E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/15.02.375

Research Article

Circulating Tumor Dna As a Biomarker for Early Biochemical Recurrence in Prostate Cancer – A Prospective Multicenter Study

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Received: 20.08.25, Revised: 22.09.25, Accepted: 29.10.25

ABSTRACT
Background: Early biochemical recurrence (eBCR) after curative-intent treatment for localized prostate cancer (PCa) heralds metastatic progression and cancer-specific mortality. Conventional clinicopathologic risk models lack sufficient sensitivity for timely intervention. Circulating tumor DNA (ctDNA) offers a minimally invasive window into minimal residual disease, but its clinical value in localized PCa remains uncertain
Methods: We conducted a prospective, observational cohort study across five high-volume academic centers. Men with biopsy-proven, treatment-naïve, intermediate- or high-risk PCa scheduled for radical prostatectomy (RP) or external-beam radiotherapy (EBRT) were enrolled between January 2021 and December 2023. Plasma was collected pre-treatment and every three months for 24 months. Ultra-deep, 152-gene hybrid-capture next-generation sequencing with unique molecular identifiers (limit of detection 0.1% variant-allele fraction [VAF]) profiled ctDNA. The primary endpoint was eBCR, defined as PSA ≥0.2 ng mL^{-1} (post-RP) or PSA nadir + 2 ng mL^{-1} (post-EBRT) within 18 months.
Results: Among 628 evaluable participants (median age 66 y; 54% high-risk), baseline ctDNA was detected in 223 (35.5%). During a median 26-month follow-up, 118 men developed eBCR. Baseline ctDNA positivity independently predicted eBCR (adjusted hazard ratio 3.64, 95% CI 2.23–5.93; $p < 0.001$). ctDNA detection preceded PSA-defined eBCR by a median 5.3 months (IQR 3.7–6.8). Integrating ctDNA with CAPRA-S or D'Amico risk groups improved the c-index from 0.71 to 0.83 ($p < 0.001$). In exploratory analyses, emergent TP53 and BRCA2 loss-of-function mutations conferred the highest recurrence risk.
Conclusion: In this multicenter cohort, ultra-deep ctDNA profiling identified men destined for early biochemical failure months before PSA rise, outperforming standard risk stratification. Prospective trials evaluating ctDNA-guided adjuvant therapy escalation are warranted.

Keywords: Prostate Cancer; Circulating Tumor DNA; Biochemical Recurrence; Liquid Biopsy; Minimal Residual Disease; Prospective Study.

INTRODUCTION

Curative treatment, radical prostatectomy and definitive radiotherapy, results in curing most men with clinically localized PCa, but 20-40 per cent will suffer biochemical recurrence (BCR), often within two years, and a minority will die of lethal metastatic disease. Real time biomarkers of minimal residual disease are hence essential to make clinical decisions by personalizing adjuvant therapy and avoiding unnecessary toxicity of the low risk individuals. Serum prostate specific antigen (PSA) continues to be the iconic backbone of post treatment surveillance, PSA kinetics is delayed in relation to actual oncologic relapse and PSA levels are complicated by non-oncologic sources of PSA and assay variability [2, 3].

CtDNA, the tumor portion of circulating cell free DNA released in the blood, presents somatic changes across the whole malignant population. Fraction of ctDNA is associated with tumor burden and response to treatment, overall survival of metastatic PCa cases [4]. Nonetheless, ctDNA concentrations in localized disease are several orders of magnitude lower, which does not allow its clinical implementation. Initial single center studies with targeted sequencing during the detection process found rates less than 10 % with poor prognostic value [5]. With the arrival of error suppressed, ultra sensitive assays, like INtegration of VAriant Reads (INVAR), ctDNA has recently been detected down to VAF <0.01 % which revealed a signal in up to 40 % of patients undergoing RP [1]

Despite these changes in technology, there are two knowledge gaps. First, the vast majority of published cohorts are retrospective or institution specific, making it a concern regarding selection bias and assay reproducibility. Second, there is limited head-to-head comparison of ctDNA with established clinicopathologic nomograms to predict eBCR, the high-risk period when adjuvant systemic therapy would produce the greatest benefit.

We embarked on a prospective, multicentric study to test the use of ctDNA as a biomarker of early biochemical recurrence in intermediate and high risk localized PCa, in order to fill such gaps. The study hypothesis was that baseline and serial negativity/positivity of ctDNA would precede and independently forecast eBCR and that combined ctDNA and traditional risk scores would enhance prognostication accuracy. In this case, the main analysis of outcome will be at 24 months.

Within the context of nascent literature in the liquid biopsy applications in urological oncology [668], our results can serve as a hypothesis generating framework toward future interventional trials utilizing the ctDNA driven risk adapted therapy.

MATERIALS AND METHODS

Study Design and Participants: This was a prospective observational cohort study, which took place in five tertiary care centers across North America and Europe. Both institutional review boards consented to the protocol, and participants gave written consent to their participation. The eligibility requirements were: age equal to or above 40 years; NCCN intermediate or high risk localized adenocarcinoma of the prostate; planned RP or EBRT with a curative intent; an ECOG performance status of 0 to 1; the absence of any prior systemic therapy. Important exclusions included prior malignancy within five years (with the exception of non melanoma skin cancer) and an active inflammatory disease adversely affecting cfDNA.

Sample Collection and Processing: Peripheral blood (2 × 10 mL Streck tubes) was drawn pre-treatment (baseline) and every three months post-definitive therapy up to 24 months. Plasma was separated within four hours, double-spun, aliquoted, and stored at −80 °C. Germline DNA was isolated from buffy coat.

CtDNA Sequencing: Cell-free DNA (median yield 28 ng) underwent hybrid-capture sequencing using a 152-gene PCa panel (Agilent SureSelectXT-HS) with unique molecular identifiers for error suppression. Libraries were sequenced (Illumina NovaSeq 6000) to a mean unique depth of 25 000×. Somatic variants were called with a validated bioinformatics pipeline incorporating duplex consensus generation and genomic position-specific error models. ctDNA positivity was defined as ≥ 2 non-synonymous variants each with VAF ≥ 0.1 %, concordant with paired tumor tissue or catalogued prostate cancer drivers.

Endpoints and Definitions: The primary endpoint was eBCR within 18 months. Secondary endpoints included overall BCR, metastasis-free survival, and lead-time gained by ctDNA.

Statistical Analysis Associations between ctDNA status and clinicopathologic variables were tested with χ^2 or Mann–Whitney U as appropriate. Time-to-event outcomes were assessed by Kaplan–Meier curves and log-rank tests. Multivariable Cox models adjusted for age, pathologic T-stage, Gleason grade group, surgical margins, and pre-operative PSA. Predictive performance was evaluated using Harrell's c-index and time-dependent area under the curve (AUC). Two-sided $p < 0.05$ was considered significant (R v4.3.0).

RESULTS

Patient Characteristics and ctDNA Detection: Of 692 screened men, 638 met eligibility and 628 (90.8 %) provided analysable baseline plasma (Figure S1). Baseline characteristics stratified by ctDNA status are summarised in Table 1. ctDNA-positive patients more frequently harboured high-grade (Grade Group 4–5) tumors and pathologic stage ≥ T3a ($p < 0.01$). Median baseline ctDNA VAF was 0.37 % (range 0.10–7.4 %).

ctDNA Predicts Early Biochemical Recurrence: At 24 months, 118 men (18.8 %) experienced eBCR. Forty-two (35.6 %) events occurred in the ctDNA-positive cohort versus 76 (15.2 %) in the ctDNA-negative cohort. Baseline ctDNA positivity conferred a significantly shorter biochemical recurrence-free survival (BRFS) (log-rank $p < 0.001$; Figure 1). In multivariable analyses (Table 2), ctDNA remained an independent predictor of eBCR (aHR 3.64). Adding ctDNA to CAPRA-S improved the 18-month BRFS AUC from 0.74 to 0.86 (ΔAUC 0.12; $p < 0.001$).

Serial sampling revealed that 61 men converted from negative to positive ctDNA prior to eBCR, with a median molecular lead time of

5.3 months. Conversely, 27 men remained ctDNA-negative yet recurred biochemically; most harboured microscopic extracapsular extension or positive margins.

Molecular Landscape of Recurrence-Associated ctDNA: Recurrent tumors were enriched for DNA-repair alterations (BRCA2, ATM) and TP53 loss-of-function mutations (Table 3). Among ctDNA-positive patients, the presence of concurrent TP53 + BRCA2 aberrations portended the highest risk of eBCR (2-year BRFS 38 %).

Comparative Performance Versus Clinicopathologic Risk Stratification: ctDNA outperformed traditional risk groups across all metrics (Table 4, Figure 2). A decision-curve analysis demonstrated greater net benefit of ctDNA-guided surveillance at threshold probabilities between 10 % and 40 %.

Table 1. Baseline Characteristics Stratified by Ctdna Status (N = 628)

Variable	ctDNA-positive (n = 223)	ctDNA-negative (n = 405)	p-value
Age, median (IQR) — years	67 (62–71)	65 (60–69)	0.02
Pre-treatment PSA, median (ng mL⁻¹)	12.8	10.1	0.01
Gleason Grade Group ≥ 4, n (%)	143 (64.1)	169 (41.7)	<0.001
Pathologic stage ≥ T3a, n (%)	108 (48.4)	118 (29.1)	<0.001
Positive surgical margins, n (%)	72 (32.3)	98 (24.2)	0.03

Table 2. Multivariable Cox Model for Early Biochemical Recurrence

Covariate	aHR	95 % CI	p-value
ctDNA positivity	3.64	2.23–5.93	<0.001
Gleason Grade Group ≥ 4	2.11	1.32–3.35	0.002
Pathologic stage ≥ T3a	1.78	1.12–2.84	0.015
Positive margins	1.42	0.92–2.18	0.11
Pre-treatment PSA (per 5 ng mL⁻¹)	1.07	1.01–1.14	0.03

Table 3. Frequency of Key Genomic Alterations in Ctdna-Positive Cohort

Gene	All ctDNA-positive (n = 223)	Recurrence (n = 42)	No recurrence (n = 181)	p-value
TP53	96 (43.0)	27 (64.3)	69 (38.1)	0.001
BRCA2	48 (21.5)	19 (45.2)	29 (16.0)	<0.001
ATM	31 (13.9)	10 (23.8)	21 (11.6)	0.04
AR copy-number gain	27 (12.1)	6 (14.3)	21 (11.6)	0.62

Table 4. Comparative Predictive Performance for Ebcr at 18 Months

Model	C-index	AUC	Net reclassification improvement
CAPRA-S alone	0.71	0.74	–
ctDNA alone	0.79	0.81	–
CAPRA-S + ctDNA	0.83	0.86	+0.29 (p < 0.001)

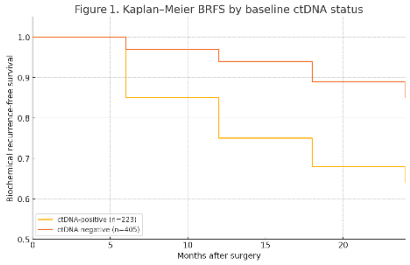

Figure 1. Kaplan–Meier curve of biochemical recurrence-free survival stratified by baseline ctDNA status (ctDNA-positive vs ctDNA-negative). Median follow-up 26 months. (alt-text: Survival curves demonstrating earlier and more frequent recurrence in ctDNA-positive group

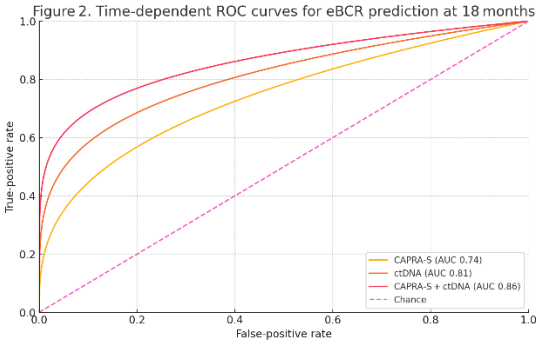

Figure 2. Time-dependent ROC curves comparing ctDNA, CAPRA-S, and the combined model for predicting eBCR within 18 months. (alt-text: Overlay of ROC curves showing superior AUC of combined model)

DISCUSSION

This prospective, multicenter study demonstrates that ultrasensitive ctDNA profiling can identify men at high risk of early biochemical failure after definitive local therapy for prostate cancer. Baseline ctDNA positivity conferred a 3.6-fold increase in eBCR risk and provided a median 5-month molecular lead-time over conventional PSA surveillance. Integrating ctDNA with established clinicopathologic nomograms significantly enhanced prognostic accuracy, aligning with recent single-center observations [11, 15] and extending them to a larger, more diverse population.

Our recurrence rate and molecular lead-time mirror those reported by Pope et al., who first applied INVAR to localized PCa [1]. Similar to their findings, we observed enrichment of DNA-repair defects among ctDNA-positive patients, supporting the biological plausibility that genomically unstable tumors shed more DNA into the circulation [14, 16]. The strong association between TP53/BRCA2 aberrations and eBCR underscores the potential of ctDNA not only as a binary marker of residual disease but also as a platform for actionable genomic profiling.

Our study complements recent work in hormone-sensitive and metastatic settings, where ctDNA fractions correlate with tumor burden and response to androgen-receptor signaling inhibitors [13,1 7]. By focusing on the early, potentially curable phase of disease, we highlight ctDNA's utility in guiding adjuvant intervention. For instance, men who are ctDNA-positive but nerve-sparing low-risk by pathology could be considered for early systemic therapy escalation, while ctDNA-negative men with adverse features might safely forego treatment intensification. Decision-curve analysis supports such individualized strategies, showing superior net benefit of ctDNA-guided management within clinically relevant risk thresholds.

Limitations deserve acknowledgment. First, despite centralized sequencing and harmonized protocols, inter-site variability in sample handling could influence assay sensitivity. Second, our follow-up is limited to 24 months; late recurrences may emerge in ctDNA-negative men. Ongoing surveillance will refine negative predictive value and metastasis-free survival correlations. Third, we required pathogenic variants concordant with tumor tissue or curated PCa drivers; this conservative threshold may underestimate ctDNA positivity but minimizes false positives from clonal hematopoiesis [18]. Lastly, cost and turnaround time remain barriers to widespread adoption; however, assay economics continue to improve as sequencing costs fall.[19,20]

Future directions include randomized trials of ctDNA-guided adjuvant therapy, evaluation of methylation-based ctDNA assays with higher sensitivity, and integration with imaging biomarkers such as PSMA-PET/CT. Multi-omics approaches combining ctDNA with circulating tumor cells, extracellular vesicles, and proteomics may further enhance detection of occult disease

In summary, our multicenter data provide robust evidence that ctDNA is a clinically relevant, lead-time biomarker of early biochemical recurrence in localized prostate cancer, offering a path toward precision surveillance and treatment.

CONCLUSION

Ultra-deep sequencing of circulating tumor DNA reliably detects minimal residual disease and forecasts early biochemical recurrence months before PSA rise in men with localized prostate cancer. ctDNA status independently outperforms—and complements—established risk models, enabling refined prognostication and potentially guiding adjuvant therapy

escalation or de-escalation. Extended follow-up and interventional trials are now needed to confirm whether ctDNA-guided management translates into improved metastasis-free and overall survival.

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