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Original Article

The Epstein criteria predict for organ-confined prostate cancer but not for minimal residual disease and outcome after radical prostatectomy

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ABSTRACT

Objective: The Epstein criteria (EC) used to select men for active surveillance do not predict biologically insignificant diseases. Minimal residual disease (MRD) is an undetected microscopic disease that remains after radical prostectomy (RP) and is a biological classification associated with the risk of treatment failure. Subtypes of MRD, the 10-year biochemical failure free survival (BFFS), and restricted mean biochemical failure free survival time (RMST) were determined and compared in EC patients treated with RP.

Material and methods: Consecutive patients with a Gleason 6 biopsy treated at a single institution were divided into those who did or did not fulfill the EC and underwent RP. One month after surgery, samples were taken for the detection of circulating prostate cells (CPCs) and bone marrow micrometastasis. MRD was defined as negative for both CPCs and micrometastasis; patients were positive for micrometastasis and CPCs separately. BFFS for up to 10 years and RMST were determined for each MRD subgroup for EC positive and negative patients.

Results: EC positive men (137/426) were significantly older ($p<0.05$) and had negative MRD, pT2 (pathologically organ confined) disease (<0.02), and lower frequency of upgrading ($p<0.02$). Of the EC positive men, 71% were MRD negative, 13% were positive for micrometastasis, and 16% were positive for CPCs with respective 10-year BFFS of 99%, 89%, and 21% (<0.001) (hazard ratio: 1.00, 1.76, 4.03, respectively) with no signficant differences between the 10-year BFFS or RMST for MRD subgroups for EC positive and negative patients.

Conclusions: EC predict pT2, MRD negative disease; however, 29% are MRD positive with a high risk of treatment failure.

Keywords: Biochemical failure free survival; epstein criteria; minimal residual disease; prostate cancer.

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Introduction

Active surveillance is an increasingly used treatment option for low-grade, small volume prostate cancer.[1] It is thought that low-grade cancers do not need immediate treatment and can be identified at the time of diagnosis on the basis of the pathological analysis of the prostate biopsy, [2] and cancers that progress while under observation may still be cured.[2]

Criteria for identifying potentially biologically insignificant tumors have been defined,[3] predicting a high likelihood of organ confined cancer and absence of biochemical failure for up to 5 years, but these Epstein criteria (EC) do not predict the presence of biologically significant disease.[4,5]

Tumor dissemination beyond the surgical field and not removed by radical prostatectomy (RP) will determine patient outcome. In pathologically organ-confined cancer treated by RP, there was a reported 4-32% relapse rate.[6,7] The inference is that tumor cells would have disseminated before surgery, implanted in distant tissues, and after a variable time period would have proliferated to cause biochemical failure. These undetected micrometastases are termed as minimal residual disease (MRD). MRD has been classified into three sub-types: two positive sub-types-those men with tumor

cells only detected in bone marrow samples and those men with tumor cells detected in the circulation [Circulating Prostate Cells (CPCs)] independent of whether bone marrow micrometastasis are present or not-and one negative subtype that includes those men who are negative for MRD.[8] The subtype of MRD determines the risk of and timing of treatment failure, is a biological classification, and explains in part the clinical heterogeneity of Gleason 6 prostate cancer.

The aim of this study was to determine in patients with a Gleason 6 prostate biopsy and treated with RP the patterns of MRD-in those men who either fulfill or do not fulfill the EC for active surveillance. The 10-year biochemical failure free survival (BFFS) and restricted mean biochemical failure free survival time (RMST) were determined and compared between MRD sub-types for patients either fulfilling or not fulfilling the EC.

Material and methods

This was a prospective study of consecutive patients with a Gleason 6 prostate biopsy and treated with RP at a single institution between January 2000 and December 2012. The patients were divided into two groups, those fulfilling the EC-defined as a prostate specific antigen (PSA) density <0.15 ng/mL, biopsy Gleason score ≤6, the presence of cancer in ≤2 of the 12 cores, and no more than 50% tumor infiltration in any core-and those not fulfilling the criteria. RP specimens were evaluated for Gleason score, extra-prostatic extension, lymph node, and seminal vesicle infiltration and margin status. Pathologically organ-confined pT2 disease was defined as the absense of extra-prostatic extension, negative infiltration of seminal vesicles, lymph nodes, and surgical margins.

Main Points:

- The Epstein criteria (EC) are used to select patients for active surveillance, predicting organ-confined prostate cancer but not biologically insignificant cancer. Men (24%) fulfilling the EC will be upgraded or upstaged after radical prostatectomy (RP).
- Minimal residual disease (MRD) classifies risk of treatment failure according to the biological characteristics of tumor cells, identifying patients who are at a risk of early and late treatment failure as well as those with an excellent prognosis.
- We report that men with EC Gleason 6 prostate cancer detected on biopsy and who choose RP had a high frequency of pT2 disease (81% versus 73%), MRD negative disease (71% versus 55%). These patients have a very low likelihood of biochemical failure 10 years after surgery (1%), implying long term cure. However, 20% of the total EC population were upgraded or upstaged, furthermore 29% had MRD, with 16% positive for circulating prostate cells, and a biochemical failure free survival of only 21% at 10 years.
- The use of the MRD classification identifies patients with high risk of early failure and those at risk for late failure independent of the EC.

Detection of MRD

One month after the surgery, blood and bone marrow samples were taken for the detection of MRD. MRD was independently evaluated with the evaluator being blinded to the clinical details.

Detection of CPCs

Venous blood (8 mL) was collected in ethylenediaminetetraacetic acid (Vacutainer®; Becton-Dickinson, Franklin Lakes, NJ, USA) and processed within 48 hours. Mononuclear cells were obtained by differential centrifugation using Histopaque 1,077 (Sigma-Aldrich, St Louis, MO, USA) and were washed and re-suspended in a 100 μL aliquot of autologous plasma. Aliquots of 25 μL were used to make slides (silanized, DAKO, Carpinteria, CA, USA). These slides were dried in air for 24 hours and fixed in a solution of 70% ethanol, 5% formaldehyde, and 25% phosphate buffered saline (PBS) (pH 7.4) for 5 minutes and finally washed three times in PBS pH 7.4.

Immunocytochemistry

CPCs were detected using a monoclonal antibody directed against PSA, clone 28A4 (Novocastro Laboratory, Newcastle, UK), and identified using an alkaline phosphatase-anti-alkaline phosphatase-based system (LSAB2, DAKO, USA) with new fuchsin as the chromogen. Positive samples underwent a second process with anti-CD45 clone 2B11 + PD7/26 (DAKO, USA) and were identified with a peroxidase-based system (LSAB2, DAKO, USA) with DAB (3,3 diaminobenzidine tetrahydrochloride) as the chromogen. CPCs were defined according to the criteria of International Society of Hemotherapy and Genetic Engineering[9] as cells that expressed PSA but not CD45; a leukocyte was defined as a cell that did not express PSA but expressed CD45 (Figures 1 and 2). A test was considered positive for CPCs when at least one cell per 8 mL of blood was detected.

Detection of bone marrow micrometastasis

Prostate tumor cells detected in bone marrow aspirates have been reported to be phenotypically different than those detected in bone marrow biopsies and may not represent true micrometastasis but rather cells circulating within the bone marrow.[10] For this reason, bone marrow biopsy touch preps were used as the sample to test for micrometastasis. The biopsy was taken from the posterior superior iliac crest to prepare four touch preps using sialinized slides (DAKO, USA) and processed as described for CPCs. A micrometastasis was defined as cells staining positive for PSA and negative for CD45 (Figures 3 and 4).

Slides for both CPCs and micrometastasis were analysed manually, stained cells were photographed using a digital camera, and from the images it was determined whether CPCs and/or micrometastasis were present.

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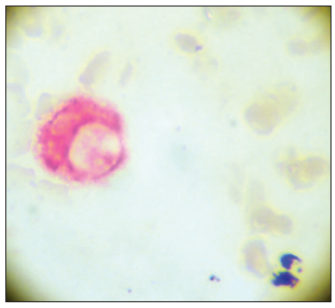

Figure 1. Circulating prostate cell expressing PSA (red) but not membrane CD45
PSA: prostate specific antigen

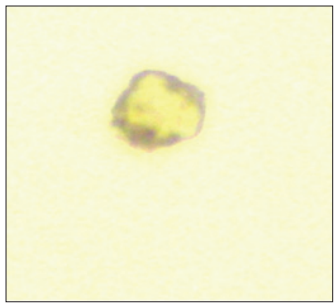

Figure 2. Leukocyte negative for expression of PSA (red) and positive for membrane CD45 (brown)
PSA: prostate specific antigen

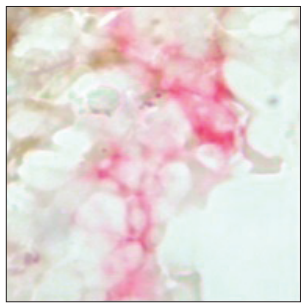

Figure 3. Bone marrow micrometastasis staining for PSA expression (red) and negative for CD45
PSA: prostate specific antigen

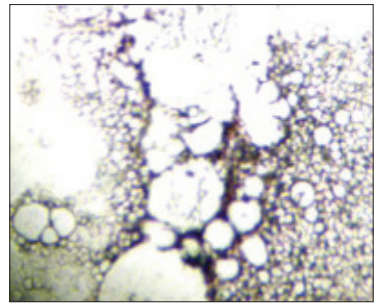

Figure 4. Bone marrow negative for cells expressing PSA (red) and positive for membrane CD45 (brown)
PSA: prostate specific antigen

Study endpoint

The primary endpoint was the presence of biochemical recurrence, and the secondary endpoint was the mean time to failure after primary treatment.

Statistical analysis

The analysis was performed using the program Stata/SE 16.0 for Windows (Stata Corp LLC). The clinical and pathological features were described as measurements of central tendency and dispersion or as proportions with their respective confidence intervals. The prognostic groups were compared using the chi-squared test for multiple proportions. A p-value <0.05 was taken to signify statistical significance, and all tests were two tailed.

For both Epstein groups, a nonparametric BFFS analysis was performed at 10 years of follow-up, to determine the BFFS proportion of patients (Kaplan-Meier) and the RMST for each MRD prognostic group.[11-13] The RMST to 10years establishes the expected time to biochemical failure during 10 years of observation.[13,14] The log-rank test was used to compare the BFFS by MRD prognostic groups.

Multivariable survival analyses using the Cox regression model, however, show that according to the proposed MRD hypothesis, the risk of biochemical failure is not proportional between groups and varies with time. This situation breaches the assumption of proportional risks for using the Cox regression model [13,14] and as such cannot be used. An alternative is the flexible parametric survival model (FP model), which permits the prediction (not descriptive like Kaplan-Meier model) of survival when there is no compliance with the proportional risk's assumption.[13-16] The FP model is a regression method in which the dependent variable is the survival for the studied outcome.

For the prediction of biochemical failure, for a follow-up time of 10 years using the MRD prognostic groups, an FP model was built using the following dummy independent variables: CPCs negative and micrometastasis positive (prognostic group B) and CPCs positive (prognostic group C).

The discrimination of a prognostic model reflects its ability to distinguish between patient outcomes. We assessed the discrimination of the FP predicted model using the Harrell's C discrimination index.[17] From the FP predicted BFFS model for up to 10 years, the RMST and survival proportion were determined for each prognostic group of MRD and similarly for the EC positive and negative subgroups.

Ethical considerations

The local ethics committee approved the study, all patients provided written informed consent, and the study fully complied with the Chilean law on patients' rights and the Declaration of Helsinki.

Results

A total of 137 men with a biopsy Gleason 6 fulfilling the EC and 338 men with a biopsy Gleason 6 not fulfilling the EC formed the study group (Table 1). Men fulfilling the EC were significantly older and had significantly more MRD negative cancer. There were no significant differences in the serum PSA or the percent of men who were upgraded between EC groups. Significantly fewer men fulfilling the EC were upstaged.

Kaplan-Meier biochemical failure free survival and restricted mean survival times

For the 112 men with pT2 EC positive and the 247 pT2 EC negative men, the BFFS were determined at 5 and 10 years for each MRD prognostic groups (Table 2, Figure 5). There were no significant differences between the two Epstein groups in terms of the biochemical failure free survival for the MRD subgroups, (log rank test 0.47).

The Kaplan-Meier BBFS curves for the three MRD subtypes show that with increasing MRD group, the 10-year BFFS

Table 1. Characteristics of 426 men with Gleason 6 prostate biopsy classified according to the EC

	EC positive n=137 pT2 surgical specimen N=112 (81%)	EC negative N=338 pT2 surgical speciemn n=247 (73%)	p
Age (years) mean (SD)	68.6 (9.4)	65.8 (7.9)	<0.05
PSA ng/mL median (IQR)	5.12 (4.50-6.68)	5.37 (4.63-6.89)	0.68
MRD prognostic groups			
A	79 (71%)	135 (55%)	<0.02
B	15 (13%)	47 (19%)	
C	18 (16%)	65 (26%)	
	pT3 or Gleason ≥7 n=25 (18%)	**pT3 or Gleason ≥7 n=91 (27%)**	**0.046**
Upgrade	8 (6%)	22 (7%)	0.89
Upstage	17 (12%)	69 (20%)	<0.02

MRD: minimal residual disease; PSA: prostate specific antigen; IQR: interquartile range; EC: Epstein criteria

decreases; however, the risk of biochemical failure varied with time in patients with only micrometastasis (Group B), increasing after 5 years (late relapse) (Figure 5). The FP survival model for the prediction of BFFS at 10 years by MRD prognostic groups, showed two degrees of freedom for the restricted cubic spline function used for the baseline hazard rate (DF2). This incorporated the following coefficients: a) CPCs negative and micrometastasis positive (prognostic group B), Hazard ratio (HR) 1.76 (p<0.01) and b) CPCs positive (prognostic group C), HR 4.03 (p<0.01). The Harrel´s C discrimination between observed and predicted BFFS was 0.91, which was considered to be very good.

Table 3 shows the RMST for the two EC groups with respect to MRD prognostic groups.

There were no significant differences between EC positive and negative groups with respect to the RMST between MRD negative patients and those with only micrometastasis, both were above 9 years. Patients positive for CPCs had a significantly shorter mean time to biochemical failure, approximately 6 years.

In summary, patients who fulfilled the EC had a significantly higher frequency of pT2 disease, higher frequency of MRD negative disease, and lower frequency of CPC positive MRD. The BFFS and RMST for the MRD subtypes was similar between EC positive and negative groups.

Discussion

As a result of screening, the number of men diagnosed with prostate cancer has significantly increased; however, many may never develop symptoms or die from their prostate cancer. Thus, it is important to identfy men who have biologically insignificant disease that will not benefit from treatment, and active surveillence would be a potential therapy. Defining biologically insignificant disease has been difficult, tumor size of <0.5cm^3 has been suggested[18]; however, the Epstein criteria[5] has been adapted for identifying men eligible for active surveillance.[19,20]

We report those men with EC Gleason 6 prostate cancer detected on biopsy and who chose RP and had a high frequency of pT2 disease (81% vs. 73%) and MRD negative disease (71% vs. 55%). These patients have a very low likelihood of biochemical failure 10 years after surgery (1%), implying long term cure. However, the EC showed a substantial underestimation of the potentially biologically significant prostate cancer of 18% between upgrading 6% and upstaging 12%. This was significantly lower than the 27% in patients with a Gleason 6 prostate biospy who did not fulfill the EC. In this group, the frequency of upgrading was similar (7%), whereas upstaging was significantly higher (20%). In addition, 29% of EC positive men with pT2 disease were MRD positive, 16% positive for CPCs and a BFFS of 19% at 10 years. Interestingly, the frequency of those patients who are only positive for bone marrow micrometastasis was similar in both groups with a 10 year biochemcial failure free survival of 89-90%.

This suggests that the biological charactersitics of the tumor dictate the prognosis and not the morphological and pathological characteristics. The EC are based on known prognostic risk factors found in the prostate biopsy and the pre-surgical serum PSA and prostate volume. However, not all cancer cells

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Table 2. Five and ten-year Kaplan–Meier observed and flexible parameter predicted biochemical failure free survivals for men with pT2 EC positive and negative prostate cancer

	EC pT2 positive		EC pT2 negative	
	5yrs KM FP	10 yrs KM FP	5yrs KM FP	10 yrs KM FP
MRD negative	100 100	99 98	100 100	98 99
mM positive	100 100	89 83	100 95	90 81
CPC positive	78 76	21 25	79 77	19 22
				Log rank p=0.47

KM: Kaplan–Meier observed biochemical failure free survival; FP: Flexible Parameter predicted biochemical failure free survival; MRD: minimal residual disease; mM: micrometastasis; CPC: circulating prostate cells; EC: Epstein criteria

Table 3. Observed and predicted RMST for the two Epstein criteria groups according to minimal residual disease subgroups

	EC positive		EC negative	
	Observed RMST	Predicted RMST	Observed RMST	Predicted RMST
MRD (-)	9.75 (9.54-9.91)	9.81 (9.69-9.95)	9.78 (9.61-9.95)	9.84 (9.71-9.93)
mM (+)	9.41 (9.10-9.72)	9.28 (8.82-9.71)	9.35 (9.03-9.67)	9.16 (8.76-9.55)
CPC (+)	6.27 (5,48-6,74)	6.08 (5.62-6,73)	6.13 (5.59-6.68)	6.11 (5.57-6.64)
All	8,24 (7.91-8.57)	8.26 (7.82-8.56)	8.19 (7.89-8.49)	8.27 (8.08-8.45)

RMST: restricted mean survival time; MRD: minimal residual disease; mM: micrometastasis positive; CPC: circulating prostate cell positive; EC: Epstein criteria

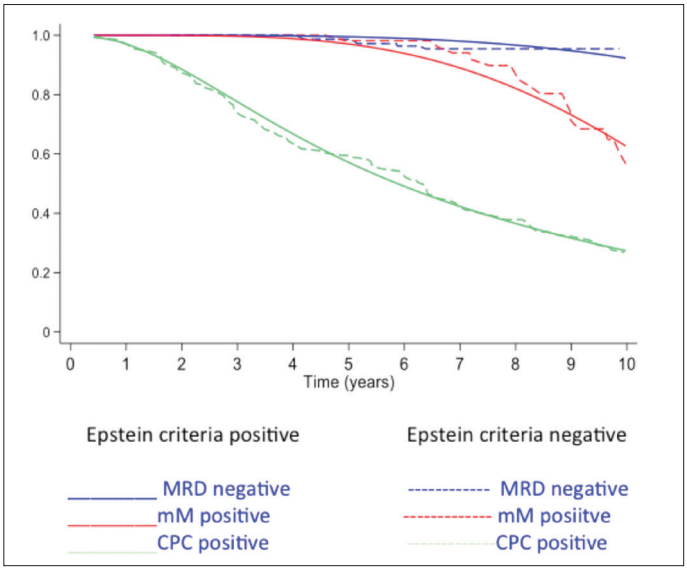

Figure 5. Ten-year Kaplan–Meier observed biochemical failure free survivals for men with pT2 Epstein criteria positive and negative prostate cancer
PSA: prostate specific antigen

are equal, and there is heterogeneity in the phenotypic expression of tumor cells in the same patient. Some tumor cells are capable of disseminating early in prostate cancer, surviving in the circulation, and implanting in distant tissues.[21] The morphological characteristics used to define Gleason score do not identify these characteristics. The presence of MRD infers that there is microdissemination beyond the tumor. In men fulfilling the EC, 29% had one type of microdissemination detected in addition to the 19% upstaged or upgraded. This represents 58 (42%) patients of the total population fulfilling the EC. In comparison, in men with a Gleason 6 prostate biopsy that did not fulfil the EC, 203 (60%) had microdissemination, upgrading, or upstaging. These observations suggest that a substantial proportion of patients considered for active surveillance are at risk of disease progression. Those men with EC do not necessarily have biologically insignificant cancer. If during the observation period, patients progress to EC negative Gleason 6, the risk of biologically significant disease significantly increases. These results suggest that RP is a very effective therapy when there is no MRD.

A significant strength of this study is that all biopsy information and pathological material was obtained, recorded, processed, and interpreted at a single centre in a standardized fashion, allowing accurate assessment of all the variables constituting the EC and pT2 prostate cancer. The study has several limitations though: The detection of micrometastasis using bone marrow

aspirations or biopsy has been previously reported, although differing antibodies have been used to identify tumor cells, anti-cytokeratin, anti-PSA, and anti-prostate specific membrane antigen (PSMA) for prostate cells. The use of reverse transcriptase polymerase chain reaction (RT-PCR) for PSA and PSMA is reported to have 10 times the sensitivity to detect tumor cells. However, detecting every cancer may not be important, patients post allogeneic bone marrow transplantation for leukemia may have very small numbers of leukemic cells detected by RT-PCR in bone marrow samples but remain in remission for many years. These leukemia cells may survive for prolonged periods before being eradicated by host defenses.[22] As such, ultra-sensitive methods to detect tumor cells may overestimate clinically important MRD in patients with solid tumors.

Although thought to be an invasive procedure, performed under sedation and local anesthesia, the risk of adverse effects is minimum, less than 0.08%.[23]

For the detection of CPCs, we used differential gel centrifugation and immunocytochemistry, acknowledging that the detection of CPCs or circulating tumor cells is method dependent. However, although the study had the disadvantage of being a single-center study, it has the advantage of an immunocytologist who has the experience and training to perform the tests. Using the EpCAM (Epithelial Cell Adhesion Molecule) based CellSearch® system, the frequency of patients positive for CPCs has been reported to be between 5% and 42% in patients with localized cancer,[24,25] with the EPISPOT assay in 42%® and the CellCollector® in 48%.[26] The differences and pitfalls of the different methods to detect CPCs have been reviewed.[27] The method we used to detect CPCs will not detect CPCs and micrometastasis that do not express PSA. However, this method has the advantage that it could be carried out in the routine laboratory of a general hospital without the need for high cost technology or highly specialized personnel.

The EC have been used to select patients for active surveillance, predicting pT2 disease. In these patients with pT2, 71% were negative for MRD and after RP had a BFFS of 99% at 10 years. However, 20% of the total EC population were upgraded or upstaged, and furthermore, 29% had MRD with 16% positive for CPCs and a BFFS of only 21% at 10 years. The use of the MRD classification identifies patients with high risk of early failure and those at risk for late failure independent of the EC criteria.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of the Western Metropolitan Health Authority.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions Concept - N.P.M.; Design - N.P.M.; Supervision - N.P.M., E.R., C.F.; Resources - N.P.M.; Materials - N.P.M., S.O., C.F., A.S., E.R.; Data Collection and/or Processing - C.F., A.S., E.R., E.G., S.O.; Analysis and/or Interpretation - N.P.M., S.O., A.S., C.F., E.R., E.G.; Literature Search - S.O., E.G.; Writing Manuscript - N.P.M., E.G.; Critical Review - C.F., A.S., E.R., S.O., E.G.; Final Approval - N.P.M., S.O., A.S., C.F., E.R., E.G.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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