# Assessment of Serum microRNA Biomarkers to Predict Reclassification of Prostate Cancer in Patients on Active Surveillance

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**Purpose:** Conventional clinical variables cannot accurately differentiate indolent from aggressive prostate cancer in patients on active surveillance. We investigated promising circulating miRNA biomarkers to predict the reclassification of active surveillance cases.

**Materials and Methods:** We collected serum samples from 2 independent active surveillance cohorts of 196 and 133 patients for the training and validation, respectively, of candidate miRNAs. All patients were treatment naïve and diagnosed with Gleason score 6 prostate cancer. Samples were collected prior to potential reclassification. We analyzed 9 circulating miRNAs previously shown to be associated with prostate cancer progression. Logistic regression and ROC analyses were performed to assess the predictive ability of miRNAs and clinical variables.

**Results:** A 3-miR (miRNA-223, miRNA-24 and miRNA-375) score was significant to predict patient reclassification (training OR 2.72, 95% CI 1.50–4.94 and validation OR 3.70, 95% CI 1.29–10.6). It was independent of clinical characteristics in multivariable models. The ROC AUC was maximized when combining the 3-miR score and prostate specific antigen, indicating additive predictive value. The 3-miR score plus the prostate specific antigen panel cutoff achieved 89% to 90% negative predictive value and 66% to 81% specificity.

**Conclusions:** The 3-miR score combined with prostate specific antigen represents a noninvasive biomarker panel with high negative predictive value. It may be used to identify patients on active surveillance who have truly indolent prostate cancer.

**Key Words:** prostatic neoplasms; microRNAs; biomarkers, tumor; watchful waiting; predictive value of tests

**Abbreviations and Acronyms**

3-miR = miRNA-223, miRNA-24 and miRNA-375
AS = active surveillance
DRE = digital rectal examination
GS = Gleason score
miRNA = microRNA
MRI = magnetic resonance imaging
NPV = negative predictive value
PCa = prostate cancer
PHI = Prostate Health Index
PSA = prostate specific antigen
PSA-DT = PSA doubling time
UHN = University Health Network

PROSTATE cancer is the most commonly diagnosed type of cancer and the fifth leading cause of cancer related deaths in men worldwide.[1] PCa is usually diagnosed on needle biopsies prompted by an abnormal PSA test and/or abnormal DRE. Since the advent of PSA screening, the incidence of PCa has risen dramatically with most newly diagnosed patients

Accepted for publication December 2, 2017.

No direct or indirect commercial incentive associated with publishing this article.

The corresponding author certifies that, when applicable, a statement(s) has been included in the manuscript documenting institutional review board, ethics committee or ethical review board study approval; principles of Helsinki Declaration were followed in lieu of formal ethics committee approval; institutional animal care and use committee approval; all human subjects provided written informed consent with guarantees of confidentiality; IRB approved protocol number; animal approved project number.

Supported by Movember PCC TAG No. 2014-01 1417 (BB), Ontario Graduate Scholarships (RSCL, FZ), Ontario Student Opportunity Trust Funds Awards (RJ, FZ) and University of Toronto Fellowships (RSCL, RJ, FZ).

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now presenting early with clinically localized (low risk) disease.[2,3] There are significant limitations to the PSA test, such as a lack of specificity and sensitivity, over diagnosis and consequently overtreatment.[4,5] In these patients at low risk complications associated with definitive interventions such as radical prostatectomy and radiotherapy typically outweigh the potential benefits.[6]

Active surveillance is a treatment option in patients with PCa who are at low risk with favorable clinical and pathological presentation. This aims to mitigate overtreatment by closely monitoring patients for cancer progression and recommending treatment only in those who show signs of disease progression. Patients are monitored through several periodic procedures, including PSA testing, DRE, prostate biopsy and more recently multiparametric MRI.[7] In recent years AS has been increasingly performed to monitor PCa. Approximately 40% of newly diagnosed patients at low risk are on AS in contemporary practices across North America, Europe and Australia.[7–10]

In AS programs 25% to 35% of cases are reclassified to higher risk status and recommended for treatment within 5 years of enrollment due to disease progression or the detection of occult high grade PCa.[11,12] Triggers for reclassification commonly include rapid PSA kinetics, GS upgrading or increased tumor volume at repeat biopsy.[7,11,12] Thus, these patients with reclassified disease receive delayed treatment. Conversely patients who harbor truly indolent PCa undergo repeat invasive biopsies, which can cause pain, bleeding and infection.[13,14]

Conventional clinical markers used in AS programs, such as PSA and GS or the percent of biopsy cores positive for cancer (number of positive cores/total cores taken) at diagnosis, cannot accurately distinguish between patients on AS who have indolent or aggressive PCa up front. Although there are several FDA (U.S. Food and Drug Administration) or CLIA (Clinical Laboratory Improvement Amendments) certified biomarkers for outcome prediction at various stages of PCa, none has been shown to estimate the risk of reclassification in AS cases.

Circulating miRNAs have emerged as potential PCa biomarkers in recent years. miRNAs are small, single strand noncoding RNAs (18 to 22 nucleotides) which regulate gene expression through hybridization to the 3′ untranslated region of target messenger RNAs to inhibit translation.[15,16] Because this class of molecules is stable and reliably detected in biofluids, they are amenable to serve as noninvasive biomarkers.[17,18] Furthermore, multiple circulating miRNAs have been shown to be associated with PCa progression or predictive of the response to therapy.[17,18]

Circulating miR-375, miR-30c, miR-223, miR-24, miR-21, miR-145, miR-141, miR-26b and let-7a have been demonstrated in multiple independent studies to be dysregulated in high grade (GS 8 or greater) or metastatic PCa compared to low grade PCa and/or nonPCa cases (supplementary Appendix, http://jurology.com/). However, to our knowledge it is unknown whether these miRNA aberrations are detectable during the early stages of PCa development such that they can distinguish up front the patients with truly indolent tumors from those who are likely to experience disease progression.

Thus, we assessed these candidate miRNAs in serum samples from patients on AS recruited from 2 independent health care institutions for the ability to differentiate indolent from aggressive PCa subpopulations as defined by reclassification status.

## MATERIAL AND METHODS

### miRNA Candidate Selection

A panel of 9 miRNAs (miR-141, miR-375, miR-21, miR-30c, miR-145, miR-26b, miR-223, miR-24 and let-7a) were selected based on a literature review of miRNA studies of the serum or plasma of patients with PCa. miRNAs were selected based on an association with PCa progression with validation in at least 1 independent cohort. The supplementary Appendix (http://jurology.com/) lists the cited studies.

### Patients and Specimens

We performed sample size calculations with G*power software (http://www.gpower.hhu.de/en.html). Assuming a 20% reclassification rate at the time of data analysis calculations were performed for 80% power and at the 2-sided 5% significance level. This generated a target sample size of 134 to 378 patients for moderate (OR 2) and low (OR 1.5) associations between the biomarker and reclassification, respectively.

Serum samples were collected with informed consent from patients on AS at 2 independent health care institutions and stored at −80C. The training cohort was retrospective and consisted of 196 patients from a larger ongoing prospective cohort study at Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada.[11,19] Samples were collected between May 2009 and June 2016. The validation cohort consisted of 133 patients who were prospectively recruited between December 2015 and February 2017 at UHN, Toronto, Canada. In each cohort all patients were treatment naïve and diagnosed with GS6 PCa.

All serum samples were collected prior to potential reclassification. Approval was obtained from the institutional research ethics boards at Sunnybrook Health Sciences Centre, UHN and Sinai Health System, Toronto, Ontario, Canada.

All patients underwent confirmatory biopsy within 1 year of diagnosis except the 10 most recently recruited patients in the UHN cohort. Patients were then monitored periodically with PSA tests, DRE, biopsy and/or MRI. Cases were reclassified upon the detection of any 1 or