Prognostic Value of Combined “Triple”-Reverse Transcription-PCR Analysis for Prostate-Specific Antigen, Human Kallikrein 2, and Prostate-Specific Membrane Antigen mRNA in Peripheral Blood and Lymph Nodes of Prostate Cancer Patients

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ABSTRACT

Purpose: We present the largest study of both peripheral blood and lymph node samples examining the utility of reverse transcription-polymerase chain reaction (RT-PCR) for established molecular markers as a diagnostic tool in the molecular staging of prostate cancer patients undergoing radical prostatectomy.

Experimental Design: Peripheral blood from 358 patients was obtained before radical prostatectomy. Corresponding obturator lymph node samples were collected from 153 of these patients. Nested RT-PCR for prostate-specific antigens (PSA), human kallikrein 2 (hK2), and prostate-specific membrane antigen (PSMA) were performed on cDNA from peripheral blood. The lymph node cDNA was analyzed for PSA und hK2 expression.

Results: RT-PCR in peripheral blood was positive in 124 (34.6%) of 358 samples for PSA, 215 (60.1%) of 358 for PSMA, and 97 (27.1%) of 358 for hK2. Comparison of positive RT-PCR rates of pT2 and pT3 tumors in corresponding peripheral blood for PSA, PSMA, and hK2 were 31.9 and 40.0%, 58.8 and 62.5%, and 26.9 and 27.5%, respectively. Histopathologically, cancer-free lymph node samples were positive in RT-PCR for PSA and hK2 in 70 (49.6%) of 141 and 89 (63.2%) of 141 of cases. All histologically positive lymph node samples (n = 12, pN+) were positive for PSA RT-PCR. PSA RT-PCR alone, as well as combined PSA/PSMA RT-PCR evaluation, in peripheral blood showed a significant association with grading. PSA RT-PCR lymph node-negative samples were significantly less likely positive in their corresponding peripheral blood RT-PCR sample.

Conclusions Although the preoperative PSA RT-PCR in peripheral blood correlated with the grading of prostate cancer, no combination of RT-PCR results using “triple” markers (PSA, hK2, PSMA) in peripheral blood and/or lymph nodes yielded additional preoperative staging information.

INTRODUCTION

Prostate cancer is the most common noncutaneous malignancy of men in the United States with approximately 198,100 cases and 31,500 deaths occurring in 2001 (1).

Despite extensive research, it remains difficult to discriminate preoperatively between patients with locally confined prostate cancer and patients with extraprostatic disease. This is demonstrated by the fact that more than one third of men with clinically localized prostate cancer have extraprostatic disease at the time of surgery (2). Extensive screening using serum prostate-specific antigen (PSA) has led to earlier detection of the disease, and most tumors are now recognized at an organ-confined stage (3). To date, a combination of digital rectal examination, PSA serum levels, and needle biopsy are the basis of preoperative staging and can help to decrease the incidence of pathologic upstaging (4). However, significant inaccuracies in preoperative staging remain. It has been known that hematogenous dissemination of tumor cells occurs in cancer as well as in in vivo models (5). Efforts to develop molecular approaches to detect disseminating tumor cells of solid tumors in peripheral blood or bone marrow, and to correlate the detection with respect to diagnosis, prognostic value, and clinical monitoring, are under investigation (6).

Two groups initially reported the use of reverse transcription polymerase chain reaction (RT-PCR) to study prostate cancer micrometastasis; tissue sources for PCR analysis included peripheral blood, bone marrow, and pelvic lymph nodes (7, 8). RT-PCR of histologically cancer-free pelvic lymph nodes from patients undergoing radical lymphadenectomy seems to be particularly promising. Potential molecular markers are PSA, prostate-specific membrane antigen (PSMA), and human kallikrein-2 (hK2). Unfortunately, the published studies have yielded conflicting and controversial results (9–11).

A major argument for the use of RT-PCR is the fact that clinically and pathologically localized prostate cancer has a significant chance of biochemical failure after radical retropubic prostatectomy, even for low-grade cancer with all margins and lymph nodes histopathologically negative. RT-PCR might de-
detect truly clinically occult metastases and may account for
disease recurrence of patients initially diagnosed with localized
cancer. A recent study failed to correlate preoperative RT-PCR
for PSA and PSMA results with biochemical failure or patho-
logic stage in 141 patients with localized prostate cancer (12).

In our study, we examined the expression of all three
established molecular markers (PSA, PSMA, and hK2) in the
peripheral blood of 358 consecutive patients for preoperative
staging and correlated the results with clinical data. In addition,
RT-PCR for PSA and hK2 was performed in corresponding 153
lymph node samples. As the hypogastric obturatory lymph
nodes are believed to be the first lymph nodes involved in
metastatic prostate cancer, we examined this lymph node station
regarding micrometastatic spread (13). We did not include
PSMA RT-PCR in our lymph node analysis because it is not
exclusively expressed in the prostate (14). Specifically, we will
address whether all three markers are superior versus a single
marker, whether lymph node RT-PCR results correlate with
clinical variables more accurately than do peripheral blood
RT-PCR data, and whether there is any association between
peripheral blood and lymph node results.

PATIENTS AND METHODS

From December 1997 to February 2000, 358 patients with
biopsy-proven prostate cancer treated at the Urology Department
of the Klinikum Offenbach were enrolled in this study. In addition,
45 healthy volunteers, without prior history of a malignancy or any
urologic disease, as well as 10 prostate cancer patients with ad-
vanced distant metastasis, were recruited in November and Decem-
ber 1997. All of the prostate cancer patients received 3 months of
neoadjuvant hormonal ablation therapy with leuprorelide acetate.
Pathologic stages were determined following the TNM
classification system (12). Grading was performed according to
WHO guidelines. Roughly, grade 1 tumors relate to Gleason score 2–4, grade 2 tumors to Gleason 5–6/7 and grade 3 tumors to Gleason 8–10. Detailed pathologic data of all of the patients included in this study are shown in Table 1.

Cell Lines. The LNCaP cell line [American Type Cell
Collection (ATCC) no. CRL 1740] is derived from a human
lymph node metastatic prostate cancer (15). It is a well-estab-
lished cell line model that expresses PSA, hK2, and PSMA. The
cells were cultured in RPMI 1640, supplemented with 4 mmol/L
L-glutamine, 10% fetal bovine serum, 100 units/mL penicillin,
and 100 μg/mL streptomycin, and served as positive control in
the sensitivity experiments.

**Isolation of Mononuclear Cells from Peripheral Blood and RNA Extraction.** The nucleated cell fraction was iso-
lated from 5 ml of whole blood in EDTA with a Percoll gradient
(Pharmacia, Freiburg, Germany) centrifugation according to the
manufacturer’s description. Total RNA was isolated with the
RNeasy kit (Qiagen, Hilden, Germany) according to the manu-
facturer’s description, immediately snap-frozen, and stored at
−70°C until further processing. Total RNA from snap-frozen
lymph node samples was extracted with TRIzol reagent (Invitro-
genics, Carlsbad, CA).

Reverse Transcription. Total RNA (1.5 μg) and 0.5 μg
of oligo d(T) (Promega, Heidelberg, Germany) in a total volume
of 11 μL were incubated for 10 min at 70°C and were imme-
diately cooled on ice. To each RNA sample, 4 μL of reaction
buffer (Promega) consisting of 250 mmol/L Tris-HCl (pH 7.5),
358 mmol/L KCl, 15 mmol/L MgCl₂, 50 mmol/L dithiothre-
tnine (DTT), 2 μL of distilled water, 1 μL of dNTP (10 mmol/L
each) (Boehringer Mannheim, Mannheim, Germany), 1 μL
(10 units) of RNase inhibitor (Boehringer Mannheim), and 1 μL
of M-MLV reverse transcriptase (Promega) were added, shortly
centrifuged and subsequently incubated at 37°C for 90 minutes.
Final cDNA was stored at −20°C or was immediately used for
amplification by PCR.

**PCR and Nested PCR.** All of the primers used in this
study were custom designed by MWG-Biotech (Ebersberg,
Germany). Introns spanning primer pairs specific for human
PSA (NM_001648) were sense, 5′-GGG TGA TCT TGC
TGG GTC GG-3′, and antisense, 5′-CCT TCT GAG GGT
GAA CTA CCT GCG-3′. The antisense primer was replaced by
5′-AGA TTC ACC CGA GCA GGT CCT CCT GCT-3′ for
nested PCR. Introns spanning primer pairs specific for human PSMA
(NM_004476) were for sense, 5′-TCA CCG GGA CTC ATG
GCT GT-3′, and antisense, 5′-GCC TGA ACG AAT TCC
AAG TCG-3′; sense (nested PCR), 5′-AGA GAA GGG TGG
AGA CCT AG-3′, and antisense (nested PCR), 5′-ACT GAA

<table>
<thead>
<tr>
<th>Table 1 Pathologic tumor stages (pT), lymph node stages (pN), and grading according to WHO classification system (N=358)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pT</td>
</tr>
<tr>
<td>pT₁ₐ</td>
</tr>
<tr>
<td>pT₁ₜ</td>
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<tr>
<td>pT₁ₚ</td>
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<td>pT₂ₚ</td>
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<td>pT₃ₚ</td>
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<td>pT₅ₚ</td>
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<tr>
<td>pT₆ₚ</td>
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<tr>
<td>pT₇ₚ</td>
</tr>
<tr>
<td>pNᵢ</td>
</tr>
<tr>
<td>pN₁ₛ₂</td>
</tr>
</tbody>
</table>

Grading
1. (well differentiated) 63 (17.6)
2. (moderately differentiated) 209 (58.4)
3. (poorly differentiated) 86 (24.0)
RESULTS

Sensitivity and Specificity of PSA-, hK2-, and PSMA-nested RT-PCR. Control RT-PCR for GAPDH confirmed the integrity of the isolated RNA in all of the samples. To test whether or not marker transcripts are constitutively expressed in prostate cancer tissues, we applied our RT-PCR assay for PSA, hK2, and PSMA to prostate cancer tissue from prostatectomy specimens in 20 patients. As expected, all of the tissues that were analyzed were positive for each of the three markers (data not shown). The sensitivity assays revealed that miRNA for PSA, hK2, and PSMA from an average of 10 LNCaP cells diluted in 10^7 PBMCs could be detected after nested RT-PCR in initially negative control PBMCs from healthy donors. These sensitivity results are comparable with the published reports for which RT-PCR was used (10).

In another control group of 10 patients with proven distant metastatic prostate cancer, 8 (80%), 7 (70%), and 9 (90%) were positive for PSA, hK2, and PSMA expression by RT-PCR in peripheral blood, respectively. Specificity testing of the markers used in the control group consisting of 45 healthy male volunteers showed false-positive results in peripheral blood RT-PCR for PSA in 3 (6.7%) of 45, for hK2 in 0 (0%) of 45, and for PSMA in 2 (4.5%) of 45.

RT-PCR Results in Peripheral Blood Samples. An overview of the general positivity rates for PSA, PSMA, and hK2 is given in Table 2. PSA-, hK2-, or PSMA-RT-PCR, as well as all possible permutations including the combination of all three markers, were not associated with pathologic T stage or N stage. For example, 76 (31.9%) of 238 patients with organ-confined T2 tumors and 48 (40%) of 120 patients with extraprostatic T3 or T4 tumors were PSA-RT-PCR positive (P = 0.13, χ^2 test), whereas 114 (34.3%) of 331 pN0 staged patients were PSA positive compared with 10 positive patients among a total of 27 pN+ patients (37.0%) in the cohort. For detailed results of all RT-PCR marker combinations in association with final T stage and N stage, see Table 3.

When comparing tumor grading with RT-PCR results, a difference (not statistically significant for single marker analysis) in peripheral blood RT-PCR positivity in low- or intermediate-grade versus high-grade patients could be observed. PSA RT-PCR was positive in 20 (31.7%) of 63 grade 1 patients, in 35 (40.7%) of 86 grade 2 patients, and in 35 (40.7%) of 86 grade 3 patients.

However, when combining PSA and PSMA-RT-PCR (only double-positive patients in peripheral blood RT-PCR are counted as valid cases in statistical analysis) a statistically significant difference appears: 11 (17.5%) of 63 grade 1 tumors, 36 (17.2%) of 209 grade 2 tumors, and 24 (27.9%) of 86 of grade 3 tumors are double positive. The comparison of grade 1/grade 2 versus grade 3 results in a P value of 0.03 in χ^2 analysis.

The combination of PSA and hK2 did not show any statistical difference in the groups, whereas a triple marker analysis resulted in a P value of 0.053 in the χ^2 test [for grade 1, 3 (4.8%) of 63; for grade 2, 12 (5.7%) of 209; for grade 3, 10 (11.6%) of 86]. Table 4 shows the comprehensive result.

RT-PCR Results in Lymph Node Samples. In the subgroup of 153 patients from whom lymph node samples could be
obtained, 141 patients (91.9%) were histopathologically staged N\textsubscript{0}, and 12 patients (8.1%) were staged lymph node positive. All 12 patients with lymph node-positive disease in the subgroup were positive in PSA RT-PCR analysis. Eight (66.7%) of 12 patients with lymph node-positive disease in the subgroup were positive for both markers (double positive), and 20 (38%) were double negative.

Among 100 of 153 patients with a tumor stage of pT\textsubscript{3} or higher, 48 (48%) were PSA positive, 25 (47%) were hK2 positive, 18 (18%) were PSA/hK2 positive, and 20 (20%) were hK2 positive.

In this subgroup, the PSA-, hK2-, and PSA/hK2 RT-PCR positivity rates of the patients with pT\textsubscript{3} or lower versus the patients with pT\textsubscript{4} or higher (n = 53) were 56 (56%) of 100 versus 26 (49%) of 53 for PSA, 68 (68%) of 100 versus 28 (54%) of 53 for hK2, and 48 (48%) of 100 versus 18 (34%) of 53 for combined PSA + hK2, respectively.

Association of Lymph Node and Peripheral Blood RT-PCR. We were especially interested in a possible association between lymph node and blood RT-PCR results in the group of patients with pN\textsubscript{1} disease. We, therefore, analyzed all of the possible marker combinations. The detailed results of this analysis, which leads to multiple permutations, are given in Table 6.

<table>
<thead>
<tr>
<th>Marker</th>
<th>RT-PCR positive n (%)</th>
<th>RT-PCR negative n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA</td>
<td>124 (34.6)</td>
<td>234 (65.4)</td>
</tr>
<tr>
<td>PSMA</td>
<td>215 (60.1)</td>
<td>143 (39.9)</td>
</tr>
<tr>
<td>hK2</td>
<td>97 (27.1)</td>
<td>261 (72.9)</td>
</tr>
<tr>
<td>PSA + PSMA</td>
<td>71 (19.8)</td>
<td>90 (25.1)</td>
</tr>
<tr>
<td>PSA + hK2</td>
<td>39 (10.9)</td>
<td>176 (49.2)</td>
</tr>
<tr>
<td>PSMA + hK2</td>
<td>65 (18.2)</td>
<td>111 (31.0)</td>
</tr>
<tr>
<td>PSA + PSMA + hK2</td>
<td>25 (7.0)</td>
<td>72 (20.1)</td>
</tr>
</tbody>
</table>

Interestingly, PSA-RT-PCR lymph node-positive patients were significantly more often positive in the blood PSA RT-PCR than were lymph node-negative patients (43.8 versus 27.9%; P = 0.05 in \chi\textsuperscript{2} analysis). A combined PSMA/PSA analysis yielded similar results: 28 (38.4%) of 73 lymph-node PSA-positive patients were positive for PSA and PSMA in their corresponding peripheral blood RT-PCR analysis, compared with 11 (16.2%) of 68 (P = 0.003, \chi\textsuperscript{2} analysis).

**Correlation of Serum PSA Values, Prostate Volume, and RT-PCR Results.** Baseline characteristics of serum PSA values and prostate volume were as follows: data were not normally distributed; hence, median values were determined, and, for statistical evaluation of possible significant differences, the Mann–Whitney U test was applied. Median preoperative serum PSA was 9.3 ng/mL (range, 0.4–25.5 ng/mL). Median prostate volume was 27.3 cm\textsuperscript{3} (range 15.0–79.0 cm\textsuperscript{3}).

Preoperative serum PSA values were significantly different in patients with pT\textsubscript{1} versus pT\textsubscript{3} tumors (8.5 ng/mL versus 13.4 ng/mL, respectively (P < 0.001, Mann–Whitney U test]), pN\textsubscript{0} versus pN\textsubscript{1} tumors (9.0 ng/mL versus 16.4 ng/mL, respectively (P = 0.004, Mann–Whitney U test), and in low-grade (grade 1) versus high grade (grade 3) tumors (8.0 ng/mL versus 13.4 ng/mL, respectively; P = 0.001, Mann–Whitney U test). Preoperative prostate volume did not show any association with the above-mentioned staging and grading data.

Furthermore, no significant differences in pre- or postoperative serum PSA values, as well as in preoperative prostate volume, were seen in association with RT-PCR results in all possible permutations (data not shown).

**DISCUSSION**

Precise preoperative clinical staging of prostate cancer remains difficult, and new prognostic factors are clearly needed (16). These factors should define the aggressive and metastatic potential of early prostate cancer and ideally predict the clinical outcome (17). A major problem in anticipating the clinical course of prostate carcinoma is highlighted by the fact that the...
frequency of detecting lymph node metastasis by routine pathologic analysis is not associated with the frequency of disease recurrence after radical prostatectomy (18). RT-PCR might be a promising tool in achieving progress in this difficult field.

The use of RT-PCR for clinical monitoring of prostate cancer patients and detection of disseminating prostate cancer has been under investigation for almost 10 years, but the published results are still controversial. Different studies have shown that RT-PCR is able to detect circulating PSA-, hK2-, and PSMA-expressing cells, not only in patients with systemic disease but also in patients with clinically localized disease (19–22).

Few studies have shown a correlation between RT-PCR and pathologic stage or prognostic value, specifically survival duration (23–26). In reports critically summarizing the literature on the use of RT-PCR to preoperatively predict extra prostatic disease and PSA recurrence after radical retropubic prostatectomy, the authors argue that additional studies regarding the clinical utility of RT-PCR in prostate cancer patients are needed (9, 27). Quantitative approaches to detect micrometastatic circulating cells, including expression levels, are thought to improve on the results (observed as simply positive or negative) obtained with standard RT-PCR assays (28). Despite a trend toward a higher PSA-RT-PCR positivity in peripheral blood in pT3 cancer patients (40.0% versus 31.9%; P = 0.13, \( \chi^2 \) analysis), in concordance with the vast majority of studies published thus far, we were not able to show that RT-PCR analysis by triple markers or any possible permutations could stratify patients with localized cancers to patients with extraprostatic disease. The fact that PSA-RT-PCR is significantly more often positive in high-grade tumors (41%) than in the group of low-(32%) and intermediate-grade cancers (33%) as well as the increase of statistical significance when analyzing a combination of PSA and PSMA-RT-PCR results (17% double positivity in low and intermediate grade versus 28% in high-grade cancers) is promising. Interestingly, a study also found an improvement of RT-PCR significance when combining PSA and PSMA RT-PCR results of peripheral blood and extracapsular penetration (24).

Encouraging results using RT-PCR to detect micrometastasis in histologically cancer-free lymph nodes were published (29, 30). Deguchi et al. (29) pioneered the use of PSA RT-PCR on lymph nodes. The number of analyzed samples was small (22 patients),

**Table 4** Peripheral blood RT-PCR results for all markers and marker combinations associated with tumor grading according to WHO classification

<table>
<thead>
<tr>
<th>Marker</th>
<th>Result</th>
<th>Grade 1 (n = 63)</th>
<th>Grade 2 (n = 209)</th>
<th>Grade 3 (n = 86)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Grade</td>
<td>Grade</td>
<td>Grade</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n) (%)</td>
<td>(n) (%)</td>
<td>(n) (%)</td>
</tr>
<tr>
<td>PSA</td>
<td>Positive</td>
<td>20 (31.7)</td>
<td>69 (33.0)</td>
<td>35 (40.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.26⁺</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.175⁺</td>
</tr>
<tr>
<td>PSMA</td>
<td>Positive</td>
<td>36 (57.1)</td>
<td>124 (59.3)</td>
<td>55 (64.0)</td>
</tr>
<tr>
<td>hK2</td>
<td>Positive</td>
<td>21 (33.3)</td>
<td>52 (24.9)</td>
<td>24 (27.9)</td>
</tr>
<tr>
<td>PSA + PSMA</td>
<td>Positive</td>
<td>11 (17.5)</td>
<td>36 (17.2)</td>
<td>24 (27.9)</td>
</tr>
<tr>
<td>PSA + PSMA</td>
<td>Negative</td>
<td>18 (28.6)</td>
<td>52 (24.9)</td>
<td>20 (23.3)</td>
</tr>
<tr>
<td>PSA + hK2</td>
<td>Positive</td>
<td>6 (9.5)</td>
<td>20 (9.6)</td>
<td>13 (15.1)</td>
</tr>
<tr>
<td>PSA + hK2</td>
<td>Negative</td>
<td>28 (44.4)</td>
<td>108 (51.7)</td>
<td>40 (46.5)</td>
</tr>
<tr>
<td>PSA + hK2</td>
<td>Positive</td>
<td>12 (19.0)</td>
<td>38 (18.2)</td>
<td>15 (17.4)</td>
</tr>
<tr>
<td>PSA + hK2</td>
<td>Negative</td>
<td>18 (28.6)</td>
<td>71 (34.0)</td>
<td>22 (25.6)</td>
</tr>
<tr>
<td>PSA + hK2</td>
<td>Positive</td>
<td>3 (4.8)</td>
<td>12 (5.7)</td>
<td>10 (11.6)</td>
</tr>
<tr>
<td>PSA + hK2</td>
<td>Negative</td>
<td>12 (19.0)</td>
<td>46 (22.0)</td>
<td>14 (16.3)</td>
</tr>
</tbody>
</table>

NOTE. P was calculated by \( \chi^2 \) test.

⁺ Grade 1 versus grade 3.

† Grade 1–2 versus grade 3.

**Table 5** RT-PCR results for PSA and hK2 in obturatory lymph nodes of the subgroup of 153 patients

<table>
<thead>
<tr>
<th>Marker</th>
<th>pN₀⁺</th>
<th>pN₁⁻</th>
<th>pN₂⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>PSA</td>
<td>70 (49.6)</td>
<td>12 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>hK2</td>
<td>89 (63.2)</td>
<td>8 (66.7)</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>PSA + hK2</td>
<td>61 (43.3)</td>
<td>8 (66.7)</td>
<td>4 (33.3%)</td>
</tr>
</tbody>
</table>

NOTE. N = 153.

⁺ For pN₀, n = 141 (92%).

† For pN₁⁻, n = 12 (8.1%).
but the authors were able to show, as we do in the present analysis, that all nodes with histopathologically confirmed metastasis were positive in PSA RT-PCR (28). Another study reported that 23 (79%) of 29 patients, pathologically free of micrometastatic cells, were positive by RT-PCR (30). An earlier report addressed the same question and found, in a retrospective analysis, 16 (44%) of 37 of lymph node-negative PSA RT-PCR patients. Our results fall in-between these last studies with 70 (49.6%) of 141 of lymph node-negative PSA RT-PCR patients.

A major criticism of all studies examining the use of RT-PCR in detection of occult metastases is that the expression of the message could result from basal illegitimate transcription of non-prostatic cells. An indicator that the high number of RT-PCR-positive lymph nodes in pN0 specimens does not result from illegitimate transcription is the fact that we found a correlation of lymph node and peripheral blood RT-PCR for PSA and combined PSA/PSMA RT-PCR. Only 11 (16.2%) of 68 of PSA RT-PCR-negative pN0 patients were double positive for PSA and PSMA in the corresponding peripheral blood sample, compared with 28 (38.4%) of 73 of lymph node-negative PSA RT-PCR patients.

This study is the first to analyze lymph node samples from a large defined patient cohort by RT-PCR for hK2 and PSA, associate these findings with clinical variables, and combine the results of three markers (PSA, PSMA, and hK2) in peripheral blood. Disappointingly, hK2 does not add any value to RT-PCR-based prostate cancer evaluation. As with all other results presented in this study, further follow-up of hK2 lymph node RT-PCR-positive patients has to be awaited. Additionally, the triple-marker evaluation in peripheral blood does not seem to be of value and revealed basically the same observation as the PSA/PSMA RT-PCR study presented recently (12).

One could argue that the results presented in this study may have been compromised by the fact that patients received neo-adjuvant antiandrogen therapy. It was found that patients who had pT2 and pT3 tumors and who were treated with neoadjuvant hormones were less likely to have circulating PSMA-positive cells in their peripheral blood, compared with those in the nontreated control group, before and after surgery (32). In concordance with this finding, it was reported that the hormone-treated group showed a significantly lower incidence of preoperative PSA mRNA positivity than did the group undergoing surgery alone (20 versus 69%, P = 0.036; ref. 33). However, a recent study that compared hormone-treated versus untreated patients did not find any difference in RT-PCR positivity in peripheral blood or in bone marrow (34). Our overall positivity rates for PSA, hK2, and PSMA do not differ much from results of other studies, in which patients have not been treated hor-

### Table 6 Association of obturator lymph node (LN) RT-PCR results with peripheral blood RT-PCR results in 141 patients whose N stage was N0

<table>
<thead>
<tr>
<th>Marker in blood RT PCR</th>
<th>Result</th>
<th>PSA positive</th>
<th>PSA negative</th>
<th>hK2 positive</th>
<th>hK2 negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n = 73</td>
<td>n = 68</td>
<td>n = 88</td>
<td>n = 53</td>
</tr>
<tr>
<td>Blood RT PCR PSA</td>
<td>Positive</td>
<td>32 (43.8)</td>
<td>19 (27.9) (P = 0.05)</td>
<td>31 (35.2)</td>
<td>20 (37.7)</td>
</tr>
<tr>
<td>PSMA</td>
<td>Positive</td>
<td>57 (78.1)</td>
<td>46 (67.6) (P = 0.16)</td>
<td>65 (73.9)</td>
<td>38 (71.7)</td>
</tr>
<tr>
<td>hK2</td>
<td>Positive</td>
<td>24 (32.9)</td>
<td>19 (27.9)</td>
<td>30 (34.1)</td>
<td>13 (24.5) (P = 0.23)</td>
</tr>
<tr>
<td>PSA + PSMA</td>
<td>Positive</td>
<td>28 (38.4)</td>
<td>11 (16.2) (P = 0.003)*</td>
<td>25 (28.4)</td>
<td>14 (26.4)</td>
</tr>
<tr>
<td>PSA + hK2</td>
<td>Negative</td>
<td>12 (16.4)</td>
<td>14 (20.6) (P = 0.2)*</td>
<td>17 (19.3)</td>
<td>9 (17.0)</td>
</tr>
<tr>
<td>PSA + hK2</td>
<td>Positive</td>
<td>13 (17.8)</td>
<td>7 (10.3) (P = 0.2)*</td>
<td>14 (15.9)</td>
<td>6 (11.3)</td>
</tr>
<tr>
<td>PSA + hK2</td>
<td>Negative</td>
<td>30 (41.1)</td>
<td>37 (54.4) (P = 0.11)*</td>
<td>41 (46.6)</td>
<td>26 (49.1)</td>
</tr>
<tr>
<td>PSMA + hK2</td>
<td>Positive</td>
<td>19 (26.0)</td>
<td>16 (23.5)</td>
<td>23 (26.1)</td>
<td>12 (22.6)</td>
</tr>
<tr>
<td>PSMA + hK2</td>
<td>Negative</td>
<td>11 (15.1)</td>
<td>19 (26.0) (P = 0.06)*</td>
<td>16 (18.2)</td>
<td>14 (26.4) (P = 0.247)*</td>
</tr>
<tr>
<td>PSA + PSMA + hK2</td>
<td>Positive</td>
<td>11 (15.1)</td>
<td>5 (7.4) (P = 0.149)*</td>
<td>11 (12.5)</td>
<td>5 (9.4)</td>
</tr>
<tr>
<td>PSA + PSMA + hK2</td>
<td>Negative</td>
<td>9 (12.3)</td>
<td>3 (4.4) (P = 0.267)*</td>
<td>13 (14.8)</td>
<td>9 (17.0)</td>
</tr>
</tbody>
</table>

NOTE. P was calculated by χ² test.

* Double positive versus not double positive, that is, positive for both markers versus not positive for both markers.
† Double negative versus not double negative, that is, negative for both markers versus not negative for both markers.
‡ All positive versus some were negative or positive.
§ All negative versus some were negative or positive.
monally. In addition, a recent publication did not see significant changes in the Kaplan–Maier analysis for biochemical relapse-free survival excluding patients with preoperative androgen-deprivation therapy (12). In summary, to date, studies that examine the influence of neoadjuvant hormonal therapy on RT-PCR testing are conflicting and not conclusive. Even if we presume a significant effect of hormonal ablation on RT-PCR studies, our study population represented a homogeneous group because all of the patients received hormonal ablation, and no systemic error in the analysis took place.

REFERENCES

Prognostic Value of Combined "Triple"-Reverse Transcription-PCR Analysis for Prostate-Specific Antigen, Human Kallikrein 2, and Prostate-Specific Membrane Antigen mRNA in Peripheral Blood and Lymph Nodes of Prostate Cancer Patients

Ralf Kurek, German Nunez, Nikolaos Tselis, et al.


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