Association between natural killer cell activity and prostate cancer: a pilot study

Jack Barkin, MD,[1] Roberto Rodriguez-Suarez, PhD,[2] Katia Betito, PhD[2]
[1]Department of Urology, University of Toronto, Humber River Hospital, Toronto, Ontario, Canada
[2]ATGen Canada Inc., Laval, Quebec, Canada

BARKIN J, RODRIGUEZ-SUAREZ R, BETITO K. Association between natural killer cell activity and prostate cancer: a pilot study. *Can J Urol* 2017;24(2): 8708-8713.

Introduction: *Natural killer (NK) cells play a significant role in tumor cell immunosurveillance. The association between the activity of NK cells and prostate cancer has previously been demonstrated using conventional research-based tests.*
Materials and methods: *The aim of the present pilot study was to study the association between NK cell activity (NKA) and prostate cancer using a simple blood test. Subjects that had previously been selected for prostate biopsy underwent a blood test for NKA using an in vitro diagnostic device (IVDD) (NK Vue, ATGen Canada Inc., Laval, QC, Canada) prior to biopsy.*
Results: *Of the 43 subjects sent for prostate biopsy, 22 were found to have prostate cancer. The test performance of the NKA IVDD, assessed using receiver operating characteristics, showed an area under the curve of 75%, a sensitivity of 57%, a specificity of 91%, a positive predictive value of 86% and a negative predictive value of 69%, with an odds ratio of 13.33. Using a cut off of 200 pg/mL for NKA, the absolute risk of having prostate cancer with NKA values below this level was found to be 86%.*
Conclusions: *This pilot study showed that subjects with low values of NKA were more likely to have a positive outcome at prostate biopsy.*

Key Words: natural killer cell activity, prostate cancer, biopsy, immunosurveillance

Introduction

Natural killer (NK) cells play a role in innate immunity and are involved in the early defense against infections and tumors.[1] NK cells orchestrate, with the adaptive immune system, the eradication of malignant cells.[2] The relationship between NK cell activity (NKA) and cancer has been known for many decades and a reduced activity has been shown to be associated with higher risk of cancer.[3]

Accepted for publication March 2017

Acknowledgments
The authors wish to thank the study subjects, the study coordinator Shelley Burton and Dr. Richard Casey for assistance in the protocol development.

Address correspondence to Dr. Jack Barkin, Department of Urology, 960 Lawrence Avenue West, Suite 404, Toronto, ON M6A 3B5 Canada

Prostate cancer is the most commonly diagnosed cancer among Canadian men, excluding non-melanoma skin cancers, and the third leading cause of death in men from cancer[4] and remains the second most frequent cause of cancer deaths in men over 65.[5] In prostate cancer patients, NK cells appear to play a significant role in tumor cell immunosurveillance. Early pilot studies had shown that NKA, as measured using the ^{51}Cr cytotoxicity assay, was significantly different in patients with advanced stages of prostate cancer (stage D) but not in patients with localized prostate cancer (stages B and C).[6] Lahat et al[7] demonstrated a significant decrease in NKA with increasing prostate cancer disease spread (stages A through D). In 1992, Kastelan and coworkers demonstrated that in prostate cancer patients in stages D0/D1 and D2 during progression of the disease, lower NKA was seen. Interestingly, in patients experiencing a stable D2 stage, normal NKA was seen.[8] The same group also confirmed the reduction in NKA in D1+D2 stages of prostate cancer.[9] In addition, they showed that such

levels, as well as prostate-specific antigen (PSA), were correlated to cancer stage. More recent work by the same group[10] showed that NKA was significantly different between healthy controls and patients with local tumor or those with disseminated disease, as well as between healthy controls and patients responding to therapy and those not responding to therapy. The changes in NKA were related to both metastatic extension of disease and tumor response to therapy, as well as to PSA levels.[10]

Of interest, in the case of prostate cancer, the hormonal milieu does not seem to affect NKA. Levels of cortisol were not correlated with NKA,[9] nor was NKA affected by treatment with pharmacological agents used (estradiol, cyproterone acetate, diethylstilbestrol and flutamide).[8]

Therefore, NKA reduction seems, according to these authors, linked to later stages of disease and the presence of circulating tumor cells. On the contrary, the presence of stable disease appears to be linked to normal levels of NKA, highlighting the role of NK cells in immunosurveillance of cancer cells.[8]

In a recent study by Koo et al[11] in prostate cancer, NKA was measured using a simple blood test involving the incubation of 1 mL of whole blood with a patented stabilized cytokine able to stimulate these cells to secrete IFN-ɣ (NK Vue), a more practical measure of NKA compared to the research-based cytotoxicity assay. In this study, NKA was significantly lower in prostate cancer patients compared to controls. Furthermore, patients with advanced stages of cancer progression showed a greater reduction in NKA, confirming the earlier work of the Kastelan group.[8,10]

The present study looked at the use of the new NKA *in vitro* diagnostic device (IVDD) in subjects selected for prostate biopsy in a community setting. Despite the excellent performance of PSA once prostate cancer has been diagnosed and treated, at the outset it remains a poor predictor of disease and there is no clear value that mandates biopsy. There is little difference in the prevalence of prostate cancer in men with values between 4.0 and 10.0 as PSA. In this range, PSA often correlates better with prostate size than with underlying disease.[12] Seventy percent (70%) of all men diagnosed with prostate cancer do not choose immediate treatment and are managed by active surveillance with regular assessments and frequent biopsies.[13] Approximately 30% of these men, with low volume Gleason 6 disease will progress to more aggressive disease and eventually accept radiation or surgery.[14] The indications for first time prostate biopsy remain a highly individualized decision as there is no established threshold of PSA that mandates action. While PSA kinetics remain a major tool used to determine the need for repeat biopsy or more targeted biopsies, the majority of men in active surveillance programs will undergo frequent prostate biopsies. Hence the broad range of positive biopsies in urologic practice and the often subjective nature behind the indications for biopsy.[14] The other concern today is that we are seeing an almost 5% incidence of significant side effects after prostate biopsy with 72% of hospital admissions being for infection related reasons.[15] Any test that might assist the physician's decision to recommend (or to not recommend) biopsy would represent a major advance in the management of this substantial cohort of men with prostate cancer.

Materials and methods

Study design

This study was an open-label, prospective, cross-sectional clinical performance study of a new IVDD for the measurement of NKA in whole blood (NK Vue, ATGen Canada Inc., Laval, QC, Canada) in subjects scheduled for prostate biopsy. Subjects were enrolled between September 2015 and May 2016. The study was approved by an independent institutional review board and followed the Canadian Institutes of Health Research Tri-Council Policy Statement, "Ethical Conduct for Research Involving Humans".[16]

Participants

Subjects were eligible for enrollment if they were males older than 18 years of age, and had been selected for a TRUS guided prostate biopsy for suspected prostate cancer (subjects with a history of prior biopsy were not excluded). Other eligibility criteria included providing informed consent for participation. Subjects could not participate if they could not understand the consent form or were unable to read it, or if they had the following conditions which have been shown to impact the activity of NK cells: an established or suspected malignancy with the exception of basal cell carcinoma, a chronic inflammatory condition requiring anti-inflammatory treatment such as rheumatoid arthritis, Crohn's or ulcerative colitis, Lupus or any connective tissue condition which, in the opinion of the investigator, might affect the patient immune response. Subjects could not participate if they had a previous diagnosis of prostate cancer or high grade PIN or if they were taking on a regular basis a 5-alpha reductase inhibitor, anti-inflammatories with the exception of low dose (81 mg) acetylsalicylic acid or testosterone replacement therapy.

Test methods

Inclusion and exclusion criteria were reviewed with the subject following signature of the consent form. A subject questionnaire was used to capture additional demographic data and subject medical history. The following information was captured for each subject: demographic data, two previous PSA values and the results of the digital rectal exam. For each subject enrolled, a dedicated tube specific to the IVDD test (NK Vue Tube) was used to collect a 1 mL sample of blood using a direct needle system (21G) from a new venipuncture point, on the day of enrollment (biopsy was usually performed within 6 weeks of the blood draw however the delay between blood collection and biopsy was not standardized). The collected tube was gently mixed and then placed into a 37°C incubator within 5 minutes of collection for a period of between 20 h and 24 h after which the supernatant was collected, transferred into a conical 1.5 mL Eppendorf tube and centrifuged at 11,500 g for 5 minutes at room temperature. Another conical 1.5 mL Eppendorf tube was then used to collect the supernatant, which was then frozen for a period up to 6 months. Prior to final analysis, the tubes were defrosted at room temperature and centrifuged at 11,500 g for 1 minute at room temperature, and the supernatant immediately loaded onto the enzyme-linked immunosorbent assay (ELISA) plates.

The IVDD blood collection tube contains a patented, stimulatory cytokine (Promoca) which stimulates the activity of NK cells during the 20 h-24 h incubation at 37°C. The NK cells secrete interferon-gamma (IFN-ɣ) into the plasma. The IFN-ɣ is quantitated with an ELISA designed to be used with the IVDD, and the level of IFN-ɣ is expressed in pg/mL. The dynamic range of the ELISA is 32 to 1,000 pg/mL. If the sample analysis showed subject values above the dynamic range, the samples were recorded as values of 1,000 pg/mL.

Statistical analysis

The comparison of NKA measured with the NKA IVDD between subjects with prostate cancer-negative prostate biopsies and those with confirmed prostate cancer was assessed using the non-parametric Wilcoxon-Mann-Whitney test. Diagnostic test performance of the NKA IVDD was assessed using receiver operating characteristics (ROC) curve analysis, to determine the ability of the test to identify prostate cancer subjects. The sensitivity, specificity, positive and negative predictive values of the IVDD for prostate cancer confirmed by prostate biopsy were calculated at the cut off value of 200 pg/mL.

TABLE 1. **Characteristics of study population**

Variable	**Subjects (n = 43) n, (%)**
Age, mean ± SD, y	66.2 ± 10.2
NKA IVDD	
< 200 pg/mL	14 (32.6)
≥ 200 pg/mL	29 (67.4)
Finding	
No prostate cancer	21 (48.8)
Prostate cancer	22 (51.2)

NKA IVDD = natural killer cell activity *in vitro* diagnostic device

Statistical analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). Statistical significance was set to $\alpha = 0.05$.

Results

Subjects were enrolled in the study from September 2015 through May 2016 at one clinical site in Toronto, Ontario, Canada, totaling 43 prostate biopsy subjects, see Table 1. Comparison of the levels of NKA between prostate biopsy negative subjects and biopsy positive (prostate cancer) subjects did not find any significant difference.

Diagnostic test performance of the NKA IVDD was assessed using ROC curve analysis. The area under the curve was found to be 0.75 (95% CI 0.58 to 0.92, $p < 0.02$), and when the data is adjusted for ethnicity, age and family history of prostate cancer, the AUC was found to increase slightly to 0.77. At the cut off of 200 pg/mL, the NKA IVDD had moderate sensitivity (57%) and high specificity (91%), as well as high positive predictive value (86%) and moderate negative predictive value (67%), see Table 2.

In the subject population studied, looking at the second PSA value only (the most recent PSA performed), 4/43 subjects had PSA values < 4 and 10/43 subjects had values ≥ 10 ng/mL (29 subjects had PSA between 4 ng/mL and 10 ng/mL). In the subgroup of subjects with PSA between 4 and 10, the sensitivity of the NKA IVDD at the cut off of 200 pg/mL was 54%, the specificity was 94%, the positive predictive value was 88% and the negative predictive value was 71% for prostate cancer, Table 3, values similar to that found in the total population.

A highly significant odds ratio for prostate cancer was found for the NKA IVDD (OR = 13.33; 95%CI 2.5-72.3) (i.e. someone with an NKA result < 200 pg/mL is more than 13 times more likely to have prostate cancer), Tables 4 and 5. For the first PSA value recorded for subjects in this study (first PSA value: OR = 1.06; 95%CI

TABLE 2. **NKA IVDD test performance (all subjects)**

NKA IVDD	Prostate biopsy negative	Prostate biopsy positive
Negative test (%) (n = 29)	20	9
Positive test* (%) (n = 14)	2	12
Sensitivity (%) (95%CI)		57.1 (34.0-78.2)
Specificity (%) (95%CI)		90.9 (70.8-98.9)
Positive predictive value (%) (95%CI)		85.7 (57.2-98.2)
Negative predictive value (%) (95%CI)		69.0 (49.2-84.7)

*positive if NKA < 200 pg/mL; NKA IVDD = natural killer cell activity *in vitro* diagnostic device

TABLE 3. **NKA IVDD test performance (PSA between 4 ng/mL and 10 ng/mL)**

NKA IVDD	Prostate biopsy negative	Prostate biopsy positive
Negative test (%) (n = 21)	15	6
Positive test* (%) (n = 8)	1	7
Sensitivity (%) (95%CI)		53.9 (25.1-80.8)
Specificity (%) (95%CI)		93.8 (69.8-99.8)
Positive predictive value (%) (95%CI)		87.5 (47.4-99.7)
Negative predictive value (%) (95%CI)		71.4 (47.8-88.7)

*positive if NKA < 200 pg/mL; NKA IVDD = natural killer cell activity *in vitro* diagnostic device

TABLE 4. **NKA IVDD test performance – odds ratio**

Test	Odds ratio	Significant
NKA IVDD (95%CI)	13.33 (2.5-72.3)	Yes
PSA – first value (95%CI)	1.06 (0.9-1.2)	No
PSA – second value (95%CI)	1.29 (1.02-1.6)	Yes

NKA IVDD = natural killer cell activity *in vitro* diagnostic device; PSA = prostate-specific antigen

TABLE 5. **Risk of prostate cancer versus NKA**

NKA (pg/mL)	Absolute risk*	Relative risk**	Odds ratio***
< 200	86%	2.76	13.33
< 300	68%	2.05	4.33
< 400	64%	1.91	3.5
< 500	52%	1.19	1.38
< 700	48%	0.97	0.94
< 1000	48%	0.97	0.94

*absolute risk = number of biopsy positive/number of total subjects biopsied; **relative risk = absolute risk at < NKA level/absolute risk at ≥ NKA level; ***odds ratio = (no. of biopsy positive at < NKA level/no. of biopsy negative at < NKA level)/(no. of biopsy positive at ≥ NKA level/no of biopsy negative at ≥ NKA level); NKA = natural killer cell activity

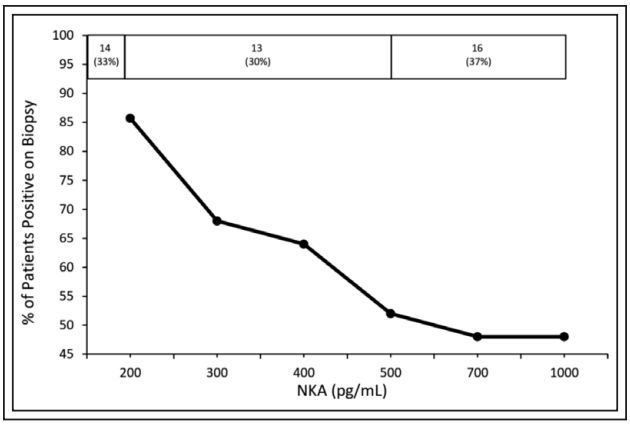

Figure 1. Risk of prostate cancer versus NKA. The proportion of subjects with prostate cancer (number of positive biopsies/total number of subjects biopsied) at different NKA (each data point). The top bar: number of subjects (%) with NKA values in the three ranges (< 200, ≥ 200 < 500, ≥ 500 ≤ 1000 pg/mL).

0.9-1.2) the results for odds ratio were not significant whereas significance was seen for the second PSA value (OR = 1.29; 95%CI 1.02-1.6). In this small number of subjects, no apparent associations were found between the NKA IVDD and Gleason score, prostate volume, PSA density, PSA velocity and with any of the demographic measurements.

The absolute risk of finding prostate cancer at different levels of NKA were calculated and are shown in Table 5 and Figure 1. Of those subjects biopsied with NKA levels below a cut off of 200 pg/mL NKA, 86% (12/14) were found to have prostate cancer, whereas the absolute risk of finding prostate cancer in those subjects with NKA levels at or above this cut off was 31% (9/29). Therefore, the relative risk of prostate cancer (i.e. the ratio of the absolute risk at NKA levels < and ≥ 200 pg/mL) was 2.76.

Discussion

The present pilot study found a very high specificity and positive predictive value for the NKA IVDD for prostate cancer in subjects selected for prostate biopsy. This study also showed that the NKA IVDD had a very high odds ratio for prostate cancer using a cut off of 200 pg/mL NKA, and showed that below this cut off, the subjects had an 86% absolute risk of having prostate cancer at biopsy.

Furthermore, when comparing the positive predictive value of the NKA IVDD to other currently used biomarkers for prostate cancer,[17,18] it appears that the NKA IVDD may be amongst the highest. In this small group of patients, we have not analyzed the combination of the NKA IVDD with other available biomarkers. That being said, this preliminary data may suggest that the NKA IVDD could justify the use of additional diagnostic tests to help determine the risk of detecting "clinically significant" prostate cancer. At this point, we have not shown that measurement of NKA is able to differentiate high grade disease from low grade disease. However, our results strongly suggest that the NKA IVDD could provide a risk assessment tool to estimate the probability of obtaining a positive biopsy.

Subsequent studies will assess the possible clinical use of an algorithm which will include NKA results along with other traditional clinical parameters. One of the limitations of this pilot study is that we were unable to rule out possible inflammation or infection in the subjects sent for prostate biopsy, either of which may have an effect on NKA and as such may have been responsible for the low negative predictive value (NPV). At this point, it is unclear what the cause of the moderate sensitivity was in this pilot study. Planned follow up studies will include other tests to detect inflammation or infection in order to determine if we can adjust the analysis to make the test more predictive for clinically significant prostate cancers (i.e. Gleason score of > 6). The intent is to also determine if there is a way to improve upon the NPV of the test.

Two recent large trials have questioned the potential role of PSA in prostate cancer screening[19] and as such, both the American and Canadian Task forces have made recommendations against PSA screening.[20,21] Doing such screening may remain appropriate in some patients and discussions with these may be warranted in order to better identify who would benefit from biopsies while reducing potential side effects. When dealing with the diagnosis of prostate cancer, we have almost adopted the approach of looking for any excuse not to do the biopsy. The concern has been the potential side effects of the biopsy, the bad press associated with overtreatment of "insignificant" cancers, the "fact" that men die with prostate cancer and not from prostate cancer. Novel tests or novel approaches to existing tests may represent important developmental tools.

As clinicians, it is our responsibility to develop such tools so that we may maximize the chances of finding cancer at biopsy, using diagnostic tests and clinical judgment in order to decide to biopsy. It is interesting to note that in this pilot study, we saw a high percentage of positive biopsies for the number done (of the 43 subjects biopsied, 22 were found to have prostate cancer). How and when we treat the cancer is another discussion.

Conclusions

The results from this pilot study were significant and show that subjects with low values of NKA were more likely to have a positive outcome at prostate biopsy. Further studies are warranted in order to escalate the NKA IVDD to a position of an easily performed, reliable non-invasive test to justify or discourage a prostate biopsy.

Disclosure

Dr. Jack Barkin has received consulting fees and study funding from ATGen Canada Inc. □

References

1. Bryceson YT, Chiang SCC, Darmanin S et al. Molecular mechanisms of natural killer cell activation. *J Innate Immun* 2011;3(3):216-226.
2. Gutkin DW, Shurin MR. Clinical evaluation of systemic and local immune responses in cancer: time for integration. *Cancer Immunol Immunother* 2014;63(1):45-57.
3. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. *Lancet* 2000;356(9244):1795-1799.
4. Canadian Cancer Society. Prostate cancer statistics. Available from URL: http://www.cancer.ca/en/cancer-information/cancer-type/prostate/statistics/?region=sk&p=1 Accessed April 3, 2017.
5. Van Hemelrijck M, Folkvaljon Y, Adolfsson J et al. Causes of death in men with localized prostate cancer: a nationwide, population-based study. *BJU Int* 2016;117(3):507-514.
6. Marumo K, Ikeuchi K, Baba S, Ueno M, Tazaki H. Natural killer cell activity and recycling capacity of natural killer cells in patients with carcinoma of the prostate. *Keio J Med* 1989;38(1):27-35.
7. Lahat N, Alexander B, Levin DR, Moskovitz B. The relationship between clinical stage, natural killer activity and related immunological parameters in adenocarcinoma of the prostate. *Cancer Immunol Immunother* 1989;28(3):208-212.
8. Kastelan M, Kraljić I, Tarle M. NK cell activity in treated prostate cancer patients as a probe for circulating tumor cells: hormone regulatory effects in vivo. *Prostate* 1992;21(2):111-120.
9. Tarle M, Kraljić I, Kastelan M. Comparison between NK cell activity and prostate cancer stage and grade in untreated patients: correlation with tumor markers and hormonal serotest data. *Urol Res* 1993;21(1):17-21.
10. Kastelan M, Kovacić K, Tarle R, Kraljić I, Tarle M. Analysis of NK cell activity, lymphocyte reactivity to mitogens and serotest PSA and TPS values in patients with primary and disseminated prostate cancer, PIN and BPH. *Anticancer Res* 1997;17(3B):1671-1675.
11. Koo KC, Shim DH, Yang CM et al. Reduction of the $CD16^{(-)}$ $CD56^{bright}$ NK cell subset precedes NK cell dysfunction in prostate cancer. *PLoS ONE* 2013;8(11):e78049.
12. Roehrborn CG, Boyle P, Gould AL, Waldstreicher J. Serum prostate-specific antigen as a predictor of prostate volume in men with benign prostatic hyperplasia. *Urology* 1999;53(3):581-589.
13. Klotz L, Vesprini D, Sethukavalan P et al. Long-term follow-up of a large active surveillance cohort of patients with prostate cancer. *J Clin Oncol* 2015;33(3):272-277.
14. Weerakoon M, Papa N, Lawrentschuk N et al. The current use of active surveillance in an Australian cohort of men: a pattern of care analysis from the Victorian Prostate Cancer Registry. *BJU Int* 2015;115(Suppl 5):50-56.
15. Nam RK, Saskin R, Lee Y et al. Increasing hospital admission rates for urological complications after transrectal ultrasound guided prostate biopsy. *J Urol* 2010;183(3):963-968.
16. Canadian Institutes of Health Research. Tri-Council Policy Statement, "Ethical Conduct for Research Involving Humans. Available from URL: http://www.pre.ethics.gc.ca/pdf/eng/tcps2-2014/TCPS_2_FINAL_Web.pdf
17. Roobol MJ, Schröder FH, van Leeuwen P et al. Performance of the prostate cancer antigen 3 (PCA3) gene and prostate-specific antigen in prescreened men: exploring the value of PCA3 for a first-line diagnostic test. *Eur Urol* 2010;58(4):475-481.
18. Parekh DJ, Punnen S, Sjoberg DD, et al. A multi-institutional prospective trial in the USA confirms that the 4Kscore accurately identifies men with high-grade prostate cancer. *Eur Urol* 2015;68(3):464-470.
19. Eckersberger E, Finkelstein J, Sadri H et al. Screening for prostate cancer: a review of the ERSPC and PLCO trials. *Rev Urol* 2009;11(3):127-133.
20. Moyer VA, U.S. Preventive Services Task Force. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2012;157(2):120-134.
21. Canadian Task Force on Preventive Health Care, Bell N, Connor Gorber S, Shane A, Joffres M, Singh H et al. Recommendations on screening for prostate cancer with the prostate-specific antigen test. *CMAJ* 2014;186(16):1225-1234.