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Natural killer cell activity and prostate cancer risk in veteran men undergoing prostate biopsy

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ABSTRACT

Background: A previous pilot study found that men with a positive prostate biopsy had low numbers of circulating natural killer (NK) cells, compared to biopsy negative men.
Methods: To confirm these data, we analyzed differences in NK cells from 94 men undergoing prostate biopsy to determine whether NK cells could predict for a positive biopsy. NK cells activity (NKA) was measured by an *in vitro* diagnostic system, with a pre-defined cut-off value for NKA at 200 pg/mL. Logistic regression and receiver operator characteristics (Area Under the Curve (AUC)) analyses were used to test the diagnostic value of NKA.
Results: The NKA test performance showed specificity of 88%, positive predictive value of 84%, sensitivity of 34%, and a negative predictive value of 41%. Among the 94 men analyzed, NKA was not significantly linked with age, race, digital rectal examination (DRE), prostate volume, PSA or biopsy grade group (all $P \geq 0.14$). In multivariable logistic regression analysis, the odds ratio (OR) of low NKA (< 200 pg/mL) for the detection of PC was 4.89, 95%CI 1.34–17.8, with a ROC area under the curve of 0.79 in all participants and increasing to 0.83 and 0.85 for the detection of PC and high-grade PC, respectively, among men with a normal DRE.
Conclusions: Men with a low NKA value had five-times higher odds of PC at biopsy. The implementation of this NKA assay in the clinic together with PSA may help to advise patients with the highest risk of PC whether, or not, to undergo a prostate biopsy.

1. Introduction

On a biological level, there are ample data to support the concept that inflammation can lead to prostate cancer (PC) development and progression [1,2]. However, how best to measure "inflammation", particularly systemic inflammation as it relates to PC is unclear. Given that complete blood counts (CBC) are often performed on patients as part of standard of care and thus are readily available in medical records, these data have been widely used in epidemiological studies. For example, a recent meta-analysis was able to identify CBC data on 16,266 men with PC, and concluded that a higher neutrophil-lymphocyte ratio (NLR) predicted worse PC outcomes, such as overall survival, particularly among men with metastatic castration-resistant PC [3]. In regards to earlier stage disease, we previously measured various CBC components in men undergoing radical prostatectomy, and found no significant associations between any CBC measures and any PC outcomes, [4] with the exception of neutrophils, which were associated with increased risk of all-cause mortality [4]. However, the value of inflammatory markers to predict the *presence* of early stage disease is not yet clear. A recent study of 223 Japanese men who all had negative prostate biopsies and were undergoing repeat biopsy found change in NLR from the first to the last biopsy was the most accurate marker to predict the presence of PC on biopsy [5]. While this highlights the potential of CBC parameters to predict cancer risk, there are other

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immune parameters that can be measured beyond CBC. Specifically, there are data that suggest natural killer (NK) cells may be more strongly related to cancer [6–8].

NK cells are cytotoxic lymphocytes of the innate immune system and play a key role in anti-tumor immunity and immunosurveillance including the production of cytokines and chemokines that mediate anti-tumor immune responses [9]. Interestingly, various studies showed the natural cytotoxicity of peripheral NK cells to be significantly lower in patients with different types of solid tumors compared to healthy individuals [10]. Several studies found an association between NK cells and different stages of PC [11–15]. Among patients with metastatic PC, greater *ex-vivo* NK cytotoxicity of PC cells correlated with longer time to castration resistance and survival [7].

The ability to rapidly and easily assess NK cell activity (NKA) is facilitated by the development of an *in vitro* diagnostic assay device (NK Vue™, ATGen, Korea), that incubates NK cells with a stimulatory cytokine and measures the amount of interferon-gamma produced [16]. NKA measured by NK VueTM negatively correlates with NLR [17]. A recent pilot study of 43 subjects undergoing a prostate biopsy found that those with a NKA $<$ 200 pg/mL were more likely to have PC at prostate biopsy (86%) compared to men with NKA $\geq$ 200 pg/mL (31%) [18]. A study from Korea using a comparable NKA assay device (Promoca®) found similar results [15], however, another study among Korean men using the NK Vue™ found no associations between NKA and PC diagnosis [19].

The objective of the current study was to test the ability of the NKA device to predict the presence of PC on biopsy in American men undergoing a prostate biopsy in a multiethnic VA cohort. We hypothesized that a low NKA value would be associated with higher risk of PC at biopsy.

2. Materials and methods

2.1. Study design and participants

After obtaining Institutional Review Board approval, men undergoing prostate needle biopsy for an elevated PSA and/or abnormal digital rectal examination (DRE) between October 2015 and July 2016 at the Durham Veterans Affairs Health Care System were recruited to participate in an ongoing study. Men were at least 18 years of age and had a PSA test within 12 months prior to enrollment. Of 100 patients recruited for this study, we excluded 3 subjects missing information on race, 2 missing data on prostate volume, and 1 patient who had a prior positive biopsy and was on active surveillance, leaving 94 patients for analysis. All men signed a written informed consent form.

2.2. Test methods

Blood was collected prior to needle biopsy and NKA in peripheral whole blood was evaluated using an NKA assay (NK Vue™, ATGen) as per the manufacturer's directions and as described in Barkin et al [18]. NKA values were expressed in pg/mL of interferon-gamma released by activated NK cells. Values outside the upper end of the standard curve were recorded as values of 1000 pg/mL. PSA was measured by the Durham VAHCS as part of standard of care.

2.3. Pathology

Biopsy tissue was assessed by a pathologist per standard of care and PC grade was abstracted from the resulting pathology report. Grade was assigned using the five grade group system where low-grade disease was defined as grade group (GG) 1 (Gleason score $\leq$6) and high-grade prostate cancer as GG 2–5 (Gleason score $\geq$7).

2.4. Statistical analysis

Statistical analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, NC). Continuous data were described as medians (interquartile range, IQR). Categorical variables were presented as frequencies and percentages. Statistical significance was set to $\alpha = 0.05$.

Patient characteristics were summarized and compared between individuals with NKA $<$ 200 pg/mL vs. $\geq$ 200 pg/mL using Wilcoxon rank sum tests for continuous variables and chi-squared tests for categorical variables. Analyses assessed whether NKA predicted PC and whether NKA added information to what was already known using standard clinical variables. We tested the diagnostic performance of 3 models for predicting PC and high-grade PC: (1) NKA alone (continuous), (2) a base model of established risk factor and detection measures including age, PSA, race, prostate volume, and DRE findings, and (3) base model + NKA (continuous). PSA and prostate volume were log-transformed prior to analysis, race was coded as black vs. non-black, and DRE was coded as normal vs. abnormal. Diagnostic test performance of the models was assessed using Receiver Operating Characteristics (ROC) curve analysis and area under the curve (AUC). Test performance analyses (sensitivity, specificity, positive and negative predictive values) were calculated at the previously defined cut-off value of 200 pg/mL [18]. Univariable and multivariable logistic regression models were used to assess the association between NKA level (continuous and categorical) and PC and high-grade PC. Multivariable models were adjusted for the variables described in the base model above. In a subgroup analysis, the multivariable analysis was repeated in men with a normal DRE to assess the performance of NKA in this group as men with an abnormal DRE are often referred for biopsy regardless of serum biomarker levels.

3. Results

3.1. Baseline characteristics

A description of baseline characteristics of patients with NKA $<$ 200 and $\geq$200 pg/mL is shown in Table 1. Among all patients, median (IQR) age of the cohort was 67 (62–69) and 68% of patients were black. Median (IQR) PSA was 6.0 (4.9–8.3) and 41% of patients had an abnormal DRE. Patient characteristics were similar between patients with NKA $<$ 200 and $\geq$200 pg/mL, except there was a higher proportion of men diagnosed with PC on biopsy in the NKA $<$ 200 pg/mL group (84% vs. 59%, p = 0.026). Sixty-two subjects had PC, and 32 did not. Of patients diagnosed with PC, 37 (60%) had high-grade PC.

3.2. Diagnostic test performance of the NKA assay

On multivariable analysis, every 100 pg/mL decrease in NKA was associated with 1.16 times increased odds of PC diagnosis (OR 1.16, 95%CI 1.01–1.32, p = 0.031; Table 2). When NKA was dichotomized at 200 pg/mL, patients with NKA $<$ 200 pg/mL had 4.89 times increased odds of PC diagnosis compared to patients with NKA $\geq$ 200 pg/mL (OR 4.89, 95%CI 1.34–17.8, p = 0.016). Associations between NKA and high-grade PC tended to be weaker on both univariable and multivariable analysis.

Using ROC analyses, the optimal cut-off in our data was calculated at a value of 218 pg/mL, very close to the pre-defined 200 pg/mL. Using $<$ 200 pg/mL as indicating a positive test, the test performance of the NKA assay showed specificity of 88%, positive predictive value of 84%, sensitivity of 34%, and negative predictive value of 41% for PC diagnosis (Table 3). When high-grade PC was treated as the outcome, the NKA assay showed specificity of 77%, positive predictive value of 48%, sensitivity of 32%, and negative predictive value 64%.

The AUC for NKA alone predicting PC was 0.61. The AUC of the base model of clinical and demographic features was 0.76, which increased to 0.79 when NKA was added to the base model (Table 4), ROC

Table 1
Baseline characteristics stratified by NKA values'.

	NKA < 200 pg/mL (N = 25)	NKA ≥ 200 pg/mL (N = 69)	Total (N = 94)	p value
Age				0.536[a]
Median	68	67	67	
Q1, Q3	62, 69	63, 69	62, 69	
Race				0.136[b]
Non-black	5 (20%)	25 (36%)	30 (32%)	
Black	20 (80%)	44 (64%)	64 (68%)	
Digital rectal exam				0.860[b]
Not suspicious for cancer	15 (60%)	40 (58%)	55 (59%)	
Suspicious for cancer	10 (40%)	29 (42%)	39 (41%)	
TRUS prostate volume (cc)				0.908[a]
Median	41	42	42	
Q1, Q3	29, 56	30.6, 56	30, 56	
PSA				0.449[a]
Median	5.4	6.2	6.0	
Q1, Q3	4.7, 7.2	5.0, 8.7	4.9, 8.3	
Biopsy result				0.026[b]
No cancer	4 (16%)	28 (41%)	32 (34%)	
Cancer	21 (84%)	41 (59%)	62 (66%)	
Grade group				0.771[b]
1	9 (43%)	16 (39%)	25 (40%)	
2-5	12 (57%)	25 (61%)	37 (60%)	
NKA value (pg/mL)				
Median	62	1000	650	
Q1, Q3	26, 138	526, 1000	177, 1000	

NKA: NK cells activity assay, Q: quartile, TRUS: transurethral ultrasound, PSA: prostate-specific antigen.
[a] Wilcoxon.
[b] Chi-Square.

curve is shown in Fig. 1. The AUC for NKA alone predicting high-grade PC was 0.55, adding NKA to the base model increased the AUC from 0.79 to 0.80 (Table 4).

3.3. Diagnostic test performance of the NKA assay in men with a normal DRE

Among the 55 men with a normal DRE, 17 patients with NKA ≥ 200 pg/mL were diagnosed with PC, while only one patient with NKA < 200 pg/mL was not diagnosed with PC. The AUC was 0.83 for PC and 0.85 for high-grade PC among men with a normal DRE. On multivariable analysis, every 100 pg/mL decrease in NKA was associated with an OR of 1.17 (95%CI 0.99–1.39) for PC, which was very similar to the OR among all men (OR = 1.16, 95%CI (1.01–1.32). However, there was a statistically significant association between NKA and high-grade PC, with every 100 pg/mL decrease in NKA associated with 1.23 times increased odds of high-grade PC (OR 1.23, 95%CI 1.00–1.51, p = 0.047).

4. Discussion

Systemic inflammation has been reported to be associated with PC diagnosis and progression [1,2], however few studies on systemic inflammatory markers and PC were conducted in early stage disease. Furthermore, only three studies previously analyzed NK cell activity for its predictive value on PC diagnosis [15,18,19], with conflicting results. We sought to test whether NK cells activity correlates with PC at biopsy using the same *in vitro* diagnostic device used in the two conflicting studies [18,19]. We found a lower NKA value was associated with higher risk of PC at biopsy, confirming the data reported by the pilot study [18]. Thus, our study confirms the use of a device to measure NKA by a simple *in vitro* test as an additional biomarker to predict PC risk in men undergoing biopsy. Moreover, this study confirms the

Table 3
Performance of NKA with a cut-point of 200 pg/mL (< 200 pg/mL indicates a positive test).

	NPV (95% CI)	PPV (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)
PC vs. no PC	41% (29%–52%)	84% (70%–98%)	88% (76%–99%)	34% (22%–46%)
High-grade PC vs. low-grade or no PC	64% (52%–75%)	48% (28%–68%)	77% (66%–88%)	32% (17%–48%)

PC: prostate cancer.

Table 2
Association between NKA value (as continuous and categorical variables) and prostate cancer or high-grade prostate cancer diagnosis.

	PC vs. no PC		High-grade PC vs. low-grade or no PC	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Univariable models				
NKA value (continuous)**	1.11 (0.99-1.25)	0.069	1.05 (0.95–1.17)	0.358
NKA value (< 200 vs. ≥ 200 pg/mL)	3.59 (1.10-11.6)	0.033	1.63 (0.64–4.10)	0.304
Multivariable models*				
NKA value (continuous)**	1.16 (1.01-1.32)	0.031	1.11 (0.98–1.26)	0.115
NKA value (< 200 vs. ≥ 200 pg/mL)	4.89 (1.34-17.8)	0.016	2.51 (0.85–7.40)	0.095

* Adjusted for age, race, PSA, prostate volume, and DRE findings.
** Modeled per 100 pg/mL decrease in NKA value.

Table 4
Area under the curve (AUC) for three different models for PC and high-grade PC diagnosis.

	PC vs. no PC			High-grade PC vs. low-grade or no PC		
	NKA alone	Base model	Base model + NKA	NKA alone	Base model	Base model + NKA
All patients	0.61	0.76	0.79	0.55	0.79	0.80

Base model: Age, race, PSA, prostate volume, and DRE findings.

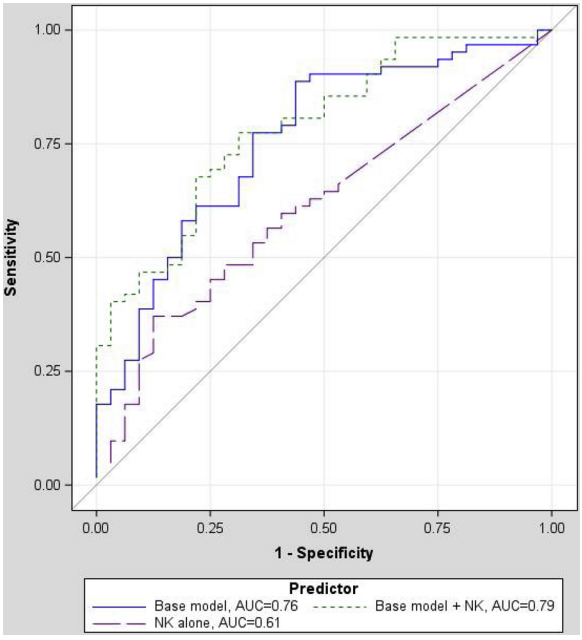

Fig. 1. ROC curves for overall PC vs. no PC for three different models.

association between NK activity and PC further supporting the link between immune function and PC. Furthermore, though we were unpowered to detect racial differences, given our cohort was predominantly black, our results suggest the NKA assay works regardless of race, although larger studies to confirm this are needed.

Our results agree with a previous pilot study of 43 Canadian men undergoing prostate biopsy, which found NKA values < 200 pg/mL were associated with an 86% risk of a positive prostate biopsy, using the same assay. Among the 94 men undergoing prostate biopsy in our study, and after accounting for age, race, PSA, prostate volume, and DRE findings, a lower NKA value (NKA < 200 pg/mL vs. ≥ 200 pg/mL) was associated with a significantly higher risk of PC at biopsy (OR: 4.89, 95%CI 1.34–17.8, p = 0.016). These results suggest that the NKA *in vitro* device could be used as a risk assessment tool to estimate the probability of a positive prostate biopsy. Although the overall increase in AUC when NKA was added to the model was modest (0.76 to 0.79), which could be due to the high-risk cohort used in this study, the fact that the predicted value improved is promising. More studies are needed to determine optimal cut-offs and the ideal patient population. Furthermore, given that the majority of our patients were black, our data suggest the NKA device works well in black men, which is an important finding given that it was only tested in predominantly white or Asian men before [15,18]. As such, the limited studies to date suggest the NKA device works regardless of race, although larger studies to confirm this are needed.

NK cells with low cytolytic activity have been correlated with increased cancer risk among different types of cancer [20]. However, until recently, NK cells have not been studied in relation to PC. One study found that a high count of NK cells in prostate tumors of men who received androgen deprivation therapy was associated with a good prognosis [21]. Furthermore, they found an inverse correlation between the density of NK cells and seminal vesical invasion [21]. While these studies suggest the prognostic value of intratumor NK cell number, a more recent study found that the NK cell number in the peripheral blood is also prognostic for PC outcomes [7]. In that study NK cells from peripheral blood of metastatic PC patients were monitored *ex-vivo* and a higher NK activity correlated with better outcomes, i.e. longer time to castration-resistant PC and survival [7]. Coupled with the prior studies [15,18], our data show that NK cell activity measured in peripheral blood of men undergoing prostate biopsy is associated with PC risk.

NK cells belong to a group of blood cells called innate lymphoid cells, which are derived from common lymphoid progenitors in the bone marrow [9]. NK cells' cytotoxic activity and cytokine production can exert anti-tumor activity, as first shown in *in vitro* and in preclinical studies [22,23]. Although little is known about NK cell infiltration of malignant tumors in humans [9], peripheral blood NK cells cytotoxicity was significantly lower in cancer patients compared to healthy individuals [10]. Our results are in agreement with these studies finding that a low NKA in men undergoing prostate biopsy was associated with higher risk of PC diagnosis.

This study has several strengths including the use of an ongoing prostate biopsy study. Although men were already scheduled for a biopsy (i.e. these were not average risk subjects), blood was collected prior to needle biopsy to measure NK cell activity in peripheral whole blood. We found the optimal NKA cut-off was 218 pg/mL, very similar to the cut-off value of 200 pg/mL that was previously described as the best level in PC using this assay, showing the consistency of results across ethnicities and populations [18]. Interestingly, analysing baseline characteristics, NKA and PSA seemed to be not associated and may be considered as independent variables suggesting NKA adds unique information above and beyond PSA.

Limitations of this study comprise the small sample size of our cohort, which included men with a high-risk for PC and with equal access to care at only one VA hospital, the Durham VA. The sample size was even smaller when men with a normal DRE were analyzed separately, which could mean the positive association found could be due to a type I error or false positive results. For all patients, immune system related conditions that may affect NK cell levels were not accounted for. Nonetheless, the strengths of the associations between NKA and a positive biopsy support previous data [15,18]. Future larger and better powered studies are needed to assess NKA test performance in a wide variety of clinical settings.

5. Conclusions

This study shows that subjects scheduled for prostate biopsy were more likely to have a PC diagnosis when NKA levels were below the cut-off of 200 pg/mL, and reinforces previous reported associations between low NKA and presence of PC [15,18]. Larger studies are needed to confirm our findings.

Authorship contribution

SJF and ACV conceived and designed the study. AMD coordinated the study. EW facilitated the acquisition of the samples. LEH performed the statistical analysis. ACV drafted the manuscript. SLS, SK, ET, JHF, and SJF revised the draft. All authors read and approved the final version.

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Declaration of Competing Interest

Some aspects of this study were funded by ATGen Canada Inc. All authors have none conflicts of interest.

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