High vs. intermediate	0.0065	1.3	1.1–1.5
		Intermediate vs. low	0.0011	1.3	1.1–1.6
3	Preoperative PSA level	>20 vs. 10–20	0.0004	1.5	1.2–1.8
		10–20 vs. 4–10	<0.0001	1.5	1.3–1.8
		4–10 vs. <4	0.0105	1.4	1.1–1.9
	cT stadium	T3a vs. T2c	0.0005	0.4	0.2–0.7
		T2c vs. T2b	0.0093	1.6	1.1-2.3
		T2b vs. T2a	0.1192	1.2	1.0–1.6
		T2a vs. T1c	0.0027	1.3	1.1–1.6
	Gleason grade prostatectomy	≥4+4 vs. 4+3	<0.0001	1.9	1.5–2.4
		4+3 vs. 3+4	<0.0001	2.4	2.1–2.9
		3+4 vs. ≤3+3	<0.0001	2.4	1.9–3.1
	3-Level DNA score	High vs. intermediate	0.0048	1.3	1.1–1.5
		Intermediate vs. low	<0.0001	1.4	1.2–1.7
4	Preoperative PSA level	>20 vs. 10–20	<0.0001	1.6	1.3–1.9
		10–20 vs. 4–10	<0.0001	1.6	1.4–1.9
		4–10 vs. <4	0.0039	1.5	1.1–2.0
	cT stadium	T3a vs. T2c	0.0039	0.5	0.3–0.8
		T2c vs. T2b	0.0105	1.6	1.1–2.3
		T2b vs. T2a	0.1871	1.2	0.9–1.5
		T2a vs. T1c	0.0014	1.4	1.1–1.6
	Gleason grade biopsy	≥4+4 vs. 4+3	<0.0001	1.6	1.3–2.0
		4+3 vs. 3+4	<0.0001	1.5	1.2–1.9
		3+4 vs. ≤3+3	<0.0001	1.7	1.4–2.0
	3-Level DNA score	High vs. intermediate	<0.0001	1.4	1.2–1.7
		Intermediate vs. low	<0.0001	1.5	1.3–1.8

NOTE: Scenario 1 contains the strongest parameters that become available only after prostatectomy, while scenario 4 includes the "minimal" parameters that are available at the time of the biopsy. Scenarios 2 and 3 include both pre- and postoperative parameters. HR, hazard ratio; CI, confidence interval.

Non-diploid DNA status was tightly linked to unfavorable cancer features, such as high Gleason grade, advanced tumor stage, and positive nodal stage. Comparable findings had been reported from the vast majority of earlier studies. Most of the larger studies analyzing at least 100 cancers reported associations with Gleason grade (13, 16, 18), other grading systems (7, 13, 16, 20), or tumor stage (13,16,18). Accordingly, DNA ploidy was also strongly associated with PSA recurrence at least in some of these studies (7, 10). Similar findings were also made in earlier and smaller studies involving fewer than 100 patients (7, 12, 17, 19,23, 32, 34). Given the undisputed link of non-diploid DNA with unfavorable tumor phenotype, it is likely that those studies on 33 (23), and 81 (24) patients failing to find associations with PSA recurrence were disturbed by low patient numbers.

Based on this obvious link of non-diploid DNA status and poor tumor phenotype and clinical outcome, ploidy measurement was earlier suggested for routine use in the diagnostic work-up of biopsies (35, 36). It was claimed that ploidy data could compensate for possible mistakes made by the pathologist in his Gleason grading, which is a very strong prognostic feature but notorious for subjectivity and interobserver

Table 3. Improvement of the accuracy of predicting biochemical recurrence in different models

		Scenario 1		Scenario 2		Scenario 3		Scenario 4	
	Model	AUC	Gain	AUC	Gain	AUC	Gain	AUC	Gain
1	Basic model	0.7669		0.7703		0.7452		0.7223	
2	+ Ploidy	0.7675	0.09%	0.7743	0.53%	0.7501	0.65%	0.7315	1.27%
3	+ PTEN/6q deletion	0.7707	0.50%	0.7819	1.52%	0.7581	1.73%	0.7420	2.72%
4	+ Ploidy and PTEN/6q deletion	0.7726	0.74%	0.7833	1.69%	0.7615	2.19%	0.7487	3.65%

NOTE: The basic model includes only the established clinicopathological prognostic parameters as defined by our four clinical scenarios. Models 2–3 include the parameters of the basic model and, in addition, the ploidy data (model 2), the deletion data (model 3), and the combination of ploidy and deletion data (model 4). AUC indicates the area under the curve in receiver-operator analysis. Gain indicates the difference (on a percentage basis) in the AUC of models 2, 3, and 4 as compared with the basic model 1.

variability (37–39). That ploidy measurement by itself cannot make Gleason grading "safe" in its capability to identify the "dangerous" cancers is, however, demonstrated by the 39.9% of Gleason $\geq$4+4 cancers (known to have a dismal prognosis), and the 55.2% of cancers with PSA recurrence, having a diploid DNA content. Given that many subtle DNA changes—such as small chromosomal deletions—are strongly linked to unfavorable prostate cancer prognosis (25, 26, 40), and considering that a loss of a small fragment of a chromosome will not become detectable by DNA flow cytometry, it is not surprising that many aggressive prostate cancers are DNA diploid.

We therefore hypothesized that adding powerful prognostic deletion markers to ploidy analysis would optimize our assay for identifying biologically aggressive prostate cancers. Deletions of PTEN and 6q15 were selected for this purpose because both of them are strongly linked to unfavorable disease outcome and also because these deletions cover relevant molecular subgroups (25–28). PTEN deletions preferably occur in ERG fusion–positive and 6q15 deletions in ERG fusion–negative cancers (25, 28). That the addition of these deletions to pure ploidy data may be advantageous for an approach to identify potentially aggressive cancers is already suggested by the much lower rate of cancers with Gleason $\geq$4+4 (now 21.1% as compared with 39.9% without considering deletions) and of cancers with PSA recurrence (now 29.0% as compared with 55.2% without considering deletions) when deletion analysis is combined with ploidy analysis. The strong additional benefit of our combined approach is further demonstrated by the striking difference in the risk of PSA recurrence in each individual subgroup defined by DNA ploidy (diploid, tetraploid, aneuploid) depending on whether or not PTEN and/or 6q15 deletions were additionally present.

Limitations of this study include the use of PSA recurrence as a clinical endpoint instead of cancer specific death, which was a rare event in our cohort, and that the analysis was performed on prostatectomy specimens instead of true core needle biopsies. We do not feel that these issues question the message of this study. Specifically, we believe that PSA recurrence is an excellent surrogate marker if it comes to the assessment of prognostic biomarkers. From our experience, all prognostic parameters predicting PSA recurrence in prostatectomy samples are also linked to other endpoints such as time to metastasis, time to cancer-related death, or unfavorable disease course in patients not undergoing potentially curative therapy. Gleason grade represents the best example for this notion. It is well accepted that high Gleason grade is associated with all possible unfavorable clinical endpoints. To our knowledge, there is no molecular or morphologic feature that was shown—in sufficiently large studies—to be robustly linked to PSA recurrence but to be unrelated to metastasis and cancer-related death. Molecular parameters that have been described to be linked to metastasis and cancer-related death in patient cohorts describing at least 100 patients with unfavorable events include p53 (41, 42), bcl-2 (41), microvessel density (41), Ki-67 (42), CD10 (43), cyclin D1 (44), telomere length (45), HER2-neu (46), Stat5a/b (47), AMACR (48), HSP-27 (49), and MDM2 (42). All these parameters have also been linked to PSA recurrence in the respective studies (44, 46–48).

In an attempt to analyze additional and possibly better clinical endpoints in prostatectomy samples, clinical subgroups were defined that reflect the "biological milestones" of cancer progression, including (i) organ-confined tumors (pT2) without relapse in long-term follow-up, (ii) "local invasive" cancers (pT3) without relapse or with permanent response to local secondary radiation in long-term follow-up, (iii) "occult systemic" disease characterized by failure of two local therapies (radical prostatectomy and secondary radiation), but no evidence of distant metastases in long-term follow-up, and (iv) metastatic disease characterized by presence or development of regional or distant metastases. These subgroups are clinically valuable as only group 1 is potentially suited for an active surveillance strategy. That our "3-level DNA score" was strongly associated with unfavorable alternative clinical endpoints (Fig. 2A) provides additional support for the potential clinical relevance of the combination from DNA ploidy, PTEN, and 6q15 deletions.

It is obvious that studies evaluating prognostic biomarkers should optimally be performed on pretherapeutic tissue samples (i.e., prostate biopsies) rather than on samples obtained after definitive therapy (i.e., prostatectomy). From a practical point of view, such analyses are hardly feasible, however. This is not only because initial biopsies from cancer patients are typically distributed among many diagnostic institutions but also because the precious needle biopsies would be exhausted after only a few studies. To mimic as much as possible the presurgical situation, we have performed multivariate statistical analyses that use parameters available before surgery in addition to statistical models using post-surgical parameters. That strong independent associations of our "3-level DNA score" with early PSA recurrence was found in all analyzed pre- and post-surgical scenarios strongly argues for this combined DNA score representing a biomarker with clinical relevance in prostate cancer. It was therefore no surprise that our DNA score also provided a benefit of prognostic information beyond an established clinical nomogram, i.e., the CAPRA-S score.

In summary, the results of our study demonstrate that measurement of the gross DNA content in combination with analyzing two specific deletions by FISH provide strong and independent prognostic information in prostate cancer.

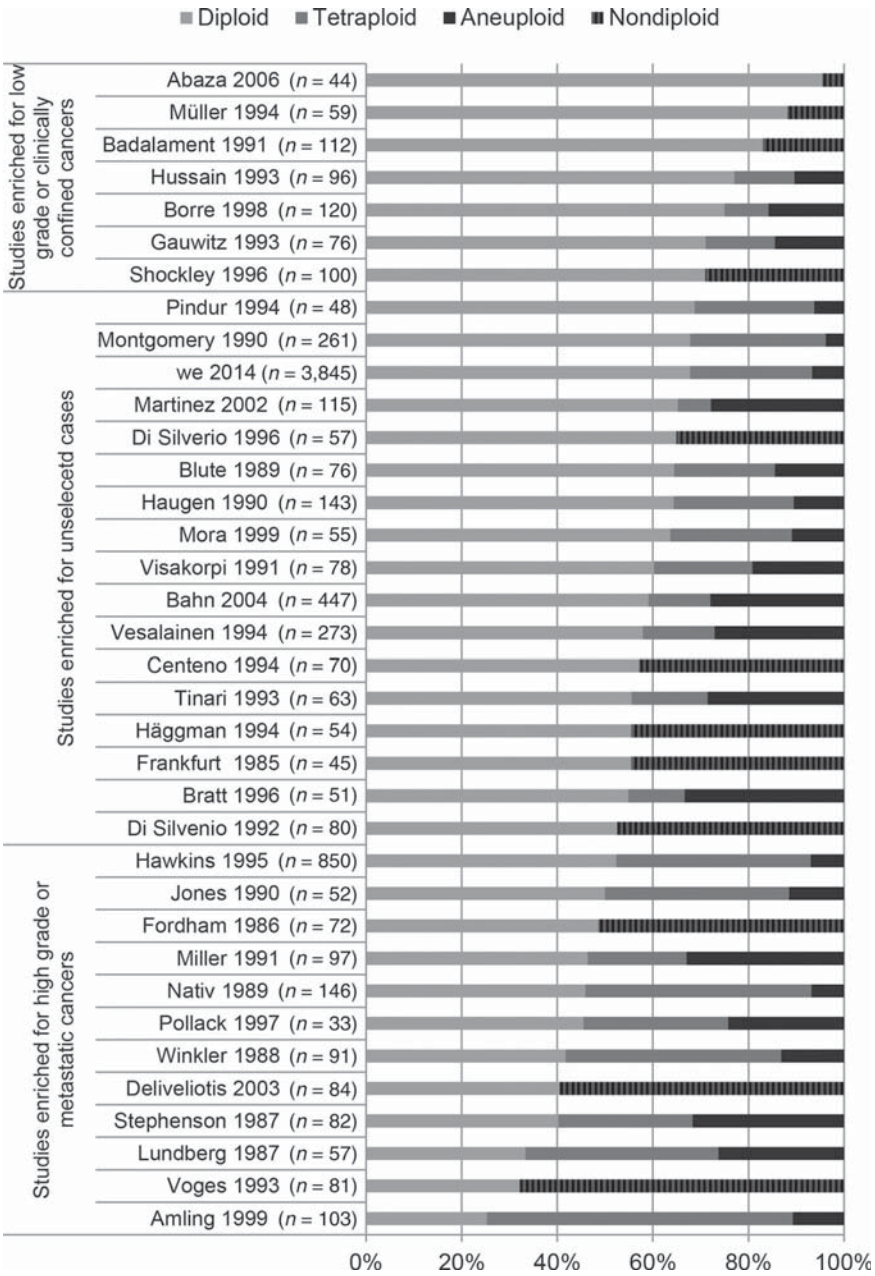

Figure 3.
Comparison of flow cytometry findings reported from different studies (7–24, 32–34, 50).

This especially holds true for the subgroup of Gleason 3+4 cancers, which is the most disputed subset with respect to a possible inclusion in active surveillance concepts. In contrast to most previously published prognostic features, e.g., based on RNA or protein expression analyses, FISH and flow cytometry provide highly reproducible yes/no answers. We thus expect that future trials utilizing this combined DNA measurement for prediction of cancer aggressiveness on pretherapeutic prostate cancer biopsies will prove strong utility of our approach.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Received March 19, 2015; revised November 17, 2015; accepted November 22, 2015; published OnlineFirst January 26, 2016.

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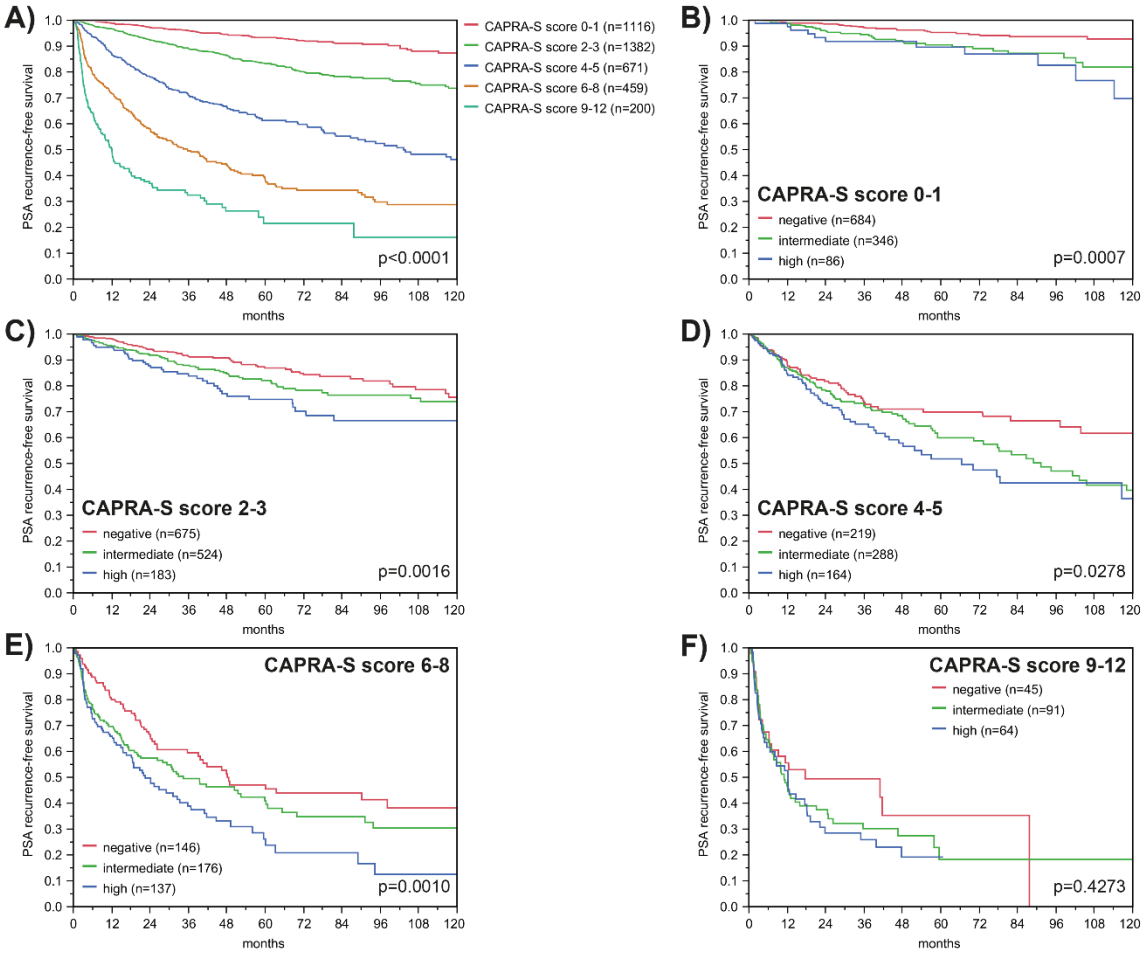
Supplementary figures



Supplementary figure 1: Benefit of the 3-level DNA score beyond the CAPRA-S nomogram for prediction of early biochemical recurrence. A) Prognostic impact of different levels of the CAPRA-S score. B)-F) Additional prognostic impact of the 3-level DNA score in subsets of cancers with B) CAPRA-S score 0-1, C) CAPRA-S score 2-3, D) CAPRA-S score 4-5, E) CAPRA-S score 6-8, F) CAPRA-S score 9-12. The 3-level DNA score combines cancers according to the following criteria: negative, DNA diploid and no deletion; intermediate, DNA diploid with deletion (of at least one of PTEN and 6q) or DNA tetraploid without deletion; high, DNA tetraploid with deletion or DNA aneuploidy.

2 Darstellung der Publikation

2.1 Einleitung

Das Prostatakarzinom ist die häufigste Krebserkrankung des Mannes und steht an dritter Stelle der krebsbedingten Todesursachen in den westlichen Industrienationen [1]. Die größte Gruppe der Prostatakarzinome ist allerdings wenig aggressiv und verursacht daher zeitlebens keine Symptome. Autopsiestudien haben gezeigt, dass die meisten Männer eher „mit“ als „an“ einem Prostatakarzinom versterben [2]. Dennoch existieren auch Tumoren, welche hoch aggressiv sind und zu einem lebensbedrohlichen Krankheitsverlauf führen [3].

Die heutigen diagnostischen Verfahren zur Früherkennung des Prostatakarzinoms bestehen aus der digital rektalen Untersuchung, dem Prostata spezifischen Antigen (PSA)-Screening im Serum, bildgebender Verfahren sowie bei auffälligen Befunden eine Ultraschall gesteuerte Biopsie der Prostata. Am Biopsiematerial erfolgt die pathologische Beurteilung des Karzinoms mittels des Gleason-Scores [4]. Der Gleason-Score beschreibt den Grad der Entdifferenzierung der Drüsenstruktur und wird in den Stufen gut differenziert (1) bis undifferenziert (5) angegeben [5].

Diese etablierten diagnostischen Methoden reichen jedoch nicht aus, um die lebensbedrohlichen Prostatakarzinome von den eher „gutartig“ verlaufenden Tumoren sicher zu unterscheiden. Die therapeutische Empfehlung, ob eine aggressive chirurgische Therapie, Radiatio oder eine aktive Überwachung des Patienten („Active Surveillance“) durchgeführt werden soll, ist daher in der momentanen Situation sehr schwierig. Aus diesem Grund entscheiden sich die meisten Männer für die Entnahme der Prostata mit dem nahezu sicheren Ausschluss eines potentiell lebensbedrohlichen Verlaufes. Dies ist allerdings mit einer signifikanten Morbidität verbunden. Zur Verminderung der Übertherapie ist ein primäres Ziel der Prostatakarzinomforschung die Etablierung von prognostisch relevanten molekularen Markern, welche die etablierten Prognoseparameter bei der Einschätzung der Aggressivität des Tumors unterstützen können. Kürzlich konnte bereits erfolgreich eine Verbesserung des „klassischen“ Gleason-Scores durch die Einführung des „quantitativen“ Gleason-Scores in der Arbeitsgruppe des Instituts für Pathologie am Universitätsklinikum Hamburg-Eppendorf etabliert werden [6]. Des Weiteren zeigen Studien, dass Deletionen als häufigste genetische Veränderungen des Prostatakarzinoms eine hohe prognostische Relevanz besitzen [7-10].

Generell kommt es im Rahmen der Akkumulation von genetischen Veränderungen in Tumorzellen oft auch zu Veränderungen des DNA-Gehalts. Bei einem Teil der Tumoren kommt es im Laufe der Tumorprogression zu einer Verdopplung des Chromosomensatzes (Tetraploidie), oft gefolgt von einem Verlust eines größeren Teiles der zugewonnenen Chromosomen (Aneuploidie). Es ist daher nicht erstaunlich, dass die Messung des DNA-Gehaltes bereits in zahlreichen Studien als potentiell prognostischer Parameter untersucht wurde [11-29]. Obwohl die Mehrzahl der durchgeführten Studien bereits zwischen 1980 und 1990 veröffentlicht wurden, gibt es immer noch Tumorentitäten in denen unklar ist, ob der DNA-Gehalt zur besseren Einschätzung des Progressionsrisikos eingesetzt werden kann. Die Bestimmung des Ploidiestatus von Tumorzellen kann mittels Durchflusszytometrie oder Bildzytometrie erfolgen. Dabei wird angenommen, dass nicht-diploide Tumoren als Surrogat für genomische Instabilität stehen, welche generell für hohe Tumoraggressivität spricht. In zahlreichen Tumorentitäten konnte nachgewiesen werden, dass tetraploide und aneuploide Tumoren deutlich mit einem ungünstigen Tumorphänotyp assoziiert sind. Auch beim Prostatakarzinom wurde vorgeschlagen, die Ploidie Bestimmung als diagnostische Methode zur Differenzierung von Patienten mit guter und schlechter Prognose zu nutzen [30, 31]. Trotz dieser Erkenntnisse fand die Bestimmung des Ploidiestatus keine Anwendung in der Diagnostik von Tumoren. Grund dafür ist vor allem, dass Methoden gefunden wurden, wie z.B. „Fluorescence in situ hybridizazion“ (FISH) oder „next-generation sequencing“ (NGS), mit denen auch kleinste zytometrisch nicht erfassbare klinisch relevante genetische Veränderungen nachgewiesen werden können.

Die Ergebnisse der Studien über den Ploidiestatus des Prostatakarzinoms variieren stark. Einige Studien mit 45 bis 850 Patienten suggerierten eine prognostische Relevanz [32-52], andere mit 33 bis 447 Patienten fanden dies nicht [53-58].

Für unsere Studie nutzten wir Tumoren aus unserer großen Hamburger Prostata Kohorte, um die potentielle prognostische Relevanz der Ploidie beim Prostatakarzinom endgültig zu klären. Darüber hinaus untersuchten wir die klinische Relevanz des Ploidiestatus in Verbindung mit zwei der häufigsten prognostisch relevanten Deletionen des Prostatakarzinoms (PTEN und 6q15). Insgesamt konnte die vorliegende Studie zeigen, dass zumindest die Kombination der Bestimmung des Ploidiestatus und des Kopiezahlstatus von PTEN und 6q15 einen viel versprechenden prognostischen Test für das Prostatakarzinom darstellt.

2.2 Material und Methoden

Patientenkollektiv und klinische Endpunkte

Für diese Studie nutzten wir Daten von 3.845 Patienten über den Kopiezahlstatus von PTEN und 6q15 aus jeweils einer „tissue microarray“ (TMA) Studie [7, 8]. Alle Patienten wurden zwischen 1992 und 2012 in der urologischen Klinik oder der Martini Klinik des Universitätsklinikums Hamburg-Eppendorf prostatektomiert. Klinische Verlaufsdaten waren für alle Patienten mit einem Median von 45 Monaten vorhanden. Ein PSA Rezidiv wurde angenommen ab einem Wert von 0,2 ng/ml. Außerdem wurden die Patienten in vier Gruppen mit dem Schwerpunkt auf klinische und biologische Parameter unterteilt: Gruppe 1 (organbeschränktes Tumorwachstum) beinhaltete 444 Patienten mit auf die Prostata beschränktem Tumorwachstum (pT2) ohne biochemisches Rezidiv innerhalb drei Jahren. Gruppe 2 (lokal invasives Tumorwachstum) beinhaltete 172 Patienten mit extraprostatischem Tumorwachstum (pT3a oder pT3b) ohne biochemisches Rezidiv innerhalb drei Jahren oder mit einem biochemischen Rezidiv, welches aber permanent auf eine sekundäre adjuvante oder Salvage-Bestrahlungstherapie angesprochen hat. Gruppe 3 (okkulte systemische Dissemination) beinhaltete 307 Patienten mit einem biochemischem Rezidiv nach zwei lokalen Therapien (radikaler Prostatektomie und sekundäre adjuvante oder Salvage-Bestrahlungstherapie), aber ohne Fernmetastasen im Langzeitverlauf. Gruppe 4 (metastatisches Tumorwachstum) beinhaltete 119 Patienten mit Fern- (Knochen- und/oder viszeralen) oder Lymphknotenmetastasen. Die restlichen 2.803 Patienten hatten nicht ausreichend klinische Informationen, um sie einer der vier Gruppen zuordnen zu können.

Durchflusszytometrie

Die Durchflusszytometrie ist eine Methode zur Analyse von Struktur und molekularen Eigenschaften von einzelnen Zellen. Für die Bestimmung der Ploidie wurden Prostatakarzinomzellen aus in Formalin fixiertem, in Paraffin eingebettetem Gewebe vereinzelt und mittels Propidiumiodide gefärbt. Der genaue Ablauf der Methode ist in der beigefügten Publikation detailliert beschrieben.

Interpretation der Durchflusszytometrieresultate

Die Ergebnisse der Messung der einzelnen Prostatatumoren lieferten Histogramme, welche den DNA Gehalt vieler Zellen eines Prostatakarzinoms darstellten. Der erste darstellbare Peak mit dem geringsten DNA Gehalt wurde als diploider Peak gewertet und bekam einen DNA Index von 1,0. Der DNA Gehalt von weiteren aufgetretenen Peaks wurde in Relation zu dem DNA Gehalt des ersten Peaks gesetzt und stellte damit den DNA Index des weiteren Peaks dar. Zeigte das Histogramm eines Tumors einen weiteren Peak mit einem DNA Index zwischen 1,8 und 2,2 mit mehr als 10% aller Zellen in diesem Bereich, wurde der Tumor als Tetraploid gewertet. Zeigte sich ein Peak außerhalb dieses DNA Index Bereiches, wurde der Tumor als Aneuploid gewertet. Wenn neben dem ersten Peak kein weiterer vorlag, wurde der Tumor als Diploid gewertet.

2.3 Ergebnisse

Insgesamt konnten pro Tumor zwischen 1.043 und 85.049 Zellkerne analysiert werden. Einen diploiden Status hatten 2.605 der 3.845 (67,8%) Tumoren. Einen abweichenden DNA Gehalt wiesen 1.240 der Tumoren auf. Davon waren 984 (79,4%) tetraploid und 256 (20,6%) aneuploid. Eine PTEN Deletion wiesen 684 (17,8%) und eine 6q15 Deletion 781 (20,3%) der Tumoren auf. Eine detaillierte Darstellung aller Ergebnisse ist in der beigefügten Publikation zu finden.

Die wesentlichen Ergebnisse sind:

1. Ein veränderter DNA Gehalt ist mit einem schlechten Phänotyp des Prostatakarzinoms assoziiert.
2. Die Wahrscheinlichkeit eines PSA Rezidivs steigt von diploiden über tetraploiden zu aneuploiden Tumoren an.
3. Fast die Hälfte aller Tumoren mit einem schlechten Phänotyp weisen einen diploiden Status auf. Durch die Kombination der Ploidie mit den Deletionen von PTEN und 6q15 reduziert sich dieser Anteil auf um die Hälfte.
4. Der Nachweis einer Deletion steigert die Wahrscheinlichkeit des PSA Rezidivs sowohl in diploiden als auch tetraploiden und aneuploiden Tumoren.

5. Die prognostische Aussagekraft des kombinierten DNA Status ist unabhängig von den etablierten prä- und postoperativen Prognoseparametern.

2.4 Diskussion

In der vorliegenden Studie konnte mittels DNA Zytometrie in 6,8% ein aneuploider und in 25,6% ein tetraploider Status in den untersuchten Prostatakarzinomen nachgewiesen werden. Diese Ergebnisse stehen im Einklang mit vorherigen Studien, die ebenfalls den Ploidie Status mittels DNA Zytometrie an nicht selektierten Prostatakarzinomen untersuchten [33, 35, 36, 38, 40, 41, 48, 53, 59, 60]. Einen deutlich höheren Anteil an nicht-diploiden Tumoren von 39,7 bis 74,8% fanden lediglich Studien, die insbesondere Tumoren mit fortgeschrittenem Stadium oder mit einem hohen Gleason-Score untersuchten [35, 36, 39, 44-46, 49, 53, 57, 58, 61, 62]. Studien, die Karzinome in frühen Stadien untersuchten, fanden einen geringeren Anteil an nicht-diploiden Tumoren [32, 41, 43, 47, 56, 63-65].

Der nicht-diploide DNA Status war deutlich assoziiert mit ungünstigen Tumoreigenschaften, wie z.B. einem hohen Gleason-Score, einem fortgeschrittenen Tumorstadium und der Lymphknotenmetastasierung. Vergleichbare Ergebnisse wurden in den meisten früheren Studien gefunden, die mindestens 100 Tumoren untersuchten [33, 37, 41, 42, 45, 47, 59, 64]. In diesen Studien konnte eine Assoziation zwischen nicht-diploiden Tumoren und dem Gleason-Score, anderen Gradingsystemen oder dem Tumorstadium nachgewiesen werden. Des Weiteren zeigte der Ploidie Status in einigen dieser Studien eine starke Assoziation mit der Wahrscheinlichkeit eines PSA Rezidivs [37, 64]. Dies konnte allerdings auch in Studien gezeigt werden, die sehr kleine Patientenkohorten untersuchten [34, 36, 38, 46, 57, 61, 63].

Aufgrund des Zusammenhangs zwischen dem nicht-diploiden DNA Status und einem schlechten klinischen Verlauf, wurde die Messung des Ploidie Status bereits als Methode für die Routinediagnostik am Biopsie Material vorgeschlagen [30, 31]. Es besteht die Hoffnung, dass die Kenntnis über den Ploidie Status die subjektive Aussagekraft des Gleasongradings zumindest partiell ausgleicht. Der Gleason-Score ist der beste prognostische Parameter beim Prostatakarzinom [6]. Allerdings unterliegt das Grading durch den Pathologen einer hohen Subjektivität, die zu einer

starken Interobserver-Variabilität führen kann [66-68]. Die Messung des Ploidie Status kann allerdings mit Sicherheit das Gleasongrading nicht ersetzen. Dies zeigt sich insbesondere dadurch, dass 40% der Tumoren mit einem Gleason-Score ≥4+4, welche Bekannterweise eine schlechte Prognose haben, und 55% der Tumoren mit einem PSA Rezidiv einen diploiden Status haben. Unter der Berücksichtigung, dass mittels der DNA Zytometrie nicht alle chromosomalen Veränderungen detektiert werden können, ist dieses Ergebnis nicht besonders überraschend.

Das Prostatakarzinom ist insbesondere durch kleine chromosomale Deletionen von hoher prognostischer Relevanz charakterisiert [7, 8, 10]. Diese chromosomalen Veränderungen können mittels Zytometrie nicht nachgewiesen werden. Dies erklärt, warum die meisten aggressiven Prostatatumoren diploid sind. Basierend auf dieser Tatsache entstand die Hypothese, dass zur Identifizierung der biologisch aggressiven Tumoren eine kombinierte Untersuchung des Ploidie Status und der prognostisch relevanten Deletionen ideal wäre. Um diese Hypothese zu prüfen, wurden die Deletionen von PTEN und 6q15 ausgewählt, da diese eine starke Assoziation zu einem schlechten klinischen Verlauf aufweisen [7, 8, 10, 69]. Zusätzlich repräsentieren sie zwei relevante Subgruppen des Prostatakarzinoms. PTEN Deletionen sind deutlich mit der TMPRSS2:ERG Fusion assoziiert [7], während Deletionen von 6q15 vermehrt in ERG negativen Tumoren auftreten [8]. Dass die Kombination Ploidie/Deletionen von Vorteil ist, zeigt die deutlich geringere Rate an Tumoren mit einem hohen Gleason-Score und einem PSA Rezidiv im Gegensatz zur Ploidie alleine. Durch die Kombination mit dem Deletionsstatus reduzierte sich der Anteil an „normalen“ Tumoren von 40% auf 21% (Gleason ≥4+4) bzw. von 55% auf 29% (PSA Rezidive). Der eindeutige Vorteil dieses kombinierten Prognosetestes zeigt sich vor allem bei der genauen Betrachtung der Wahrscheinlichkeit des PSA Rezidives in den einzelnen Subgruppen. Hier zeigt sich, dass die prognostische Relevanz des diploiden-, tetraploiden- und aneuploiden Status von der Abwesenheit oder Anwesenheit der PTEN bzw. 6q15 Deletion abhängt.

Eine theoretische Einschränkung dieser Studie ist die Verwendung des PSA Rezidivs als Studienendpunkt statt des tumorspezifischen Todes. Der tumorspezifische Tod ist ein gängiger Studienendpunkt bei den meisten Tumorentitäten. Beim Prostatakarzinom kann dieser jedoch nicht verwendet werden, da er nur selten vorkommt (weniger als 5% der Patienten) [70]. Des

Weiteren wurden die Analysen an Proben aus Prostatektomiepräparaten durchgeführt und nicht an den für die Therapieentscheidung wichtigen Stanzbiopsien. Wir sind jedoch der Meinung, dass diese „Einschränkungen“ die Aussagekraft unserer Studie nicht limitieren. Das PSA Rezidiv ist ein hervorragender Parameter zur Bestimmung der prognostischen Aussagekraft von molekularen Markern. Alle Prognoseparameter, die mit einem frühen PSA Rezidiv zusammenhängen, sind ebenfalls mit zahlreichen anderen biologischen Parametern des Prostatakarzinoms assoziiert. Dazu zählen der Zeitpunkt der Metastasierung, der tumorspezifische Tod sowie der schlechte Krankheitsverlauf von Patienten, bei denen die Ektomie keinen kurativen Ansatz hatte. Der Gleason-Score ist das beste Beispiel dafür. Es ist allgemein anerkannt, dass ein hoher Gleason-Score mit all diesen biologischen Parametern bzw. klinischen Endpunkten korreliert [6, 71]. Unserer Kenntnis nach gibt es keine molekularen oder morphologischen Veränderungen beim Prostatakarzinom, für die in großen Studien eine eindeutige Assoziation zum PSA Rezidiv, aber nicht der Metastasierung oder dem tumorspezifischen Tod nachgewiesen werden konnte. Es gibt außerdem zahlreiche Studien mit mindestens 100 Patienten, die einen Zusammenhang zwischen verschiedenen molekularen Parametern und der Metastasierung bzw. dem tumorspezifischen Tod zeigen konnten [72-84]. Dazu zählen z.B. p53, bcl-2, Ki-67, CD10, Cyclin D1, Telomer Länge, HER2-neu, Stat5a/b, AMACR, HSP-27 und MDM2, welche alle mit einem frühen PSA Rezidiv assoziiert sind [74, 75, 77, 78, 81, 85-94].

Um potentiell bessere klinische Endpunkte für die Analyse zu nutzen, wurden von uns vier klinische Gruppen definiert, die die wesentlichen biologischen Charakteristika der Tumorprogression beschreiben. Gruppe 1: Lokal begrenztes Tumorwachstum (pT2) ohne biochemisches Rezidiv im weiteren klinischen Verlauf, Gruppe 2: Über die Prostata hinausgehendes Tumorwachstum (pT3) ohne biochemisches Rezidiv oder mit reversiblem PSA Rezidiv nach lokaler Bestrahlung, Gruppe 3: Okkult systemische Tumoren ohne Anzeichen für Metastasen und ohne Ansprechen auf die folgende Therapie, sowie Gruppe 4: Lymphogene und oder hämatogene Metastasierung. Diese vier Gruppen sind klinisch hoch relevant. Insbesondere die Gruppe 1 kennzeichnet Patienten, die für die „Active Survaliance“ Strategie in Frage kommen. Dass unser „3-level DNA score“ einen starken Zusammenhang mit den ungünstigen klinischen Endpunkten zeigte, weist deutlich

auf die hohe klinische Relevanz der kombinierten Analyse aus DNA Status und Deletion Status hin.

Es ist offensichtlich, dass Studien zur Validierung von prognostischen Biomarkern Optimalerweise an prätherapeutischen Gewebeproben (Prostatabiopsie) durchgeführt werden sollten und nicht an Gewebeproben, die nach der Therapie gewonnen wurden. Dies ist in der Praxis jedoch häufig schwer durchzuführen. Grund dafür ist, dass die initialen Biopsien der Tumorpatienten häufig in unterschiedlichen Instituten untersucht werden. Dies und die Tatsache, dass das Biopsiematerial nur in geringer Menge vorliegt, führt dazu, dass an diesem Gewebematerial nur wenige Analysen durchgeführt werden können. Um die präoperative Situation so gut wie möglich darzustellen, haben wir unterschiedliche multivariate Analysen durchgeführt, die sowohl prä- als auch postoperative Parameter berücksichtigen. Dass in diesen Analysen unser „3-level DNA score“ sowohl in den prä- als auch in den postoperativen Szenarien für die Vorhersage des frühen PSA Rezidivs geeignet ist, spricht deutlich dafür, dass dieser Score eine hohe klinische Relevanz beim Prostatakarzinom hat. Basierend darauf ist es nicht überraschend, dass unser „3-level DNA score“ auch in dem etablierten klinischen Nomogramm (CAPRA-S score) prognostische Informationen liefert.

Insgesamt zeigen die Ergebnisse unserer Studie, dass die Messung des DNA Gehaltes in Kombination mit zwei spezifischen Deletionsloci („3-level DNA score“) ein unabhängiger prognostischer Marker beim Prostatakarzinom ist. Dies trifft insbesondere für die Gruppe der Tumoren mit einem „günstigeren“ Gleason-Score (3+4) zu, welche potentiell für die „Active Survaliance“ Strategie geeignet sind. Im Gegensatz zu den meisten früher vorgeschlagenen prognostischen Parametern, die auf der Messung der mRNA oder Proteinexpression basieren, führen sowohl die FISH, als auch die Durchflusszytometrie zu sehr gut reproduzierbaren „ja“ oder „nein“ Antworten. Aus diesem Grund sind wir der Meinung, dass unser „3-level DNA score“ in der Zukunft als Marker zur Vorhersage der Tumoraggressivität an den prätherapeutischen Prostatabiopsien hervorragend geeignet ist.

2.5 Zusammenfassung

Ein nicht-diploider DNA Status wurde als potentieller prognostischer Parameter beim Prostatakarzinom diskutiert. In unserer Studie analysierten wir die klinische Relevanz der DNA Ploidie in Kombination mit den prognostisch relevanten Deletionen PTEN und 6q15 an 3.845 Prostatakarzinomen. Von diesen Tumoren waren 67,8% diploid, 25,6% tetraploid und 6,8% aneuploid. Deletionen von PTEN zeigten 17,8% und von 6q15 20,3% der Karzinome. Der veränderte DNA Gehalt und die Deletionen waren assoziiert mit einem hohen Gleason-Score, einem fortgeschrittenen Tumorstadium und positivem Lymphknotenstatus ($p<0,0001$ für alle). Die Wahrscheinlichkeit eines frühen PSA Rezidivs stieg von den diploiden über die tetraploiden zu den aneuploiden Tumoren an ($p<0,0001$ für alle). Allerdings hatten 40% der Patienten mit einem Gleason-Score ≥4+4 und 55% der Patienten mit einem PSA Rezidiv Tumoren mit einem diploiden Status. Durch die Kombination mit der PTEN und/oder 6q Deletion reduzierte sich dieser Anteil auf 21% (Gleason ≥4+4) und 29% (PSA Rezidiv). Die prognostische Relevanz der kombinierten Deletion und Ploidie Analysen („3-level DNA score“) wurde zudem sowohl in einer univariaten als auch in einer multivariaten Analyse geprüft. Das Risiko eines frühen PSA Rezidivs wurde in den diploiden ($p<0,0001$) und auch in den tetraploiden ($p<0,0001$) sowie den aneuploiden ($p=0,0049$) Tumoren durch die Anwesenheit der Deletionen erhöht. In der multivariaten Analyse zeigte der „3-level DNA score“ eine hohe prognostische Relevanz unabhängig von den etablierten prä- und postoperativen Prognoseparametern. Insgesamt zeigen die Ergebnisse der vorliegenden Arbeit, dass die kombinierte Analyse des DNA Gehaltes aus dem Ploidie Status und den spezifischen chromosomalen Veränderungen eine hohe klinische Relevanz beim Prostatakarzinom hat.

2.6 Abstract

Aberrant DNA content has been discussed as a potential prognostic feature in prostate cancer. We analyzed the clinical significance of DNA ploidy in combination with prognostic relevant deletions of PTEN and 6q15 in 3,845 prostate cancers. The DNA status was diploid in 67.8%, tetraploid in 25.6% and aneuploid in 6.8% of tumors. Abnormal DNA content was linked to high Gleason score, advanced tumor stage, and positive nodal stage ($p<0.0001$ each). Deletions of PTEN and 6q15 occurred in 17.8% and 20.3% of tumors, and were linked to high Gleason score and advanced tumor stage ($p<0.0001$ each). The risk of PSA recurrence increased significantly from diploid to tetraploid and from tetraploid to aneuploid DNA status ($p<0.0001$ each). However, 40% of patients with Gleason score ≥4+4 and 55% of patients with PSA recurrence had diploid cancers. This fraction decreased to 21% (Gleason ≥4+4) and 29% (PSA recurrence) if PTEN and/or 6q deletion data were added to ploidy data to identify cancers with an aberrant DNA status. The significance of combining both deletions and ploidy was further demonstrated in a combined recurrence analysis. Here, presence of deletions increased the risk of PSA recurrence in diploid ($p<0.0001$), tetraploid ($p<0.0001$), and aneuploid cancers ($p=0.0049$). Multivariate modeling including preoperatively and postoperatively available parameters identified the “combined DNA status” as a strong independent predictor of poor patient outcome. It is concluded, that a combinatorial DNA content analysis involving general (ploidy) and specific events (deletions) have the potential for clinical utility in prostate cancer.

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differentiation aggravate adverse prognostic parameters in prostate cancer and are characterized by negligible inter-observer variability. World J Urol. 26(3)**:**243-250.

3 Erklärung des Eigenanteils an der Publikation

Eigenanteil

Ich habe die Durchflusszytometrie zur Bestimmung des DNA Gehalts in den Prostatatumoren in Zusammenarbeit mit der Arbeitsgruppe um PD Dr. Ronald Simon am Institut für Pathologie des Universitätsklinikums Hamburg Eppendorf etabliert.

Für die Forschungsarbeit habe ich über 6.000 Prostatakarzinome mittels Durchflusszytometrie analysiert. Von diesen Karzinomen hatten 3.845 ebenfalls einen FISH Status für Deletionen von PTEN und 6q15. Mit diesen Karzinomen führte ich alle statistischen Analysen für die Forschungsarbeit durch. Für die Publikation habe ich die Literaturrecherche betrieben, alle Abbildungen und Tabellen erstellt und eine erste Version des Manuskripts geschrieben. Des Weiteren war ich an der Fertigstellung der finalen Version des Papers beteiligt.

Anteil der Ko-Autoren

- Konzept und Design der Studie: Ronald Simon, Guido Sauter, Thorsten Schlomm
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4 Publikationen und Präsentationen

Publikationen

Clinical Utility of Quantitative Gleason Grading in Prostate Biopsies and Prostatectomy Specimens.

Sauter G, Steurer S, Clauditz TS, Krech T, Wittmer C, Lutz F, Lennartz M, Janssen T, Hakimi N, Simon R, von Petersdorff-Campen M, Jacobsen F, von Loga K, Wilczak W, Minner S, Tsourlakis MC, Chirico V, Haese A, Heinzer H, Beyer B, Graefen M, Michl U, Salomon G, Steuber T, Budäus LH, Hekeler E, Malsy-Mink J, Kutzera S, Fraune C, Göbel C, Huland H, Schlomm T. Eur Urol. 2016 Apr;69(4):592-8. doi: 10.1016/j.eururo.2015.10.029.

The Combination of DNA Ploidy Status and PTEN/6q15 Deletions Provides Strong and Independent Prognostic Information in Prostate Cancer.

Lennartz M, Minner S, Brasch S, Wittmann H, Paterna L, Angermeier K, Öztürk E, Shihada R, Ruge M, Kluth M, Koop C, Wilczak W, Krech T, Lebok P, Wittmer C, Heinzer H, Steuber T, Adam M, Huland H, Graefen M, Haese A, Simon R, Sauter G, Schlomm T. Clin Cancer Res. 2016 Jun 1;22(11):2802-11. doi: 10.1158/1078-0432.CCR-15-0635.

Integrating Tertiary Gleason 5 Patterns into Quantitative Gleason Grading in Prostate Biopsies and Prostatectomy Specimens.

Sauter G, Clauditz T, Steurer S, Wittmer C, Büscheck F, Krech T, Lutz F, Lennartz M, Harms L, Lawrenz L, Möller-Koop C, Simon R, Jacobsen F, Wilczak W, Minner S, Tsourlakis MC, Chirico V, Weidemann S, Haese A, Steuber T, Salomon G, Matiu M, Vettorazzi E, Michl U, Budäus L, Tilki D, Thederan I, Pehrke D, Beyer B, Fraune C, Göbel C, Heinrich M, Juhnke M, Möller K, Bawahab AA, Uhlig R, Huland H, Heinzer H, Graefen M, Schlomm T. Eur Urol. 2017 Jan 20. pii: S0302-2838(17)30030-1. doi: 10.1016/j.eururo.2017.01.015.

Präsentationen

The combination of DNA ploidy status and PTEN/6q15 deletions to provide strong and independent prognostic information in prostate cancer.

Lennartz M, Minner S, Kluth M, Brasch S, Wittmann H, Paterna L, Heinzer H, Simon R, Sauter G, Schlomm T. 2015 ASCO Annual Meeting Chicago. Poster

5 Danksagung

Mein herzlicher Dank gilt vor allem Herrn Prof. Dr. Guido Sauter. Seit dem Tag, an dem wir uns das erste Mal trafen, hatte er immer ein offenes Ohr für meine Belange und unterstützte meine Arbeit durch konstruktive Kritik. Dadurch und durch die Möglichkeit, an dem Institut für Pathologie meine Doktorarbeit machen zu dürfen, konnten wir das Projekt schnell und erfolgreich abschließen.

Außerdem danke ich Herrn PD Dr. Ronald Simon, welcher stets ein sehr freundlicher, zugewandter und hilfreicher Rechenpartner war. Danke auch an Frau Dr. Martina Kluth für die hilfreichen Erklärungen und perfekten FISH Daten.

Dem Team aus Katja Angermeier, Rami Shihada, Mingu Ruge, Sophie Brasch, Hilko Wittmann und Leonard Paterna danke ich für die gute Zusammenarbeit und die vielen gemeinsamen arbeitsreichen Stunden im Labor.

Zum Schluss möchte ich noch meiner Familie danken, meiner Mutter Gisela Lennartz, meinen Schwestern Caroline und Christine Lennartz, die mir immer sehr geduldige Zuhörerinnen waren.

Ein ganz besonderer Dank gilt meinem Vater, Herrn Dr. Ernst Lennartz jun. und meinem Großvater, Herrn Dr. Ernst Lennartz sen., ohne die ich niemals zur Medizin gekommen wäre.

6 Lebenslauf

Entfällt aus datenschutzrechtlichen Gründen.

7 Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.

Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: .....