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Early Postoperative Peripheral Blood Reverse Transcription PCR Assay for Prostate-specific Antigen Is Associated with Prostate Cancer Progression in Patients Undergoing Radical Prostatectomy[1]

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ABSTRACT

Preoperative peripheral blood reverse transcription-PCR (RT-PCR) for prostate-specific antigen (PSA) [RT-PCR-PSA] is not associated with an increased risk of progression after radical prostatectomy. We tested the hypothesis that early postoperative peripheral blood RT-PCR-PSA would detect prostate cancer cells persisting in the circulation that would be associated with disease progression. The study group consisted of 145 consecutive patients who underwent radical prostatectomy for clinically localized disease (median follow-up, 54.5 months) for whom pre- and postoperative peripheral blood samples were available. RT-PCR-PSA was performed on preoperative and postoperative peripheral blood specimens. Pre- and postoperative RT-PCR-PSA were positive in 27% and 12%, respectively, of patients. Most (64%) preoperative RT-PCR-PSA-positive patients converted to a negative RT-PCR-PSA status after prostate removal ($P < 0.001$). Whereas preoperative RT-PCR-PSA was not associated with prostate cancer characteristics or outcome, a positive postoperative RT-PCR-PSA assay was associated with extracapsular extension ($P = 0.044$) and seminal vesicle involvement ($P = 0.024$). Furthermore, postoperative RT-PCR-PSA was an independent predictor of disease progression ($P = 0.027$). In patients who experienced disease progression, postoperative RT-PCR-PSA was associated with a more aggressive pattern of failure ($P = 0.005$). Whereas a significant number of patients with clinically localized prostate cancer have prostate cells detectable preoperatively by RT-PCR-PSA circulating in their blood, most of these cells are clinically insignificant because the majority of these patients convert to RT-PCR-PSA-negative status and maintain disease-free status after prostate removal. In contrast, postoperative RT-PCR-PSA detection of prostate cells in the peripheral blood is associated with established markers of aggressive prostate cancer and is an early independent predictor of disease progression, presumably because of an association with established micrometastatic disease.

INTRODUCTION

RT-PCR[3] has been shown to identify very small numbers of disseminated prostatic cells (1). However, the biological significance and clinical significance of these cells are highly variable. Few groups have found a significant association between preoperative peripheral blood RT-PCR-PSA and pathological prostate cancer stage (2–6), development of overt metastases (7), or progression-free survival (8, 9), whereas the majority of authors have failed to demonstrate any clinically significant role for preoperative RT-PCR-PSA (10–13). Interestingly, studies have reported that most patients with positive peripheral blood or bone marrow RT-PCR-PSA assay results convert to a negative result after prostate removal (11–15), suggesting that most of these circulating cells lack the capacity for survival and progression to clinical metastases after radical prostatectomy. We hypothesized that patients with circulating cells detected by RT-PCR-PSA *after* radical prostatectomy would be more likely to harbor occult metastases that would be associated with prostate cancer progression, despite effective local control of disease. Therefore, to determine the relationship between pre- and postoperative peripheral blood RT-PCR-PSA and risk for prostate cancer progression, we performed this assay on patient matched pre- and postoperative specimens obtained for a large cohort of patients with clinically localized prostate cancer who underwent radical prostatectomy and who had long-term follow-up.

MATERIALS AND METHODS

Patient Selection and Sample Acquisition. Between December 1994 and November 1995, 214 men underwent radical prostatectomy for clinically localized prostatic adenocarcinoma by surgeons of the Scott Department of Urology at The Methodist Hospital (Houston, TX). One hundred and forty-five of the 214 patients who had their 6–8 week follow-up visit at our department (thus having pre- and postoperative peripheral blood samples available) were included in our study. Institutional review board-approved informed consent for the collection of clinical data, as well as serum and prostatic tissue samples, was obtained for all patients. No patient was treated preoperatively with either hormonal or radiation therapy, and none had any secondary cancer. Serum PSA was measured by the Hybritech Tandem-R assay (Hybritech, Inc., San Diego, CA). We retrospectively measured postoperative ultrasensitive serum PSA levels in frozen archived specimens (IMMULITE Third Generation PSA assay; Diagnostic Products Corp., Los Angeles, CA), obtained 6–8 weeks after radical prostatectomy, in 16 of the 18 patients who had a positive postoperative RT-PCR-PSA assay result and serum available. Clinical stage was assigned by the surgeon, according to the 1992 tumor-node-metastasis (TNM) system.

Peripheral blood specimens were collected into Vacutainer CPT 8-ml tubes containing 1 ml of 0.1 M sodium citrate anticoagulant (Becton Dickinson, Franklin Lakes, NJ) after a preoperative overnight fast on the morning of the day of surgery, at least 4 weeks after transrectal guided needle biopsy of the prostate. Postoperative plasma samples were collected in all patients between 6 and 8 weeks after surgery during their first postoperative visit on which serum PSA was also measured.

Pathological Examination. All prostatectomy specimens were examined pathologically at our institution by a single pathologist (T. M. W.), who was blinded to clinical outcome. The radical prostatectomy specimens were processed by whole-mount technique, and pathological parameters were evaluated in a manner described previously (16). Total tumor volume was computed by computerized planimetry from the whole-mount sections from 60 of the 120 prostatectomy patients.

Postoperative Follow-Up. Patients were generally scheduled to have a digital rectal examination and serum PSA evaluation starting 6 weeks postoperatively, every 3 months for the first postoperative year, semiannually from the second through the fifth year, and annually thereafter. Biochemical progression was defined as a sustained elevation, on two or more occasions, of

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[3] The abbreviations used are: RT-PCR, reverse transcription-PCR; PSA, prostate-specific antigen; RT-PCR-PSA, RT-PCR for PSA.

PSA > 0.2 ng/ml. In 28 of the 33 patients experiencing biochemical progression, a staging evaluation including bone scan, ProstaScint scan, and/or PSA doubling time calculation was performed before administration of salvage radiation or hormonal therapy. Four (3%) patients had lymph node-positive disease discovered at the time of surgery, and the prostate was not removed. These patients were categorized as failures from the day after surgery. Seven (5%) patients received adjuvant radiation therapy before biochemical progression because of positive surgical margins. Four of them subsequently experienced PSA relapse and were categorized as having progression from the date of the first value > 0.2 ng/ml, whereas the remaining three were censored on the date of the last follow-up examination. Postprogression serum PSA doubling time was calculated for patients who had biochemical progression, and at least three PSA measurements after the date of progression using the following formula: $DT = \ln(2) \times T/[\ln(\text{final PSA}) - \log(\text{initial PSA})]$; where DT is the serum PSA doubling time, T is the time interval between the initial and final PSA level, final PSA is the presalvage treatment PSA level, and initial PSA is the PSA level noted at the time of the postoperative biochemical recurrence (17).

Fourteen of the patients who experienced disease progression were treated at the Methodist Hospital with external beam radiation therapy limited to the prostatic fossa as described previously (18). A complete response to salvage radiation therapy was defined as the achievement and maintenance of an undetectable serum PSA level (19, 20). Radiation therapy was considered to have failed if the postradiation serum PSA levels did not fall to and remain at an undetectable level.

RNA Preparation, Reverse Transcription Reaction, cDNA Synthesis, Oligonucleotide Primers, and PCR. The RT-PCR assay (21) and the PSA primers spanning 710 bp (2) have been described previously. Briefly, the upstream primer sequence was 5′-GATGACTCCAGCCACGACCT-3′, and the downstream sequence was 5′-CACAGACACCCCATCCTATC-3′. The PCR was performed for 35 cycles consisting of the following steps: denaturation at 94°C for 1 min; annealing at 66°C for 1 min; and extension at 72°C for 2 min. Both gel analysis and a second PCR reaction amplifying the glyceraldehyde-3-phosphate dehydrogenase housekeeping gene were used to assess mRNA integrity. Internal negative control reactions for the RT-PCR were performed using all of the reagents as for the experimental samples, but with lymphoblast RNA in each of the assays. None of the assays exhibited a signal from the internal negative control. Internal positive control reactions for the RT-PCR were performed using PSA cDNA. RT-PCR results were scored in a blinded fashion to clinical outcome.

Statistical Analysis. Fisher's exact test was used to evaluate the association of RT-PCR-PSA results with clinical and pathological features. Differences in variables with a continuous distribution across RT-PCR-PSA categories were assessed using the Mann-Whitney U test. The McNemar test was used to evaluate changes in a repeated measures situation. The Kaplan-Meier method was used to calculate survival functions, and differences were assessed with the log-rank statistic. Multivariable survival analysis was performed with the Cox proportional hazards regression model. Preoperative PSA level had a skewed distribution and was therefore modeled with a log transformation in the Cox models. Clinical stage was evaluated as T_1 *versus* T_2. Biopsy and radical prostatectomy Gleason sum were evaluated as grade 2–6 *versus* grade 7–10. Statistical significance in this study was set as $P < 0.05$. All reported Ps are two-sided. All analyses were performed with SPSS statistical package (SPSS version 10.0 for Windows).

RESULTS

Association of Pre- and Postoperative RT-PCR-PSA with Clinical and Pathological Characteristics of Prostate Cancer. Clinical and pathological characteristics of 145 prostatectomy patients and association with pre- and postoperative peripheral blood RT-PCR-PSA assay results are shown in Table 1. A positive postoperative RT-PCR-PSA assay result was associated with extracapsular extension ($P = 0.044$) and seminal vesicle involvement ($P = 0.024$), whereas preoperative RT-PCR-PSA was not associated with any characteristics of prostate cancer. The mean patient age in this study was 61.1 ± 7.7 years (median age, 62.4 years; age range, 40–73 years), and the mean preoperative PSA was 9.35 ± 6.7 ng/ml (median, 7.8 ng/ml; range, 0.2–49.0 ng/ml). There was no association between pre- or postoperative RT-PCR-PSA assay results and age at time of radical prostatectomy, preoperative PSA level, preoperative PSA density, or prostatectomy tumor volume (all Ps > 0.05).

Table 1 *Association between results of pre- and postoperative peripheral blood RT-PCR-PSA assays with clinical and pathological characteristics of 145 patients who underwent radical prostatectomy for clinically localized prostate cancer*

		Preoperative RT-PCR-PSA		Postoperative RT-PCR-PSA	
	No. of patients (%)	Positive (%)	P[a]	Positive (%)	P[a]
Total	145 (100)	39 (27)		18 (12)	
Clinical stage					
T1	67 (46)	18 (27)		8 (12)	
T2	78 (54)	21 (27)	1.000	10 (13)	1.000
Biopsy Gleason sum					
2–6	102 (70)	31 (30)		11 (11)	
7–10	43 (30)	8 (19)	0.158	7 (16)	0.440
RP[b] extraprostatic extension[c]					
Negative	102 (72)	27 (26)		9 (9)	
Positive	39 (28)	11 (28)	0.835	9 (23)	0.044
RP seminal vesicle involvement[c]					
Negative	131 (93)	36 (27)		14 (11)	
Positive	10 (7)	2 (20)	1.000	4 (40)	0.024
RP surgical margin[c]					
Negative	114 (81)	31 (27)		13 (11)	
Positive	27 (19)	7 (26)	1.000	5 (19)	0.340
RP Gleason sum[c]					
2–6	82 (58)	23 (28)		9 (11)	
7–10	59 (42)	15 (25)	0.848	9 (15)	0.457
RP lymph node metastases					
Negative	140 (97)	38 (27)		17 (12)	
Positive	5 (3)	1 (20)	1.000	1 (20)	0.490
RP DNA ploidy[d]					
Diploid	67 (56)	19 (28)		5 (8)	
Aneuploid	53 (44)	14 (26)	0.840	9 (17)	0.135

[a] Fisher's exact test.
[b] RP, radical prostatectomy.
[c] Radical prostatectomy extraprostatic extension, seminal vesicle involvement, margin status, and Gleason sum were unavailable for four patients, who did not undergo a prostatectomy because of grossly positive pelvic lymph nodes at the time of surgery.
[d] Radical prostatectomy DNA ploidy was unavailable for 25 patients.

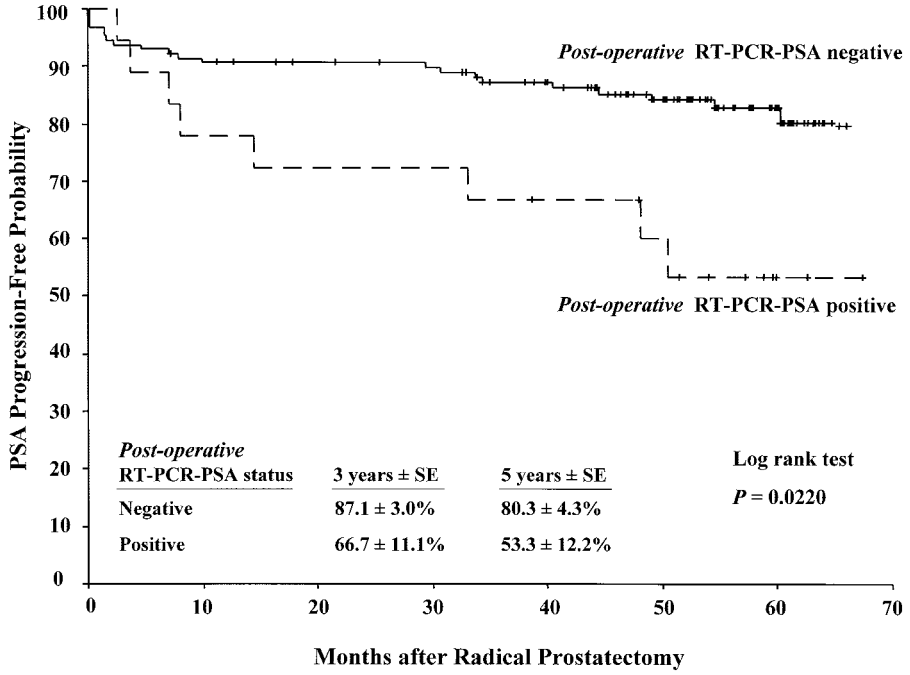

Fig. 1. Kaplan-Meier estimates of PSA progression-free probability for 145 patients with clinically localized prostate cancer treated with radical prostatectomy stratified by results of the postoperative RT-PCR-PSA assay.

Association of Preoperative with Postoperative RT-PCR-PSA. The percentage of patients with a positive RT-PCR-PSA result decreased after prostate removal (27% preoperative positive *versus* 12% postoperative positive, $P = 0.005$). Twenty-five patients who had a positive preoperative RT-PCR-PSA assay had a negative postoperative RT-PCR-PSA assay; 14 patients who had a positive preoperative assay also had a positive postoperative assay, and 4 patients who had a negative preoperative result had a positive result postoperatively.

Association of Pre- and Postoperative RT-PCR-PSA with Prostate Cancer Progression. Overall, 23% of patients (33 of 145 patients) had cancer progression with a median postoperative follow-up of 54.5 months. Using the log-rank test, we found that patients with a positive postoperative RT-PCR-PSA assay were at increased risk of prostate cancer progression ($P = 0.0220$; Fig. 1). The median time to prostate cancer progression in postoperative RT-PCR-PSA-negative and -positive patients was 32.0 months (range, 0.1–70.1 months) and 8.0 months (range, 0.1–50.4 months), respectively. However, preoperative RT-PCR-PSA was not associated with prostate cancer progression ($P = 0.7221$).

On univariable Cox proportional hazards regression analysis, a postoperative positive RT-PCR-PSA result, extraprostatic extension, seminal vesicle involvement, a positive surgical margin, and radical prostatectomy Gleason sum ≥ 7 were associated with the risk of prostate cancer progression ($P = 0.023$, $P = 0.049$, $P = 0.047$, $P < 0.001$, and $P = 0.045$, respectively; Table 2). In a postoperative multivariable model, postoperative RT-PCR-PSA, surgical margin status, and prostatectomy Gleason sum were all associated with disease progression ($P = 0.027$, $P < 0.001$, and $P = 0.044$, respectively; Table 2) when adjusted for preoperative PSA, extraprostatic extension, and seminal vesicle involvement.

Association of Pre- and Postoperative RT-PCR-PSA with Characteristics of Patients Who Experienced Disease Progression. Of the 33 radical prostatectomy patients who experienced disease progression, 4 patients underwent abortive radical prostatectomy because of lymph node-positive disease discovered at the time of surgery and were considered to have aggressive failure. In addition, 10 patients were presumed to have aggressive disease failure because of the results of a metastatic work-up (lymph node involvement, $n = 1$; positive bone or prostascint scan, $n = 3$), because their PSA doubling times were less than 10 months (median, 6.2 months; range, 2.3–8.6 months; $n = 9$), and/or because they failed to respond to local salvage radiation therapy ($n = 9$). Nineteen patients were presumed to have nonaggressive disease failure because their PSA doubling times were greater than 10 months ($n = 16$; median, 20.1 months; range, 13.3–317.4 months), and/or because they achieved a complete response to local salvage radiation therapy ($n = 5$). Early postoperative RT-PCR-PSA was positive in one patient who developed nonaggressive failure and seven patients with aggressive failure ($P = 0.005$). However, preoperative RT-PCR-PSA was not associated with aggressive prostate cancer progression ($P = 0.422$).

Table 2 *Univariable and multivariable Cox regression analysis of postoperative features for the prediction of prostate cancer progression in patients undergoing radical prostatectomy for clinically localized disease*

	Univariable			Multivariable		
Variable	Hazard ratio	*P*	95% CI[a]	Hazard ratio	*P*	95% CI
Postoperative RT-PCR-PSA	3.647	0.023	1.035–6.113	2.884	0.027	1.130–7.362
Preoperative PSA levels[b]	1.395	0.231	0.809–2.405	0.703	0.243	0.388–1.271
Extraprostatic extension	2.354	0.049	1.103–4.839	1.191	0.716	0.463–3.062
Seminal vesicle involvement	2.848	0.047	1.114–7.813	1.090	0.896	0.299–3.966
Surgical margin status	4.328	<0.001	2.075–9.029	5.730	<0.001	2.563–12.809
RP Gleason sum[c]	3.167	0.045	1.019–4.607	2.450	0.044	1.022–5.870

[a] CI, confidence interval.
[b] Preoperative PSA levels were logarithmically transformed.
[c] Radical prostatectomy (RP) Gleason sum was categorized as grade 2–6 *versus* grade 7–10.

Association of Pre- and Postoperative RT-PCR-PSA with Ultrasensitive PSA. Of the 145 patients included in our study, 141 underwent radical prostatectomy, whereas surgery was aborted in 4 patients who had lymph node-positive disease discovered at the time of surgery. Of the 141 patients, none had detectable PSA (<0.2 ng/ml) 6–8 weeks after prostatectomy as determined by the standard assay used by our department during that period (Hybritech Tandem-R assay). With the current availability of ultrasensitive PSA assays that can reliably detect PSA levels down to a lower level of detection of 0.009 ng/ml (IMMULITE Third Generation PSA assay; Diagnostic Products Corp.), we retrospectively measured postoperative ultrasensitive serum PSA levels in frozen sera, obtained 6–8 weeks after radical prostatectomy, in 16 of the 18 patients who had a positive postoperative RT-PCR-PSA assay result and archived serum available. One of these 16 patients had metastases to pelvic lymph node identified in the surgical specimen and failed with detectable and rising PSA (>0.2 ng/ml) 14 months after surgery. Whereas his 6 weeks postoperative PSA level was recorded as undetectable (<0.2 ng/ml), the PSA was 0.069 ng/ml when measured in frozen archived serum using the IMMULITE Third Generation PSA assay, a PSA level that is associated with prostate cancer progression in our current practice. The remaining 15 patients had an ultrasensitive PSA level of ≤0.02 ng/ml (median, 0.009 ng/ml; range, 0–0.020 ng/ml), well within the disease-free limit of our current practice.

DISCUSSION

The availability of third generation PSA assays that provide reliable results down to a lower limit of detection of 0.009 ng/ml has shortened the time to reliable detection of prostate cancer progression compared with older PSA assays. Nonetheless, a significant number of patients with an undetectable or clinically insignificant PSA level (<0.03–0.05 ng/ml) 6 weeks after surgery, as measured with an ultrasensitive assay, will still eventually develop prostate cancer progression. We found that a positive RT-PCR-PSA mRNA performed 6–8 weeks after radical prostatectomy could identify patients who were at higher risk for the development of eventual disease progression, despite undetectable levels of PSA protein in the serum as measured using an ultrasensitive assay. Therefore, postoperative RT-PCR-PSA, which is highly sensitive as reflected by a reliable detection of as few as 5 copies of PSA cDNA and 1 LNCaP cell in 10^6 cultured lymphoblasts, (10), was an earlier predictor of disease progression than an ultrasensitive PSA assay.

Newer therapeutic modalities such as chemo- and immunotherapy for prostate cancer may delay progression or improve survival in men who are no longer curable with local therapy due to the early dissemination of micrometastatic disease. Early identification of patients destined to fail with distant disease would spare them the morbidity and cost of adjuvant or salvage local therapy that would be ineffective. Furthermore, following general principles of oncology, these newer therapies are likely to be most effective when initiated while the burden of metastatic disease is small. Early postoperative RT-PCR-PSA appears to be a candidate assay that can help identify patients at high risk for clinical progression to metastatic disease at the earliest sign of advanced disease, when the tumor burden is smallest. For example, 15 of the 16 patients who had a positive postoperative RT-PCR-PSA assay result and archived serum available had undetectable ultrasensitive PSA levels (median, 0.009 ng/ml; range, 0–0.02 ng/ml), but 6 of them experienced prostate cancer progression a median of 7.5 months after surgery. This assay may also prove useful to monitor the response to therapy in patients without any other clinical evidence of metastases.

In agreement with most studies (10, 11, 12, 13, 22), except one (8), we found no association between preoperative RT-PCR-PSA and prostate cancer outcome. Interestingly, all four patients with a negative preoperative but positive postoperative RT-PCR-PSA assay later developed progression of their disease. We and others (12, 21) have shown that tumor cell dissemination is a relatively early phenomenon in prostate cancer, with even low-volume and -stage prostate tumors intermittently releasing cells into the systemic circulation. However, in order for these disseminated cells to develop into overt clinical metastases, further genetic alterations essential to the metastatic process are necessary. Whereas circulating prostate cancer cells need to traverse additional obstacles along the multistep metastatic process to establish clinical significant metastatic disease, the simple persistent presence in the circulation of prostate cancer cells after prostate tumor removal appears to be an ominous harbinger of disease progression. In agreement with previous studies (11–15), we found a 64% decrease in the percentage of patients with a positive preoperative RT-PCR-PSA result after surgery. Using serial postoperative samples up to 7 years after radical prostatectomy, Ellis *et al.* (12) found that most cases who were initially positive remained positive or converted to negative within a 1-year period after prostate removal; cases who were initially negative generally remained negative but were occasionally noted to have positive results. Because we did not systematically assay postoperative samples after the 6–8-week period, we do not know whether some of our patients who had a positive assay would have converted to a negative assay result, but our rate of RT-PCR-PSA positivity at 6–8 weeks was similar to that of Ellis *et al.* (12). This, in addition to the finding that the postoperative but not the preoperative RT-PCR-PSA result was an independent predictor of prostate cancer progression, supports the hypothesis that the prostate often sheds biologically indolent and/or clinically insignificant cells, which achieve only transient survival and are no longer detected once the prostate is removed. Less commonly, some circulating tumor cells that have the potential for causing later progression of the disease persist in the circulation. Whereas the optimal time for sampling of these clinically significant circulating cells remains to be determined, postoperative sampling 6–8 weeks after prostate removal yielded enough power to detect clinically significant disseminated prostate cancer cells.

Postoperative RT-PCR-PSA was associated with aggressive prostate cancer progression suggestive of early occult metastatic disease present at time of surgery. This argument is supported by the considerably shorter time to progression in patients with a positive RT-PCR-PSA assay compared with those with a negative assay (8.0 *versus* 32.0 months). Postoperative failure within 2 years or less of surgery has been shown to predict the development of clinically evident metastases (23). Pound *et al.* (23) have found that half of patients with clinically localized prostate cancer who sustain PSA failure within 2 years after radical prostatectomy progress to distant metastatic disease within 5 years after such PSA failure. In our study, 63% of the patients who had positive RT-PCR-PSA assay and experienced disease progression had their failure within 14 months. This extremely short time to progression of patients with a positive RT-PCR-PSA assay suggests that these patients were already on the threshold of clinical progression at time of surgery.

Whereas we found that 45% of patients with positive postoperative RT-PCR-PSA result experienced prostate cancer progression, 19% of patients with negative postoperative RT-PCR-PSA result also experienced disease progression. In addition, whereas half of patients who experienced an aggressive disease progression had a positive postoperative RT-PCR-PSA result, the other half had a negative postoperative RT-PCR-PSA result. Furthermore, postoperative RT-PCR-PSA was positive in only one of five patients with metastases to regional lymph nodes. These data suggest that other mechanisms of disease

dissemination such as via the lymph nodes and possibly bone marrow, which may not be detected by postoperative peripheral blood RT-PCR-PSA, most likely also play an important part in prostate cancer progression in some patients (12, 24, 25).

In our experience, preoperative RT-PCR-PSA was not a useful tool for guiding therapy or prognosticating outcome in patients with clinically localized prostate cancer, and therefore it has limited clinical utility. In contrast, our study clearly demonstrates a biological and statistically significant association between a positive postoperative peripheral blood RT-PCR-PSA assay and biochemical progression after radical prostatectomy. Postoperative peripheral blood RT-PCR-PSA seems to detect early low-volume occult metastases that are at the threshold of clinical progression. Prospective studies using a more objective assay (*e.g.*, real-time quantitative PCR format) are required to confirm these results, to construct a potentially better prognostic nomogram that incorporates additional validated biomarkers of early metastases and aggressive disease progression, and to establish recommendations for adjuvant and salvage therapies.

REFERENCES

1. Moreno, J. G., Croce, C. M., Fischer, R., Monne, M., Vihko, P., Mulholland, S. G., and Gomella, L. G. Detection of hematogenous micrometastasis in patients with prostate cancer. Cancer Res., *52:* 6110–6112, 1992.
2. Katz, A. E., Olsson, C. A., Raffo, A. J., Cama, C., Perlman, H., Seaman, E., O'Toole, K. M., McMahon, D., Benson, M. C., and Buttyan, R. Molecular staging of prostate cancer with the use of an enhanced reverse transcriptase-PCR assay. Urology, *43:* 765–775, 1994.
3. Olsson, C. A., de Vries, G. M., Raffo, A. J., Benson, M. C., O'Toole, K., Cao, Y., Buttyan, R. E., and Katz, A. E. Preoperative reverse transcriptase polymerase chain reaction for prostate specific antigen predicts treatment failure following radical prostatectomy. J. Urol., *155:* 1557–1562, 1996.
4. Ennis, R. D., Katz, A. E., de Vries, G. M., Heitjan, D. F., O'Toole, K. M., Rubin, M., Buttyan, R., Benson, M. C., and Schiff, P. B. Detection of circulating prostate carcinoma cells via an enhanced reverse transcriptase-polymerase chain reaction assay in patients with early stage prostate carcinoma. Independence from other pretreatment characteristics. Cancer (Phila.), *79:* 2402–2408, 1997.
5. Zhang, Y., Zippe, C. D., Van Lente, F., Klein, E. A., and Gupta, M. K. Combined nested reverse transcription-PCR assay for prostate-specific antigen and prostate-specific membrane antigen in detecting circulating prostatic cells. Clin. Cancer Res., *3:* 1215–1220, 1997.
6. Grasso, Y. Z., Gupta, M. K., Levin, H. S., Zippe, C. D., and Klein, E. A. Combined nested RT-PCR assay for prostate-specific antigen and prostate-specific membrane antigen in prostate cancer patients: correlation with pathological stage. Cancer Res., *58:* 1456–1459, 1998.
7. Mejean, A., Vona, G., Nalpas, B., Damotte, D., Brousse, N., Chretien, Y., Dufour, B., Lacour, B., Brechot, C., and Paterlini-Brechot, P. Detection of circulating prostate derived cells in patients with prostate adenocarcinoma is an independent risk factor for tumor recurrence. J. Urol., *163:* 2022–2029, 2000.
8. de la Taille, A., Olsson, C. A., Buttyan, R., Benson, M. C., Bagiella, E., Cao, Y., Burchardt, M., Chopin, D. K., and Katz, A. E. Blood-based reverse transcriptase polymerase chain reaction assays for prostatic specific antigen: long term follow-up confirms the potential utility of this assay in identifying patients more likely to have biochemical recurrence (rising PSA) following radical prostatectomy. Int. J. Cancer, *84:* 360–364, 1999.
9. Okegawa, T., Nutahara, K., and Higashihara, E. Preoperative nested reverse transcription-polymerase chain reaction for prostate specific membrane antigen predicts biochemical recurrence after radical prostatectomy. BJU Int., *84:* 112–117, 1999.
10. Andrews, B., Shariat, S. F., Kim, J. H., Wheeler, T. M., Slawin, K. M., and Lerner, S. P. Preoperative plasma levels of interleukin-6 and its soluble receptor predict disease recurrence and survival of patients with bladder cancer. J. Urol., *167:* 1475–1481, 2002.
11. Oefelein, M. G., Ignatoff, J. M., Clemens, J. Q., Watkin, W., and Kaul, K. L. Clinical and molecular followup after radical retropubic prostatectomy. J. Urol., *162:* 307–310, discussion 310–311, 1999.
12. Ellis, W. J., Vessella, R. L., Corey, E., Arfman, E. W., Oswin, M. M., Melchior, S., and Lange, P. H. The value of a reverse transcriptase polymerase chain reaction assay in preoperative staging and followup of patients with prostate cancer. J. Urol., *159:* 1134–1138, 1998.
13. Shariat, S. F., Gottenger, E., Nguyen, C., Song, W., Kattan, M. W., Andenoro, J., Wheeler, T. M., Spencer, D. M., and Slawin, K. M. Preoperative blood reverse transcriptase-PCR assays for prostate-specific antigen and human glandular kallikrein for prediction of prostate cancer progression after radical prostatectomy. Cancer Res. *62:* 5974–5979, 2002.
14. Straub, B., Muller, M., Krause, H., Schrader, M., Goessl, C., Heicappell, R., and Miller, K. Detection of prostate-specific antigen RNA before and after radical retropubic prostatectomy and transurethral resection of the prostate using "LightCycler"-based quantitative real-time polymerase chain reaction. Urology, *58:* 815–820, 2001.
15. Gelmini, S., Tricarico, C., Vona, G., Livi, L., Melina, A. D., Serni, S., Cellai, E., Magrini, S., Villari, D., Carini, M., Serio, M., Forti, G., Pazzagli, M., and Orlando, C. Real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) for the measurement of prostate-specific antigen mRNA in the peripheral blood of patients with prostate carcinoma using the taqman detection system. Clin. Chem. Lab. Med., *39:* 385–391, 2001.
16. Wheeler, T. M., and Lebovitz, R. M. Fresh tissue harvest for research from prostatectomy specimens. Prostate, *25:* 274–279, 1994.
17. Schmid, H. P., McNeal, J. E., and Stamey, T. A. Observations on the doubling time of prostate cancer. The use of serial prostate-specific antigen in patients with untreated disease as a measure of increasing cancer volume. Cancer (Phila.), *71:* 2031–2040, 1993.
18. Shariat, S. F., Shalev, M., Menesses-Diaz, A., Kim, I. Y., Kattan, M. W., Wheeler, T. M., and Slawin, K. M. Preoperative plasma levels of transforming growth factor β1 (tgf-β1) strongly predict progression in patients undergoing radical prostatectomy. J. Clin. Oncol., *19:* 2856–2864, 2001.
19. Kattan, M. W., Fearn, P. A., Leibel, S., and Potters, L. The definition of biochemical failure in patients treated with definitive radiotherapy. Int. J. Radiat. Oncol. Biol. Phys., *48:* 1469–1474, 2000.
20. Leventis, A., Shariat, S., Kattan, M., Butler, E., Wheeler, T., and Slawin, K. Prediction of response to salvage radiation therapy in patients with prostate cancer recurrence after radical prostatectomy [erratum appears in J. Clin. Oncol., *19:* 2109, 2001]. J. Clin. Oncol., *19:* 1030–1039, 2001.
21. Slawin, K. M., Shariat, S. F., Nguyen, C., Leventis, A. K., Song, W., Kattan, M. W., Young, C. Y., Tindall, D. J., and Wheeler, T. M. Detection of metastatic prostate cancer using a splice variant-specific reverse transcriptase-polymerase chain reaction assay for human glandular kallikrein. Cancer Res., *60:* 7142–7148, 2000.
22. Gao, C. L., Maheshwari, S., Dean, R. C., Tatum, L., Mooneyhan, R., Connelly, R. R., McLeod, D. G., Srivastava, S., and Moul, J. W. Blinded evaluation of reverse transcriptase-polymerase chain reaction prostate-specific antigen peripheral blood assay for molecular staging of prostate cancer. Urology, *53:* 714–721, 1999.
23. Pound, C. R., Partin, A. W., Eisenberger, M. A., Chan, D. W., Pearson, J. D., and Walsh, P. C. Natural history of progression after PSA elevation following radical prostatectomy. J. Am. Med. Assoc., *281:* 1591–1597, 1999.
24. Lange, P. H., and Vessella, R. L. Mechanisms, hypotheses and questions regarding prostate cancer micrometastases to bone. Cancer Metastasis Rev., *17:* 331–336, 1998.
25. Melchior, S. W., Corey, E., Ellis, W. J., Ross, A. A., Layton, T. J., Oswin, M. M., Lange, P. H., and Vessella, R. L. Early tumor cell dissemination in patients with clinically localized carcinoma of the prostate. Clin. Cancer Res., *3:* 249–256, 1997.