

Minimal Residual Disease Defines the Risk and Time to Biochemical Failure in Patients with Pt2 and Pt3a Prostate Cancer Treated With Radical Prostatectomy: An Observational Prospective Study

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Purpose: To compare Gleason score (GS), pathological stage, minimal residual disease (MRD) and outcome after prostatectomy radical for prostate cancer.

Patients and Methods: 290/357 men with GS 6 or 7 and pT2 or pT3a disease treated with radical prostatectomy participated. Blood and bone marrow were obtained one month after surgery. Circulating prostate cells (CPCs) were detected using differential gel centrifugation and immunocytochemistry with anti-PSA, micro-metastasis was detected using immunocytochemistry with anti-PSA. Biochemical failure free survival (BFFS) and restricted mean survival times (RMST) were calculated according to GS and stage. MRD was classified as negative, patients only positive for micro-metastasis and patients positive for CPCs; BFFS and RMST were calculated according to MRD sub-type.

Results: GS7 (HR 3.03) and pT3a (HR 3.68) cancers were associated with a higher failure rate, shorter time to failure and associated with CPC positive MRD ($p < 0.001$), while G6 and pT2 with MRD negative disease ($p < 0.001$). Men with CPC (+) MRD were at high risk of early treatment failure; 15% BFFS at 10 years, RMST 3.0 years. Men positive for only micro-metastasis were at risk of late failure, 50% BFFS at 10 years, RMST 8.0 years compared with MRD negative patients; 80% BFFS at 10 years, RMST 9.0 years.

Conclusion: The sub-type of MRD identifies Gleason 6 pT2 patients with a poor prognosis and Gleason 7 pT3a patients with a good prognosis and could be used to classify men according to personal risk characteristics for the use of adjuvant treatment.

Keywords: biochemical failure; circulating prostate cells; micro-metastasis; minimal residual disease; prostate cancer

INTRODUCTION

After radical prostatectomy for prostate cancer, biochemical failure occurs in 15-40% of patients, and is associated with the surgical Gleason score and pathological stage. Extra prostatic extension (EPE) of the tumour is an adverse prognostic risk factor, defining pT2 from pT3a disease⁽¹⁾ and therefore between organ confined and specimen confined disease. It has been suggested that pT3a patients should be classified into focal capsular penetration and non-focal penetration as biochemical failure free survivals are different^(2,3); however, all cases of EPE are classified as pT3a disease in the American Joint Committee on Cancer, seventh edition staging manual⁽⁴⁾.

In both pT2 (organ confined) and pT3a margin negative (specimen confined) all the tumor has been removed at surgery, however there is a difference in prognosis. The simplest explication would be an erroneous pathological classification, which may explain some cases but

not the majority⁽⁵⁾. The second possibility is that cancer have disseminated beyond the prostate and thus outside the surgical field at the time of operation. The residual tumor cells that remain after local or systemic therapy in patients with no signs of clinical disease is termed minimal residual disease (MRD). The presence of MRD will depend on the characteristics of the primary tumor and the ability of cancer cells to disseminate, implant and survive in distant tissues. Two types of MRD have been described⁽⁶⁾, in patients with circulating prostate cells or tumor cells (CPCs) detected in the blood there is an increased frequency of early treatment failure^(7,8). Whereas in patients with tumor cells detected only in bone marrow samples there is an association with late failure^(7,9).

We present a prospective, observational long-term follow up study of the effect of the sub-types of MRD on the outcome of radical prostatectomy monotherapy in

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Table 1. Clinical and pathological findings according pathological stage.

Variable	pT2 n=192	pT3a margin negative n=78	pT3a margin positive n=20	P-value (two tail)
Age, years mean \pm SD	65.0 \pm 8.2	66.2 \pm 9.0	67.0 \pm 8.8	03708 ^a
PSA, ng/mL Median; IQR	5.21; 1.68	6.37; 5.07	6.66; 6.59	< 0.001 ^a
Gleason score greater than 6 n (%)	25 (13%)	39 (50%)	12 (60%)	< 0.001 ^b
Biochemical failure n (%)	49 (26%)	53 (68%)	17 (85%)	< 0.001 ^b

Abbreviations: IQR= interquartile range; PSA= serum total prostate specific antigen; a Kruskal-Wallis test; b Pearson's chi-squared test.

men with Gleason 6 and 7 and pT2 and pT3 margin negative prostate cancer. A small group of men with EPE and positive surgical margins who did not undergo adjuvant therapy was used as a control group with adverse prognostic features.

PATIENTS AND METHODS

Study population

A single center, prospective observational study of men who underwent radical prostatectomy as mono-therapy for prostate cancer between 2000 and 2008, and the acquisition of follow up data was concluded in December 2017. Pre-treatment PSA and age at surgery were registered; The pathological study of the surgical piece was performed by dedicated genitourinary pathologists according to the Gleason system (pre-2005) and the pathological stage was defined according to the Partin criteria⁽¹⁰⁾. Extra-capsular extension was defined as a specimen with cancer cells in contact with the prostatic capsule and classified as positive or negative, sub-division into focal and non-focal capsular penetration was not used. Positive surgical margins were defined as one with cancer cells in contact with the inked surface of the specimen.

Patients were classified as pT2 (organ confined), pT3a negative surgical margins (specimen confined)

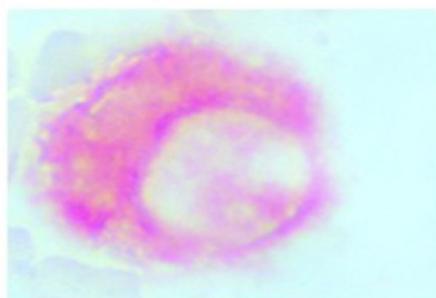
and pT3a positive margin. All men had a nadir PSA post-surgery of < 0.01ng/mL.

Exclusion Criteria: Previous treatment or consideration for treatment with androgen blockade or radiotherapy; Infiltration of the seminal vesicles and/or regional lymph nodes with cancer or a positive bone scan; Men with Gleason 8 and 9 cancer.

Serial total PSA levels were monitored three monthly for the first year and six monthly thereafter. Biochemical failure was defined as a serum PSA > 0.2ng/mL on two separate occasions. The biochemical failure free survival time was defined as the time from surgery to the time of a post-surgery PSA of > 0.20ng/mL or to the time of the last follow up. MRD detection was independently evaluated with the evaluators being blinded to the clinical details.

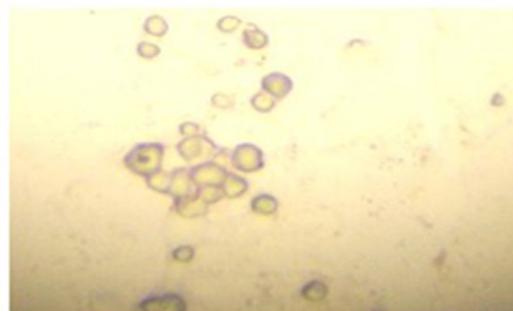
Procedures

a) Detection of secondary circulating prostate cells: one-month post-surgery an 8mL venous blood sample was taken and mononuclear cells were obtained by differential centrifugation using Histopaque 1,077 (Sigma-Aldrich, USA). The cells were used to make slides (silanized, DAKO, USA), air dried for 24 hours and fixed in a solution of 70% ethanol, 5% formaldehyde, and 25% phosphate buffered saline (PBS) pH 7.4. Immunocytochemistry: CPCs were detected using a



Circulating prostate cell

PSA (red) membrane CD45
negative



Leukocytes (negative PSA

membrane CD45 positive (brown)

Figure 1. Circulating tumour cell and leukocyte.

Table 2. Survival proportion and restricted mean survival Time (RMST) at 10 years for biochemical failure observed from use curves Kaplan-Meier, on 290 Men Treated by Radical Prostatectomy for Prostate Cancer.

Variable	Survival proportion Kaplan-Meier % (95% CI)	RMST Kaplan-Meier a years (95% CI)	
Group	pT2 n=192	Gleason score 6 n=167 72.0 (62.5 to 79.6)	9.2 (8.9 to 9.5)
		Gleason score 7 n=25 26.1 (5.2 to 54.4)	6.5 (5.1 to 8.0)
	pT3a margin negative n=78	Gleason score 6 n=39 28.1 (10.6 to 48.7)	6.7 (5.7 to 7.8)
		Gleason score 7 n=39 12.1 ^b (3.5 to 26.2)	4.0 (3.2 to 4.9)
	pT3a margin positive n=20	Gleason score 6 n=8 18.8 ^c (1.1 to 53.5)	3.7 (2.9 to 4.4)
		Gleason score 7 n=12 16.7 ^d (2.7 to 41.3)	2.8 (2.1 to 3.5)

Abbreviations: %=percentage; CI= confidence interval; a The RMST is the area under the Kaplan-Meier survival curve, determined by the numerical integration; b at 9.08 years last time not censored observed; c at 5.08 years last time not censored observed; d at 4.33 years last time not censored

monoclonal antibody directed against PSA, clone 28A4 (Novocastro Laboratory, UK), and identified using an alkaline phosphatase-anti alkaline phosphatase based system (LSAB2, DAKO, USA), with new fuchsin as the chromogen. Samples positive for PSA staining cells were incubated with anti-CD45 clone 2B11 + PD7/26 (DAKO, USA) and cells identified with a peroxidase based system (LSAB2, DAKO, USA) with DAB (3,3 diaminobenzidine tetrahydrochloride) as the chromogen. A CPC was defined as expressing PSA but not CD45 and a leukocyte as expressing CD45 but not PSA (**Figure 1**)⁽¹¹⁾. A test was considered positive when at least 1 cell/8mL of blood was detected.

b) Bone marrow biopsy: it has been reported that prostate tumor cells detected in bone marrow aspirates are phenotypically different than those prostate cells detected in bone marrow biopsies and may not represent “true” micro-metastasis but rather cells circulating within the bone marrow⁽¹²⁾. For this reason, bone marrow biopsy “touch preps” were used as the sample to test for micro-metastasis.

Patients were sedated with intravenous midazolam and a bone marrow biopsy, using local anesthetic, was taken from the posterior superior iliac crest one month after surgery. Four “touch preps” using salinized slides (DAKO, USA) were prepared and processed as described for CPCs, a micro-metastasis was defined as

cells staining positive for PSA and negative for CD45. Evaluations: Patients were divided into three groups: pT2, pT3a (margin negative), pT3a (margin positive) and further subdivided into; Group A negative for both CPCs and micro-metastasis patients (without evidence of MRD); Group B CPC negative, micro-metastasis positive; Group C CPC positive with or without bone marrow micro-metastasis detected.

Study end point: The primary study end point was the presence of biochemical failure and secondary end point mean time to failure after primary treatment.

Statistical analysis

The analysis was performed using the program Stata (Stata/SE 15.0 for Windows, Copyright 1985-2017 StataCorp LLC). Descriptive statistics were used to describe the results. The variables pT2, PT3a margin negative and pT3a margin positive) were compared for age, total serum PSA, pathological Gleason score and MRD (Group A, B and C). The Kruskal–Wallis test was used to test whether samples originate from the same distribution. A p value < .05 was taken to signify statistical significance and all tests were two tailed⁽¹³⁾.

For the whole cohort, a nonparametric survival analysis⁽¹³⁾ was performed to establish the survival proportion of Kaplan-Meier (KM) and restricted mean survival time (RMST) for the biochemical failure during the ten-year follow-up period⁽¹⁴⁾. The RMST establishes the expect-

Table 3. Minimal residual disease according to pathological stage:

Characteristic	pT2 n=192	pT3a margin negative n=78	pT3a margin positive n=20	P-value two tail
CPC (-) and mM (-) n (%)	114 (60%)	22 (28%)	3 (15%)	< 0.001 ^a
CPC (-) and mM (+) n (%)	39 (20%)	11 (14%)	4 (20%)	.487
CPC (+) n (%)	39 (20%)	45 (58%)	13 (65%)	< 0.001 ^a

Abbreviations: CPC, circulating prostate or tumor cells; mM, micro-metastasis
a Pearson's chi-square test with Marascuilo procedure for post hoc analysis pT2 versus pT3a margin negative and pT2 versus pT3a margin positive.

Table 4. Survival proportion and restricted mean survival Time (RMST) at 10 years for biochemical failure observed (Kaplan-Meier) and Predicted (Flexible Parameter Model) according to the following classification criteria: a) EPE, b) MRD and c) Gleason score greater than 6; on 290 Men Treated by Radical Prostatectomy for Prostate Cancer

Classification Criteria			Observed survival		Predicted RMST	Predicted
Stage	MRD	Gleason Score	Kaplan-Meier % (95% CI)	FPM % (95% CI)	Kaplan-Meier a years (95% CI) (95% CI)	FPM years (95% CI)
pT2 n=192	CPC negative / mM negative	6 n=108	97.8 (91.3-99.5)	90.5 1	9.9 (9.7-10.0)	9.7 (9.5-9.9)
		7 n=6	100 ^b	82.4 (65.1-91.7)	5.6 ^j 1.9 (1.2-3.0)	9.5 (9.1-9.9)
	CPC negative / mM positive	6 n=31	64.1 (38.7-81.2)	72.1 3.3 (57.5-82.5)	9.4 (9.0-9.8) (1.4-7.4)	9.2 (8.7-9.6)
		7 n=8	75.0 (12.8-96.1)	53.2 6.3 (29.4-72.2)	9.8 (9.6-10.1) (2.5-15.8)	8.5 (7.6-8.3)
	CPC positive	6 n=28	16.6 (5.3-33.3)	24.1 (12.0-38.5)	7.0 9.5 (6.0-7.9) (3.7-24.7)	6.3 (5.5-7.2)
		7 n=11	9.01 ^c (0.5-33.3)	6.4 (0.9-20.0)	2.7 18.4 (1.9 to 3.5) (6.5-52.1)	4.7 (3.5-5.9)
pT3a margin negative n=78	CPC negative / mM negative	6 n=13	88.9 ^d (43.30-98.4)	81.2 2.1 (63.4-90.0)	8.5 (7.5 to 9.6) (1.3-3.3)	9.4 (9.0-9.9)
		7 n=9	45.7 (6.9-79.5)	66.9 4.0 (41.2-83.3)	6.8 (4.2 to 9.4) (2.4-6.6)	9.0 (8.3-9.7)
	CPC negative / mM positive	6 n=11	33.3 (7.8-62.3)	50.7 6.8 (27.7-70.0)	8.6 (7.7-9.5) (2.7-17.2)	8.4 (7.5-9.2)
		7 n=0	Not observed	26.9 (8.6-49.6)	Not observed 13.1 (5.2-33.2)	7.3 (6.1-8.5)
	CPC positive	6 n=15	10.0 (0.8-33.5)	5.2 (0.9-15.5)	3.9 19.8 (2.5-5.3) (7.2-54.5)	4.5 (3.5-5.6)
		7 n=30	5.0 (0.5-18.9)	0.3 (0.1-2.3)	3.4 38.2 (2.6 to 4.2) (13.8-105.5)	3.1 (2.6-3.7)
pT3a margin positive n=20	CPC negative / mM negative	6 n=1	Not determined	67.0 4.0 (36.9-85.1)	Not determined (8.2-9.8)	9.0 (8.2-9.8)
		7 n=2	50.0 ^e (0.6 to 91.0)	46.1 7.7 (14.1-73.4)	2.6 (2.0 to 3.3) (3.9-15.2)	8.2 (6.9-9.5)
	CPC negative / mM positive	6 n=2	100% ^f	27.1 (5.8-55.0)	5.8 ^j 13.0 (4.7-36.2)	7.3 (5.9-8.8)
		7 n=2	50% ^g (0.6-91.0)	8.0 (0.4-31.7)	3.5 25.2 (2.2-4.7) (9-70.6)	6.0 (4.3-7.7)
	CPC positive	6 n=5	20.00 ^h (0.8-58.2)	0.3 (0.1-5.1)	3.1 38.1 (2.3-4.0) (12.5-115.7)	3.1 (2.2-4.1)
		7 n=8	12.5 (0.7-42.3)	0.1 (0-0.3)	2.6 73.5 (1.7-3.5) (24.0-226.0)	2.3 (1.8-2.8)

Abbreviations: MRD, minimal residual disease, CPC, circulating prostate cells; mM, micro-metastasis, %,percentage; CI, confidence interval; FPM, flexible parameter model.

^aThe RMST is the area under the Kaplan-Meier survival curve, determined by the numerical integration; ^b at time 5.58 years not observed events; ^c 5.76 years last time not censored observed; ^d at 9.08 years last time not censored observed; ^e at 2.17 years last time not censored observed; ^f at time 5.08 years not observed events; ^g at 4.33 years last time not censored observed; ^h 3.41 years last time not censored observed; ⁱ 3.33 years last time not censored observed; ^j confidence interval not determined, there are no patients with biochemical failure. FPM=flexible parameter model; HR=hazard ratio



Figure 2. Bone marrow sample positive and negative for PSA expressing micro-metastasis.

ed time from surgery to biochemical failure during the total observation period⁽¹⁵⁾. Patients were classified according: a) pathological stage), b) MRD sub-type, and c) Gleason score > 6 and the KM and RMST determined, and the results compared using the log-rank test. A flexible parametric survival model (FP model) was used to predict the survival proportion, RMST and the hazard ratio as there was no compliance with the proportional risk assumption (Cox model)⁽¹⁶⁾. The discrimination of a prognostic model reflects its ability to distinguish between patient outcomes, for which the Harrell's C discrimination index was used⁽¹⁷⁾. From the FP model for biochemical failure to ten years, the RMST, hazard ratio and survival proportion were established according to the following classification criteria a) pathological stage, b) MRD and c) Gleason score > 6. Ethical considerations: The study was approved by the local ethics committee and in complete agreement with the Declaration of Helsinki. All patients provided written informed consent.

RESULTS

357 men underwent radical prostatectomy; 67 fulfilled exclusion criteria leaving 295 men in the study group. The median follow up time was 6.7 years (IQR: 5.9 years; range 1-15 years). The mean age was 65 ± 8.5 years and a median PSA of 6.9 ng/mL (IQR 2.8). **Table 1** shows the findings according to pathological stage of the patients. The serum PSA at the time of diagnosis,

frequency of Gleason score 7 and frequency of biochemical failure were significantly higher with increasing pathological stage.

Kaplan-Meier survival (KM) curves and RMST time to biochemical failure according to pathological stage and Gleason score:

The KM proportion for biochemical failure free survival at ten years of follow-up for the whole cohort was 7.6 years (95% CI: 7.2 to 8.0 years). The biochemical failure free survival and time to failure significantly decreased with increasing pathological stage and a higher Gleason score ($p < 0.01$ log rank test) (**Figure 3**). Compared to baseline risk of failure (Gleason 6 pT2), univariate hazard ratios (HRs) were: Gleason score 7 HR 3.03 (IC: 1.99 -4.60; $p < 0.01$), pT3a margin negative HR 3.68 (95% IC: 2.37-5.71; $p < 0.01$) and pT3a margin positive HR 7.63 (95% IC: 4.03- 14.44; $p < 0.01$). Multi-variate HR were Gleason score 7 2.12 (95% CI: 1.76-2.57), pT3a margin negative 2.31 (95% CI: 1.94-2.79) and pT3a margin positive 5.32 (95% CI: 4.16-8.73) respectively.

There was agreement between the predicted survival (according to the final model of Cox) versus observed survival (model Kaplan-Meier) (Figure 3) with a Harrell's C discrimination index of 0.77 (95% IC: 0.74 to 0.81), considered as a good fit.

In summary, the results are consistent with the known risk factors for treatment failure, higher Gleason score and pathological stage (organ confined and speci-

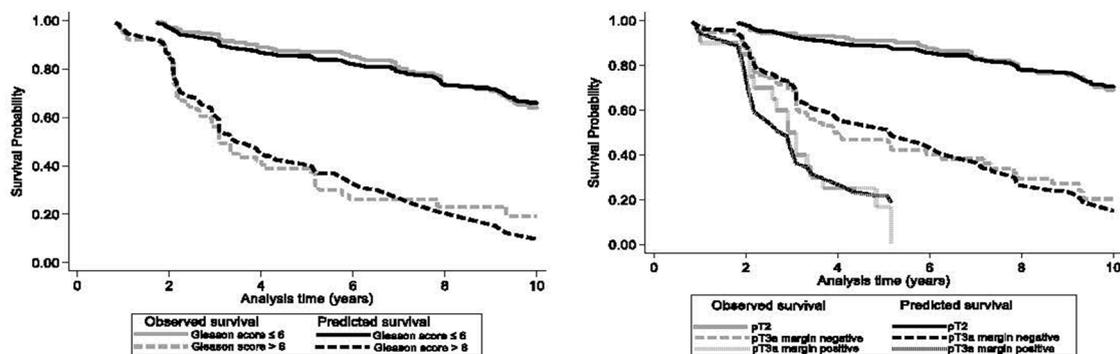


Figure 3. Comparing predicted (Cox final model) versus observed survival (Kaplan-Meier Survival) by Gleason score 7 and Pathological Stage, pT2, pT3a margin negative and pT3a margin positive for biochemical failure free progression at 10 years on 290 subjects Treated with Radical Prostatectomy for Prostate Cancer

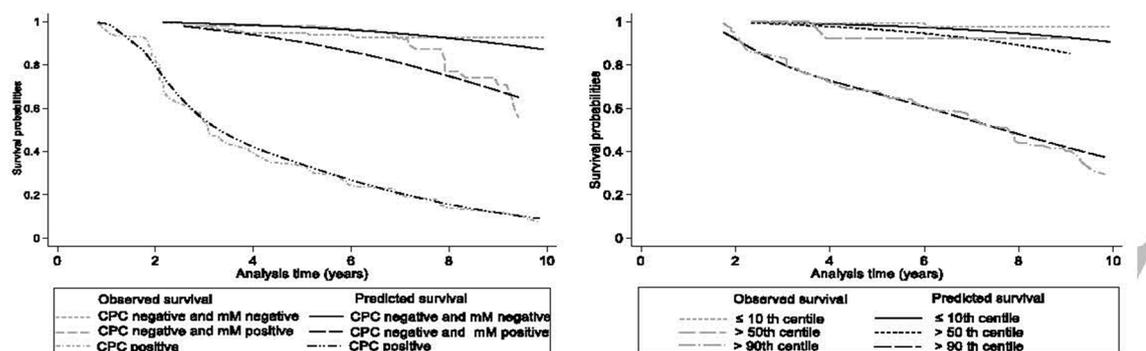


Figure 4. Kaplan Meier survival curves according to minimal residual disease.

Abbreviations: CPC, circulating prostate cells, mM, micro-metastasis, CI, confidence interval.

men-confined cancer) and represents a typical prostate cancer population.

Kaplan-Meier survival curves and RMST time to biochemical failure according to pathological stage and Gleason score and minimal residual disease:

For each pathological stage the minimal residual disease was assessed, (Table 2), as may be predicted, MRD negative patients were significantly more frequently found in patients with pT2 disease, those with CPC positive MRD were significantly more frequently found in pT3a disease. However, the frequency of CPC negative MRD was not significantly associated with pathological stage.

Classifying the patients according to MRD subtype, and where the number of patients permits this analysis, the presence of CPCs signified a significantly poorer biochemical failure free survival and shorter time to failure, and associated with increasing Gleason score and pathological stage. However, patients MRD negative, independent of pathological stage had better biochemical failure free survival and longer time to treatment failure, even those patients with pT3a margin positive (Table 3). Patients with micro-metastasis positive MRD (Group B) had a different pattern of failure, although with a lower biochemical failure free survival the time to failure was significantly longer than those patients CPC (+). Those patients MRD micro-metastasis positive have a four to five years of excellent prognosis but afterwards there is increasing late failure, in other words the risk of failure was not constant with time. (Figure 4).

The non-parametric comparison of survival by groups: a) pathological stage, b) MRD and c) Gleason score 7 showed differences with statistical significance (p value < 0.01 for log-rank test). For the whole cohort, the Kaplan-Meier survival curves for the three MRD subgroups were not parallel, which differed from the two survival curves based on Gleason score and pathological stage alone. Testing for a cohort interaction between Gleason score, pathological stage and MRD category showed a significant difference ($p < 0.05$), which implies that the risk of biochemical failure is not constant, and changes with time.

The flexible parametric survival model using the following coefficients of variables: a) pT3a margin negative: 0.73 (p -value: 0.003), b) pT3a margin positive: 1.39 (p -value < 0.0001) c) CPC negative/micro-metastasis

positive: 1.18 (p -value: 0.005), d) CPC positive: 3.16 (p -value < 0.0001) and e) Gleason score 7: 0.66 (p -value: 0.003). This FP final model considered subjects with: pT2, CPC: negative/micro-metastasis negative and Gleason score 6 as the group basal.

There was agreement when comparing the predicted FP model with the observed survival (Kaplan-Meier Survival) with a Harrell's C discrimination index of 0.91 showing an excellent fit between observed and predicted models. (Figure 4).

The predicted survival proportions, RMSTs and hazard ratios (group basal: subjects with: pT2, MRD negative and Gleason score 6) for the FP final model according to pathological stage, MRD and Gleason score are shown in Table 3.

As can be seen the HR when using Gleason score and pathological stage alone;

HR Gleason 7 3.03, pT3a margin negative 3.68 and pT3a margin positive gives a very different risk classification. As can be seen from Table 3 patients with pT3a margin negative G6 tumours have a better-predicted outcome than pT2 Gleason 6 patients with only bone marrow micro-metastasis. Similarly, patients with pT3a margin negative G7 tumours and negative for MRD had a better-predicted outcome than pT2 Gleason 6 patients with CPCs detected. Sub-classifying the patients using MRD, Gleason score and pathological stage suggests that not all Gleason 6 or 7 and not all pT2 and pT3a cancers have the same risk of treatment failure.

DISCUSSION

Classification of patients following radical prostatectomy according to the risk of treatment failure is important in the management of prostate cancer. The identification of patients who may or may not benefit from adjuvant therapy, such as radiotherapy or androgen deprivation therapy is essential. That Gleason 7 tumors or those patients with higher pathological stage cancers had a higher risk of treatment failure, as seen in this study is not surprising. The study has its limitations; it was started in 2000 and we maintained the old Gleason 7 score rather than 3 + 4 and 4 + 3⁽¹⁸⁾ accepting that some patients classified as Gleason 7 would be classified as Gleason 3 + 4 and that some Gleason 7 would be Gleason 4 + 3. Secondly, the small number of patients in some of the subgroups limits the number

of conclusions, and this can be seen in the form of the wide confidence intervals. However, the fact that statistical significant differences were detected implies real differences between patient populations. A multi-centre study with a much larger number of patients would overcome this limitation and essential before establishing concrete conclusions.

The few patients with pT3a margin positive cancer were included as a bad prognosis group; only 20/82 (24%) of pT3a margin positive patients did not undergo adjuvant treatment, this group of patients were treated between 2000 and 2004. In the recruitment stage patients with pT3a margin negative disease were observed after radical prostatectomy; studies published covering this era, reported acceptable cancer control and radiation therapy did not impact the appearance of metastasis or survival although it did delay time to biochemical failure and improve local control⁽¹⁹⁾.

PSA could be considered as a marker for minimal residual disease; post radical prostatectomy a level of over 0.2ng/ml is used to define treatment failure and to consider additional treatment. At these levels the patient normally does not have clinical symptoms, however the PSA level does not determine whether there is local or systemic residual disease. In this context, CPCs do not differentiate between local and systemic disease, whereas micro-metastasis in the bone marrow represent systemic disease. The use of bone marrow biopsies to evaluate the presence of micro-metastasis is more invasive than the use of blood tests. However, a three-year annual survey reported only 0.07% of patients reported side effects⁽²⁰⁾ and significantly less than those occurring after prostate biopsy⁽²¹⁾.

CPC detection is method dependent; methods using anti-EpCAM (Epithelial Cell Adhesion Molecule) such as CellSearch[®] detected CPCs in only 25% of men with localized cancer and failed to distinguish between healthy controls and men with prostate cancer⁽²²⁾. In contrast, using an anti-Ber-4 and telomerase based method; CPCs were detected in 80% of men with localized prostate cancer⁽²³⁾. Similarly using a size-based filtration method, CPCs were detected in 34% of men compared with only 18.6% using the CellSearch system⁽²⁴⁾. We used a simple differential gel centrifugation method to enrich CPCs and standard immunocytochemistry to detect them; the limitation of this method is the lack of external validation. This method used to detect CPCs has been internally validated at our centre, we acknowledge that there is variability in inter and intra observer evaluation, however used as a positive/negative test the results show a clinical utility.

The key points of the results of this study are the following: a) stratifying patients according to the subtypes of MRD goes beyond Gleason score and pathological stage. Although for the three types of MRD the outcome of Gleason 7 patients is worse than Gleason 6 patients, and similarly patients with pT3a margin negative cancer worse than those with pT2 cancer, not all Gleason 6, 7 and pT2 and pT3a behave in a similar fashion. This implies that the worse prognosis for Gleason 7 and pT3a patients in general is due to a higher frequency of MRD CPC positive patients.

Independent of the subtype of MRD, Gleason 7 patients had a worse prognosis and shorter time to treatment failure. The implication is that Gleason 7 cancer cells are inherently more aggressive than Gleason 6 tumour

cells. However independent of the mechanism of tumour dissemination, there is a subgroup of Gleason 7 patients MRD negative with an excellent prognosis. More recently, a 30 gene mRNA expression signature improved predictions of indolent and lethal outcome of men with Gleason 7 prostate cancer, independent of whether the Gleason score was 3 + 4 or 4 + 3, for both types there were indolent and lethal variants⁽²⁵⁾. The differing sub-types of MRD represent different biological potentials of cancer cells and may help to differentiate between indolent and lethal forms of cancer, even in patients with the same Gleason score and pathological stage. Morphological analysis of the cancer does not assess the biological potential of the tumour.

b) the time kinetics of treatment failure differs between Gleason 6 and Gleason 7 tumours. In Gleason 7 the risk of early failure is significantly higher than in Gleason 6 cancer. However, by ten years post prostatectomy the risk of future failure had decreased to be the same as MRD negative patients. In contrast with Gleason 6 cancer there was a constant failure risk. This suggests that the biological characteristics and behaviour of Gleason 6 and 7 tumour cells are different. This pattern has been reported previously, patients with adverse pathological findings at surgery, Gleason score ≥ 7 , higher pre-surgery PSA levels had a high initial risk of failure which rapidly decreased to almost zero, while those with low Gleason scores and T2 disease had fairly constant progression rates for up to ten years⁽²⁶⁾.

Patients CPC positive had a significantly higher biochemical failure rate and shorter time to failure suggesting a more aggressive form of MRD. Although this simple system of MRD classification allows risk stratification of prostate cancer patients, the future molecular characterisation of these tumour cells may allow for individualized treatments that are more effective, potentially reveal targets to prevent relapse and avoid overtreatment of patients with indolent MRD. There is a clinical need to delineate the patients with indolent MRD as they present a different biological and thus clinical process, which may require different treatment strategies.

CONCLUSIONS

Within the limitations of the study, the results suggest that the differences in treatment failure between Gleason 6 and Gleason 7 and pT2 and pT3a cancer patients can be explained by the phenotypic characters of the tumour cells, which give rise to differing patterns of MRD and in the different clinical patterns of relapse. Patients MRD negative or an "indolent" pattern may thus avoid overtreatment whereas those with CPC positive disease and a high risk of early relapse may benefit from early adjuvant treatment. This would need to be confirmed with larger scale randomized long-term trials.

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CONFLICT OF INTEREST

Dr Murray reports having received consultancy fees from Viatar CTC Solutions.

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