Circulating Mitochondrial Nucleic Acids Have Prognostic Value for Survival in Patients with Advanced Prostate Cancer

Niven Mehra,† Maarten Penning,‡ Jolanda Maas,§ Nancy van Daal,‡ Rachel H. Giles,¶ and Emile E. Voest¶

Abstract

**Purpose:** Advanced prostate cancer represents a heterogeneous disease entity with differences in clinical behavior, response to therapy, and survival. We assessed whether we could distinguish poor from good prognosis patients at presentation in our clinic by means of quantifying circulating cell-free mitochondrial and genomic nucleic acids in plasma.

**Experimental Design:** We collected plasma from 75 prostate cancer patients and from 14 subjects with benign disease. Nucleic acids were isolated, and mitochondrial DNA (mtDNA; 16S rRNA), mitochondrial RNA (mtRNA; cytochrome c oxidase subunit 1), and genomic DNA (U1A DNA) transcripts were quantified by real-time amplification. An association between cell-free nucleic acids and metastasis, prostate-specific antigen doubling time, and hemoglobin levels was determined. Multivariate Cox proportional hazard and survival estimation studies were done.

**Results:** We show that elevated mtDNA and mtRNA levels are present in plasma of prostate cancer patients with a poor 2-year survival (P = 0.02 and 0.003, respectively). Cancer patients with high plasma mitochondrial nucleic acids, using a calculated optimal cutoff point, show a decreased survival compared with patients with low levels (35% versus 73% cumulative survival for mtDNA and 21% versus 73% for mtRNA). Multivariate analysis indicates that mtRNA is an independent predictor of 2-year survival.

**Conclusions:** Quantification of plasma mitochondrial nucleic acids may be used to recognize patients with a poor prognosis. In advanced prostate cancer patients, mtRNA seemed the strongest predictor of overall survival and an independent prognostic factor for cancer-related death. Amplification of mitochondrial nucleic acids shows increased sensitivity and specificity over genomic DNA as diagnostic and prognostic marker in prostate cancer patients.

Prostate cancer is the most common cancer among men, with approximately 230,000 new annual cases in the United States alone. About 10% to 20% of men with prostate cancer present with metastatic disease, and in many others, metastases develop despite treatment with surgery or radiotherapy. For the group with distant metastasis, 5-year survival is 34%, and consequently, treatment is predominantly palliative (1). Advanced prostate cancer embodies a heterogeneous disease entity with varying clinical behavior, and therefore, distinguishing the population at high risk for treatment failure and death would allow for better disease prognostication and patient decision making.

Quantification of circulating plasma nucleic acids has been suggested as a diagnostic marker for cancer (2–5), although recent studies have focused on the quantification of nucleic acids before and during treatment in previously diagnosed cancer patients. A decrease in genomic nucleic acids in plasma seems to be related with a response to treatment (6–9) and/or an association with disease-free and overall survival (9–11). Elevated circulating nucleic acids in plasma of cancer patients are considered to originate from lysis (12), apoptosis (13, 14), necrosis (15), and/or spontaneous active release from (circularizing) tumor cells (13, 16). It has been shown that alterations in the circulating DNA match the mutations present in the tumor, indicating that at least part of these elevated circulating nucleic acids originate from tumor cells (17). The nucleic acids present in plasma are bound to the surface of blood cells (18, 19), proteins (20), nuclear complexes (21), or apoptotic bodies (11), which enhance stability and provide protection from nucleases. Recent evidence has also shown the presence of stable RNA in the plasma, serum, and saliva of cancer patients (11, 22, 23).

Most reports have focused on quantification of genomic nucleic acids in plasma, but plasma may also contain extrachromosomal mitochondrial nucleic acids. A single cell can contain hundreds to thousands of mitochondrial DNA (mtDNA) copies as opposed to two copies of genomic DNA. Although mtDNA levels previously have been quantified in plasma of healthy volunteers (24), no reports measured mtDNA in the plasma of cancer patients. One recent report

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quantified mtDNA in the saliva of patients with head and neck cancer and healthy controls and found a significant increase of mtDNA levels in cancer patients and a correlation between mtDNA and tumor stage (25).

In this report, we assessed whether we could use real-time quantification of mitochondrial nucleic acids in plasma to determine prognosis in advanced prostate cancer patients at presentation in our outpatient clinic. Here, we provide evidence that mitochondrial nucleic acids are increased in prostate cancer patients with a poor prognosis and that measuring mitochondrial nucleic acids can be used to evaluate survival probabilities independent of current prognostic factors.

Materials and Methods

Characterization of study patients and healthy volunteers. Blood samples were collected from advanced prostate cancer patients visiting the outpatient clinic of the Departments of Medical Oncology and Urology, University Medical Center Utrecht (Utrecht, the Netherlands) from October 2001 to July 2003. The study was approved by the hospital Ethics Committee, and written informed consent was obtained from all patients. Male control subjects (n = 14) were patients referred by general practitioners to the Urology outpatient clinic with the differential diagnosis of prostate cancer and whom were later diagnosed having benign prostate hyperplasia, prostatitis, or benign urethral or bladder pathology. Patient follow-up ended July 2005.

The following patient characteristics were recorded at the time of blood collection: age, presence of metastasis, hemoglobin count, and prostate-specific antigen (PSA). The median referral PSA in the prostate cancer group was 31.0 ng/mL (interquartile range, 3.4-556.5) and 1.5 ng/mL (interquartile range, 0.7-2.8) in the benign group. PSA doubling time (PSAdt) was calculated by assuming first-order kinetics and by using two additional PSA measurements after their initial visit, separated by a minimum of 3 months, or until the patients started chemotherapeutic and/or radiotherapeutic treatment. An increase in PSA was defined as >0.2 ng/mL change from previous measurement. The median referral PSA in the prostate cancer group was 31.0 ng/mL (interquartile range, 3.4-556.5) and 1.5 ng/mL (interquartile range, 0.7-2.8) in the benign group. PSA doubling time (PSAdt) was calculated by assuming first-order kinetics and by using two additional PSA measurements after their initial visit, separated by a minimum of 3 months, or until the patients started chemotherapeutic and/or radiotherapeutic treatment. An increase in PSA was defined as >0.2 ng/mL change from previous measurement. Dichotomized PSAdt (<3 or >3 months PSAdt) was used as a surrogate end point for prostate cancer—specific mortality as previously described (26). PSAdt was retrospectively determined for 59 of 75 patients. Most prostate cancer patients had hormone-refractory prostate disease.

Plasma isolation. After blood collection with BD Vacutainer tubes with sodium citrate (BD Biosciences, Mountain View, CA), the samples were directly placed on ice. Plasma was isolated from blood of patients within 2 h by centrifugation at 1,700 relative centrifugal force for 15 min. The plasma was carefully removed, without disturbing theuffy coat, aliquoted, and stored at −80°C until further use. The plasma was spun once before storage (defined as one-spin plasma). We analyzed the batch of samples collectively after a median of 26.6 months of storage (interquartile range, 23.2-34.5 months). The samples were thawed at room temperature and centrifuged at 3,400 relative centrifugal force for 15 min to remove aggregates formed in the freezethawing process before nucleic acid isolation.

Nucleic acid sequence-based amplification. For the quantification of mitochondrial and genomic nucleic acids, we used a one-tube, real-time detection and quantification method based on nucleic acid sequence-based amplification. Nucleic acids were isolated from 100 µL of the supernatant plasma, and standard nucleic acid sequence-based amplification reactions were done as previously described (27). The amplified mtDNA transcript encodes 16S rRNA, and the mitochondrial RNA (mtRNA) transcript encodes *cytochrome c oxidase subunit 1*. In selected primer regions, no mutations are described for prostate cancer3 (28). *Cytochrome c oxidase subunit 1* nucleic acid sequence-based amplification primer sequences are AATTTCTAATACGACTCACTATAGGG (molecular beacon, stem sequence shown in bold). 16S rRNA primers are GAATCTTAACTGACACTCAATATGGGAAGAAA-GCCCGGTCTCGCCATCTTAA (primer P1, T7 italicized), GAATCTCA-GTCCGTTCTCA (primer P2), and GTGACGTTATATCCTCCTTTAGGTAAC (molecular beacon, stem sequence in bold). The amplified genomic transcript encodes *UIA*. The primer set included in this gene was reported (27). To avoid (co)amplification of mtRNA in the mtDNA amplification assay, primers were designed to span a RNA splice site with one primer in the 3′-end of 16S rRNA and the other in the tRNA just downstream of the mitochondrial transcription terminator. As MspI digestion and denaturation was not done in the mtRNA amplification assay, mtDNA could not be amplified.

A dilution series of target sequence (plasmid containing mtDNA and genomic target sequence and *in vitro*—translated RNA for mtRNA target sequence) was amplified, and the time points at which the reactions became positive (the time to positivity) were plotted against the input amounts of nucleic acids. This way, a calibration curve was created that could be used to determine the amount of target sequence present in patient plasma samples. The average of duplicate amplifications was considered as the value for a sample. If the difference between duplicate amplifications was >0.5 log value, the amplification for that sample was repeated. Due to repeated differences between duplo measurements of both mtDNA and mtRNA, 3 of 89 samples were excluded from further analysis. For genomic *UIA* DNA measurements, 83 of 89 samples were available.

Cell culture. PC-3 prostate cancer cells (American Type Culture Collection, Rockville, MD) were cultured in RPMI 1640 (Life Technologies, Invitrogen, Breda, the Netherlands) supplemented with penicillin, streptomycin, glutamate, and 5% FCS. For generation of PC-3 cells devoid of mitochondria, cells were cultured in the presence of 50 ng/mL ethidium bromide, 50 µg/mL uridine, and 100 µg/mL pyruvate. Approximately 300,000 cells were lysed in Lysis buffer and frozen at −80°C until nucleic acid isolation and quantification.

Statistical analysis. For differences in median copy number of mtDNA and mtRNA between survivors and nonsurvivors after a 2-year follow-up period, patients with and without metastasis, patients with high or low PSAdt, and patients with cancer or benign disease, the nonparametric Mann-Whitney test was used. For correlative testing of mtDNA copies, mtRNA copies, patient age, PSA, and hemoglobin levels, the Spearman’s correlation coefficient was used.

A receiver operating characteristic curve was constructed to discriminate survivors from nonsurvivors and to discriminate patients with cancer from patients with benign disease using mtDNA or mtRNA copy numbers. For cancer prognostics and diagnostics, the cutoff value with the highest sensitivity and specificity was used and selected in all subsequent analyses. To give an idea of mtDNA and mtRNA as strong predictors of survival, we calculated survival prediction results of dichotomous mtDNA and mtRNA based on a variety of cutoffs. The results indicated that the choice of cutoff does not influence the relevance of mtDNA and mtRNA as predictors of patient outcome.

Differences in survival between cancer patients with mitochondrial nucleic acids below or above selected cutoff values were evaluated according to the method of Kaplan and Meier. The log-rank statistic was used to assess differences between both arms of the survival curves.

To identify independent factors influencing survival, multivariate risk factor assessment was done using the Cox proportional hazards model. Variables included in the model were presence of metastasis, hemoglobin count, PSA, and mtDNA or mtRNA. The laboratory variables hemoglobin, PSA, and PSAdt were not determined in 21, 7, and 16 cases, respectively; therefore, correlative testing and Cox proportional hazards analysis for these variables were done on <75 prostate cancer cases.

All results were analyzed using Statistical Package for the Social Sciences software (version 12.0.1). Error bars depicted are SEs of the mean. Interquartile ranges shown are from 25th to 75th percentile. Values of <0.05 (two sided) were considered significant.

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Results

Validation of real-time nucleic acid sequence-based amplification assay for mtDNA and mtRNA transcripts. We first assessed linearity of the assay by creating a calibration curve of the mtDNA and mtRNA target sequence. The transcripts encoding 16S rRNA and cytochrome c oxidase subunit 1 were quantified, and a standard curve of mtDNA and mtRNA input in relation to time to positivity was generated (Fig. 1A). We next tested the specificity of our assay by quantification of mitochondrial nucleic acids of the PC-3 prostate cancer cell line treated with the intercalating agent ethidium bromide, which at low concentrations preferentially inhibits the synthesis of mtDNA, mtRNA, and mitochondrial proteins (29). After 4 weeks of culture, no mitochondrial nucleic acids were detectable (Fig. 1B). The growth rate and viability in respiratory-deficient PC-3 cells was not affected when the cells were cultured in medium supplemented with pyruvate and uridine to enable ATP generation through glycolysis (data not shown).

Relating mitochondrial and genomic nucleic acids with clinical and laboratory variables. We isolated nucleic acids from the plasma of 89 patients with prostate cancer and benign disease (patient characteristics are shown in Table 1) and quantified the transcripts encoding 16S rRNA, cytochrome c oxidase subunit 1, and U1A DNA. In Table 2, the median copy numbers are shown for mitochondrial and genomic nucleic acids in all samples measured. Median copies of mtDNA and mtRNA in plasma were respectively ~3,000-fold and ~700-fold higher than genomic DNA. We then evaluated whether extracellular mtDNA in plasma was related with extracellular mtRNA and found a strong correlation \( n = 86; r = 0.73, P < 0.001 \). However, no significant correlation was found between genomic and mitochondrial nucleic acids. Subsequently, we examined the relation between mtDNA and hemoglobin count \( n = 58; r = -0.29, P = 0.028 \) and PSA levels \( n = 74; r = 0.27, P = 0.021 \). mtRNA was also significantly correlated with PSA levels \( n = 74; r = 0.31, P = 0.007 \) but not with hemoglobin count \( n = 58; r = -0.25, P = 0.064 \). There was no significant relation between mitochondrial nucleic acids in plasma and patient age \( n = 69; r = -0.20, P = 0.10 \) for mtDNA and \( r = -0.16, P = 0.19 \) for mtRNA. There were also no significant differences in levels of mtDNA or mtRNA in patients with metastasized disease compared with patients with locally advanced cancer \( P = 0.31 \) and 0.26, respectively), between patients with androgen-dependent or androgen-independent prostate cancer \( P = 0.67 \) and 0.86, respectively), or between patients with high or low PSAdt \( P = 0.09 \) and 0.89, respectively). No significant associations were found between plasma U1A DNA and the clinical and laboratory variables tested (data not shown).

High plasma mitochondrial nucleic acids in poor prognosis patients. We determined whether we could recognize patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign prostate disease</td>
<td>14 (15.7)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>75 (84.3)</td>
</tr>
<tr>
<td>Metastasis</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>51 (68.0)</td>
</tr>
<tr>
<td>No</td>
<td>24 (32.0)</td>
</tr>
<tr>
<td>Hormone refractory</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>61 (81.3)</td>
</tr>
<tr>
<td>No</td>
<td>14 (18.7)</td>
</tr>
<tr>
<td>Survival</td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>26 (34.7)</td>
</tr>
<tr>
<td>Deceased, tumor related</td>
<td>43 (57.3)</td>
</tr>
<tr>
<td>Deceased, nontumor related</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Unknown/lost to follow-up</td>
<td>5 (6.7)</td>
</tr>
<tr>
<td>Survival (mo)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>28.1</td>
</tr>
<tr>
<td>Interquartile range (2%-75%)</td>
<td>8.9-35.7</td>
</tr>
</tbody>
</table>

Fig. 1. Validation of real-time amplification mitochondrial nucleic acids. A, calibration curve of the transcripts encoding 16S rRNA (mtDNA (dotted line)) and cytochrome c oxidase subunit 1 (mtRNA (solid line)). B, PC-3 prostate cancer cells were cultured with ethidium bromide (EtBr) for 4 wks, and every week, mtDNA (dotted line) and mtRNA (solid line) transcripts were quantified.
with poor prognosis at presentation in our outpatient clinic by quantification of circulating cell-free mitochondrial nucleic acids in plasma. After a median follow-up time of 28.4 months after inclusion, 57% of all patients with advanced prostate cancer had died due to progression of their tumor. We assessed whether poor prognosis patients, based on 2-year follow-up data, had elevated mitochondrial nucleic acids. At presentation in our outpatient clinic, nonsurvivors had a 3.8-fold increase in mtRNA compared with survivors (P = 0.003; nonsurvivors: median copies, 16,038; interquartile range, 5,097-48,544 copies; survivors: median copies, 4,183; interquartile range, 2,284-8,579 copies). Plasma mtDNA levels were also increased in nonsurvivors, with a 2.6-fold increase compared with survivors (P = 0.02; nonsurvivors: median copies, 16,590; interquartile range, 30,765-189,131 copies; survivors: median copies, 23,275; interquartile range, 14,372-59,249 copies). In contrast, plasma U1A levels were not increased in poor prognosis patients (P = 0.71).

**Survival prediction based on levels of plasma mitochondrial nucleic acids.** We subsequently assessed whether we could discriminate survivors from nonsurvivors based on dichotomized mtDNA and mtRNA variables. The area under the receiver operating characteristic curve and sensitivity and specificity for selected mtDNA and mtRNA cutoff points are shown in Table 3. We assessed whether patients with plasma nucleic acid levels above 26,000 copies mtDNA and 10,500 copies mtRNA had significant differences in 2-year survival compared with patients with lower levels. Kaplan-Meier estimates showed significant differences for patients both with high and low mtDNA and mtRNA (Fig. 2A and B). Cumulative 2-year survival for patients with elevated mtDNA levels was 35%, whereas survival of patients with low levels was 73%. Cumulative survival after 2 years for patients with high and low mtRNA levels was 21% and 73%, respectively. Median survival for patients with elevated mtDNA and mtRNA was only 13.1 and 11.8 months, respectively.

**mtRNA is an independent predictor of survival.** We evaluated whether circulating plasma mtDNA or mtRNA were predictors of patient survival and whether they had additive value when combined with established prognostic markers. We analyzed the relationship of metastasis status, PSAdt, and hemoglobin count with patient outcome in our advanced cancer population and found that all variables were significantly associated with cancer-related death by univariate analysis. Dichotomized mtDNA and mtRNA were assessed together with these variables in a multivariable model to establish the independent properties of mtDNA and mtRNA in prostate cancer prognostics. In addition, we evaluated whether mtDNA or mtRNA was the stronger predictor. When testing mtRNA against all variables, hemoglobin count, PSAdt, and mtDNA were all significant independent predictors of survival [hazard ratio (HR), 0.45; 95% confidence interval (95% CI), 0.36-0.68; HR, 0.32; 95% CI, 0.23-0.43; and HR, 2.87; 95% CI, 1.09-7.57, respectively; Table 4]. When testing mtDNA, only hemoglobin count and PSAdt were significant independent predictors for 2-year survival (HR, 0.41; 95% CI, 0.23-0.74 and HR, 0.26; 95% CI, 0.10-0.68, respectively).

**Comparison of plasma mtDNA, mtRNA, and genomic DNA levels in prostate cancer patients and control subjects.** Next, we analyzed plasma from patients with benign disease of the prostate and urinary tract as control subjects. The median mtDNA copies per 100 μL plasma for prostate cancer patients were 49,193 (interquartile range, 18,683-109,485 copies) and 19,037 (interquartile range, 13,515-24,744 copies) for benign controls. Median mtRNA copies were 9,321 (interquartile range, 3,507-25,435 copies) and 3,161 (interquartile range, 2,284-7,340 copies) for prostate cancer patients and benign controls, respectively. When we compared the levels of nucleic acids in cancer patient plasma and the 14 benign controls, we found a significant increase of both mtDNA and mtRNA (Mann-Whitney, P = 0.005 and 0.029, respectively) but not for U1A DNA (P = 0.25). We assessed how well mtDNA or mtRNA plasma levels could discriminate prostate cancer from benign disease by receiver operating characteristic analysis. The area under the receiver operating characteristic curve for mtDNA-based diagnosis of prostate cancer patients was 0.76 (95% CI, 0.63-0.90) and 0.70 (95% CI, 0.55-0.85) for mtRNA. Selected cutoff points were 26,000 copies for mtDNA and 5,400 copies for mtRNA. Sensitivity and specificity for identification of prostate cancer from subjects with prostatitis, benign

### Table 2. Total nucleic acid level in plasma

<table>
<thead>
<tr>
<th>Sample</th>
<th>Genomic DNA</th>
<th>mtDNA</th>
<th>mtRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>Median</td>
<td>10.5</td>
<td>34,938</td>
</tr>
<tr>
<td></td>
<td>Interquartile</td>
<td>1.1-25.6</td>
<td>16,304-80,502</td>
</tr>
<tr>
<td>Benign disease</td>
<td>% detectable</td>
<td>82</td>
<td>100</td>
</tr>
<tr>
<td>Median</td>
<td>4.3</td>
<td>19,037</td>
<td></td>
</tr>
<tr>
<td>Interquartile</td>
<td>0.6-24.5</td>
<td>13,515-24,744</td>
<td></td>
</tr>
<tr>
<td>% detectable</td>
<td>85</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Median</td>
<td>12.8</td>
<td>49,193</td>
</tr>
<tr>
<td>Interquartile</td>
<td>1.2-25.7</td>
<td>18,683-109,485</td>
<td></td>
</tr>
<tr>
<td>% detectable</td>
<td>83</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Genomic and mitochondrial copy number in patient samples.

### Table 3. Selected prognostic cutoff values in prostate cancer patients

<table>
<thead>
<tr>
<th>Type</th>
<th>NA</th>
<th>Cutoff</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>AUC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>mtDNA</td>
<td>26,000</td>
<td>80</td>
<td>58</td>
<td>65</td>
<td>73</td>
<td>0.70</td>
<td>0.58-0.81</td>
</tr>
<tr>
<td></td>
<td>mtRNA</td>
<td>10,500</td>
<td>69</td>
<td>79</td>
<td>77</td>
<td>71</td>
<td>0.72</td>
<td>0.60-0.83</td>
</tr>
</tbody>
</table>
prostate hyperplasia, and other benign disorders of the urogenital organs were 67% and 92% for mtDNA and 63% and 77% for mtRNA, respectively.

**Discussion**

Of the patients who died within 2 years after presentation, 80% had elevated levels of mtDNA and ~70% had elevated mtRNA copies in their plasma compared with survivors. Cumulative 2-year survival was markedly decreased for patients with high levels of circulating mitochondrial nucleic acids. We therefore consider circulating mitochondrial nucleic acids as a novel prognostic marker for advanced cancer patients.

In the first report evaluating circulating nucleic acids (30), the authors found a 90% decrease in plasma DNA of patients following radiotherapy, and persistent high or increasing plasma DNA was associated with poor prognosis. Other groups have since reported an association of high plasma DNA levels with poor prognosis or a lack of response to treatment (6, 9). mtDNA encodes for proteins of the respiratory chain and cells can contain hundreds to thousands of mtDNA copies per cell instead of two copies of genomic DNA. Here, we show that amplification of mitochondrial nucleic acids in plasma has an advantage over genomic nucleic acids. We report that elevated levels of mitochondrial nucleic acids measured at presentation in our clinic were already predictive for patient outcome. Whether mitochondrial nucleic acids can also be used as a follow-up marker during therapy is currently being assessed in a prospective study.

As it has been shown that nucleic acids can be released from cells actively or passively (cell death), one would speculate that both RNA and DNA end up extracellular. Previous reports have shown that WBCs possess nucleic acid-binding receptors on their outer membranes and internalize and degrade nucleic acids into oligonucleotides (31). In this study, we find a correlation between mtDNA and mtRNA copies and between mitochondrial nucleic acids and PSA levels. This may indicate that the elevated nucleic acids have a direct relationship with tumor cell burden (32) and/or that paraneoplastic characteristics, such as enhanced protease activity, may cause a decrease in the nucleic acid–binding capacity to circulating blood cells (18). However, retrospective studies are exploratory and may not reflect true associations and should therefore be treated with caution. Nevertheless, mtRNA is a stronger predictor of patient outcome than mtDNA, and only mtRNA is an independent prognostic factor in multivariate analysis. Whether mtRNA stability is specifically enhanced in poor prognosis patients has yet to be determined.

In the first publication on circulating plasma nucleic acids in 1977, the authors describe an increase of DNA in the plasma of cancer patients compared with healthy controls (30). Since then, several groups have shown an increase in circulating plasma nucleic acids in cancer patients and show a sensitivity and specificity of 85% and 73% in identifying prostate cancer from benign disease (3). However, a similar study could not confirm the potential value of plasma nucleic acids as a

![Fig. 2. Kaplan-Meier estimates of overall survival. Cumulative 2-yr survival of prostate cancer patients with high (bold line) and low (faint line) circulating cell-free mtDNA (A) and mtRNA (B) levels at presentation.](image)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Metastasis</td>
<td>1.89 (0.41-8.79)</td>
</tr>
<tr>
<td>Hemoglobin count</td>
<td>0.41 (0.23-0.74)</td>
</tr>
<tr>
<td>PSA dt</td>
<td>0.26 (0.10-0.68)</td>
</tr>
<tr>
<td>mtDNA*</td>
<td>2.58 (0.83-8.07)</td>
</tr>
<tr>
<td>Metastasis</td>
<td>1.84 (0.40-8.57)</td>
</tr>
<tr>
<td>Hemoglobin count</td>
<td>0.45 (0.26-0.80)</td>
</tr>
<tr>
<td>PSA dt</td>
<td>0.32 (0.13-0.81)</td>
</tr>
<tr>
<td>mtRNA*</td>
<td>2.87 (1.09-7.57)</td>
</tr>
</tbody>
</table>

*mtDNA cutoff, 26,000 copies.  
*mtRNA cutoff, 10,500 copies.

![Table 4. Multiple Cox proportional hazards analyses for survival](image)
diagnostic tool in prostate cancer screening (33). In addition, quantification of circulating plasma RNA does not seem to have enough discriminatory power as a screening tool for cancer (34). In this study, we show that prostate cancer patients have an ~3-fold increase in mtDNA and a 2-fold increase in mtRNA copy number compared with the benign controls. However, the sensitivity and specificity are not sufficient to warrant further studies as a diagnostic tool.

To conclude, we show a strong increase in plasma mitochondrial nucleic acids in prostate cancer patients with poor prognosis. Quantification of mitochondrial nucleic acids in plasma could be a valuable predictor of prognosis in advanced hormone-refractory relapsed prostate cancer patients, and their potential should be further evaluated in a large prospective multicenter study.

References

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