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Review Article

The Role of Natural Killer Cells in the Management of Prostate Cancer. A Systematic Review

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Abstract

Purpose: The role of Natural Killer (NK) cells in the management of Prostate Cancer (PCa) is still not fully understood. It has been argued that measurement of NK cells in a blood sample may be used as a reliable, minimally invasive tool for PCa screening and evaluation of treatment effect and survival. The purpose of this systematic review was to search the current literature for evidence on the potential role of NK cells in the management of prostate cancer.

Patients and Methods: We reviewed the literature on NK cells in relation to PCa patients. A systematic review using Pub Med and scientific meeting records was carried out from February to May 2021 according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). A total of 32 full-text papers were identified.

Conclusion: Current evidence supports the hypothesis that NK cell assays might become an effective tool for screening, diagnostics, staging, and prediction of outcome in patients with PCa, but further investigation is needed to elucidate the role of NK cell phenotypes. Also, prospective studies on the implementation of NK cell assays as a supplement in personalized approaches to PCa management are required.

Keywords: Natural killer cell; Prostatic neoplasm; mCRPC; Biomarker; Immunological fingerprint; Personalized medicine

Introduction

Prostate Cancer

Prostate Cancer (PCa) is the second most common non-cutaneous neoplasm and the 5th most common cause of cancer-related deaths (7%) in men worldwide [1]. PCa is a heterogeneous disease with most cases progressing slowly [2].

Measurement of serum Prostate-Specific Antigen (PSA) is the cornerstone in early detection and monitoring of PCa [3]. Since its introduction in the late 1960s, PSA has dramatically shifted PCa staging, with most cases diagnosed as a prostate-confined tumor [4]. Nevertheless, there is still no consensus about the cutoff for an elevated PSA [5]. With a cutoff of four ng/ml the positive predictive value of PSA in the diagnosis of PCa varies widely from 25 to 40% [6] with a sensitivity and specificity of around 20% and 65%, respectively [7]. In the mid-1980s there was a rapid rise in the PCa incidence due to the introduction of PSA testing, but the recent implementation of more conservative PSA testing recommendations has led to a decline in the diagnosis of new cases of PCa [8].

The conventional diagnostic tool for PCa is Transrectal Ultrasound (TRUS) guided biopsy [9]. This procedure still has many clinical challenges, including being invasive, having side effects, and the risk of false-negative results [10]. Biopsy targeted by Magnetic Resonance Imaging (MRI) seems to overcome the pitfalls of TRUS-guided biopsy and improves diagnostic accuracy [11].

The risk of recurrence after curatively intended management of PCa depends mainly on the disease stage at initial diagnosis. Such stratification is usually based on the clinical Tumor, Node and Metastasis stage (TNM), PSA levels, and biopsy Gleason Score (GS) [2].

Radical prostatectomy and radiotherapy are the ultimate treatments for localized and locally advanced PCa [12] while Androgen Deprivation Therapy (ADT) is a backbone in the medical treatment for both high-risk localized PCa and the metastatic setting [13]. In almost all patients with incurable PCa the disease will eventually progress to the Castration-Resistant (CRPC) stage with 35% developing metastases and ultimately dying within two to four years [14].

There is accumulating evidence that an impaired immune response is an essential factor in the pathogenesis of PCa [15]. Both preclinical and clinical studies have explored the role of inflammation in PCa development and progression. Due to the correlation between immune system activity and management of cancer, many efforts are ongoing to elucidate the impact of immune biomarkers in relation to early diagnosis and optimal treatment approaches in different cancer types, including PCa.

Natural Killer Cells

The innate and acquired immune systems protect the host from foreign pathogens by differentiating between "self" and "non-self" antigens [16]. Natural Killer (NK) cells are innate effector lymphocytes that differ from B and T cells. Among all circulating lymphocytes, 10-15% are considered to be NK cells [17].

Human NK cells are identified by the absence of a Cluster of

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Differentiation 3 (CD3) and the presence of CD56. They can be subdivided into different populations based on the expression of CD16 and CD56 ($CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$) [18]. The NK cells can be broadly divided into a $CD56^{dim}$ cytotoxic subset, which represents the majority of NK cells in the blood, and a $CD56^{bright}$ cytokine producer subset [19]. Functionally, $CD56^{bright}$ NK cells are immature but can differentiate into $CD56^{dim}$ with high cytotoxic potential [16].

The effective function of NK cells depends on the balance between activating receptors such as NK cell Group 2 member D (NKG2D) and inhibitory receptors like KIR (killer cell immunoglobulin-like receptors) [19,20], following ligation with stress ligands and *Major Histocompatibility Complex* (MHC) class I molecules, respectively [21]. Natural Killer Cell Activity (NKA) can also be enhanced by interleukin-2(IL-2), and interferons and can be suppressed by suppressor T cells, suppressor monocytes, and prostaglandins.

Characterization of the target molecule(s) and the mechanisms by which NK cells recognize their target structure(s) are unanswered questions that have attracted considerable attention [22].

Natural Killer Cells in Cancer

The NK cells are effector cells that spontaneously lyse normal cells infected by microbial pathogens and a variety of syngeneic and allogeneic tumor cells. As part of the innate immune system, NK cells can recognize the absent or low expression of class I Human Leukocyte Antigen (HLA) molecules on target cells and lyse them directly. The NK cells can also recognize and bind to the NK recognition structure of the tumor cells, which leads to the releasing of NK Cytotoxic Factor (NKCF) and tumor cell lysis [23]. Another mechanism of NK-mediated target cell lysis is Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) through activation of the CD16 receptor [24]. NKA also depends on the recycling capacity of NK cells after lysis of the tumor cell and can be augmented by interferon [17].

NK cells play a critical role in tumor immune surveillance by cytotoxic activity, cytokine production [25], and directly inducing the death of tumor cells by secreting perforin and granzymes [26]. In addition, NK cells secrete pro-inflammatory cytokines, which leads to stimulation of the adaptive immune response to neutralize the escape mechanisms developed by tumor cells [15]. In patients with solid tumors, NKA is depressed and may be associated with aberrant immune regulation, which leads to the development of the tumor.

Major mechanisms associated with impaired NK cell functioning in cancer patients are down regulation of lytic perforin and granzyme production, accompanied by reduction of degranulation capabilities [26,27]. Down-regulation of NK cell-activating receptors and inhibition of the NK cell cytotoxic function are connected with exposure of NK cells to some immunosuppressive cytokines such as transforming growth factor-beta (TGF-β) produced by tumors [28]. Additionally, the killing capability of NK cells can be affected by inhibition of the natural cytotoxicity triggering receptor 1, which is an *NK* cell-specific surface molecule of the *46* kD (NCR1/NKp46) pathway.

Natural Killer Cells in Prostate Cancer

Some studies have indicated that inflammation may play a role in the pathogenesis of PCa, an important factor of which is an impaired immune response [15] with the NK cells distributed throughout the stroma and around the glandular epithelium [29].

NKA may be applied both as a supportive diagnostic marker in addition to PSA [15] and as a prognostic factor in patients with PCa [30]. Furthermore, the immune system as well as the tumor micro environment can be affected by treatments such as ADT, which increase the number of lymphocytes, including NK cells [13,31].

It has been observed that a combination of immunotherapeutic drugs such as cancer vaccines, checkpoint inhibitors, cytokines, and pharmacotherapeutic and molecular agents enhance the immune response in PCa, and therefore, some studies hypothesized that NK cells may also be used as a therapeutic tool for the management of PCa [32].

In the present review, we elucidate the potential role of NK cells in the pathogenesis, screening, diagnostics, prognostics and prediction of treatment effect in PCa patients.

Method

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta- Analyses (PRISMA). The PRISMA flow chart was used to map the records identified, included and excluded, and the reasons for exclusion (Figure 1).

Search Strategy, Inclusion and Exclusion Criteria

To ensure a systematic and thorough course of action, the search was based on the principles of the PICO process. Searches were undertaken on Pubmed and Embase between 1 February and 10 May 2021. All studies published during 40 years prior to May 2021 noting the application of NK cells and PCa were eligible. The database searches were based on two strings differing only by the keywords related to NK cells and PCa. They were both based on the Medical Subject Heading database (MeSH) "Prostatic Neoplasms" population. The keywords were "Natural killer cell" [MeSH] and "Prostate cancer". General reviews, case reports, non-English records, preclinical studies, and non-NK cell related trials were excluded.

The titles and abstracts of records generated by the searches were screened against the predefined inclusion and exclusion criteria. Records were also screened for duplicates. If the eligibility of an article could not be determined solely based on its title and abstract, it was selected for full-text screening. The data extracted from each study were: author names, title, patient population, intervention, number of patients included, and main findings.

Entering the search strings (NK cells AND Prostate cancer) into the database generated a total of 348 records. No duplicates were identified at this stage. Thirteen further studies were identified through a manual search of the references of all articles. A total of 146 articles were selected for full-text screening of which 32 met the criteria for the final analysis. The number of articles excluded per exclusion criterion is shown in (Figure 1).

Study Characteristics

The publication dates of the 32 included papers ranged from 1980 to 2021. The sample sizes ranged from 11 to 250 patients. The mean age varied from 65 to 72 years. Key study characteristics, patient

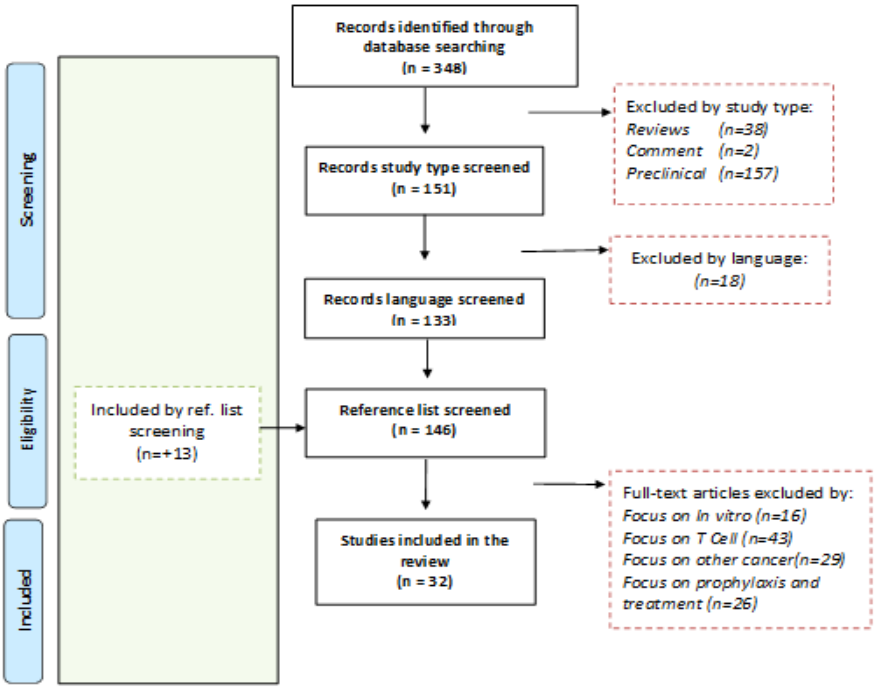

Figure 1: PRISMA flow diagram of the systematic review.

demographics, and outcomes are summarized in (Tables 1, 2 and 3).

Results and Discussion

Natural Killer Cells and Prostate Cancer Pathogenesis

The potential loss of NK cells, both in number and function, at the neoplastic and pre-neoplastic stages of tumorigenesis and in the induction and progression of cancer, including PCa, has been discussed in many studies [33].

In this section we mention 14 papers discussing the role of NK cells in PCa pathogenesis (Table 1). Five papers focused on NK cell subtypes and argued that distorted subpopulation, including reduction of $CD56^{bright}$ cells, may be responsible for the diminished NK function in PCa patients. It can be assumed that the decrease in these cells, which are the major sources for interferon gamma (IFN γ), leads to lower concentrations of IL-2. This may suggest therapeutic use of specific cytokines in the prevention of relapse or metastatic disease. Seven studies discussed different mechanisms of immune escape, including reduced killing capability of NK cells due to high levels of soluble MHC class I chain-related A (sMICA), while two studies focused on the reduced ability of NK cells to recognize tumors.

Decreased frequency and distorted subpopulation: Reduction in the frequency of cytotoxic $CD56^{dim}$ NK population (peripheral and intratumoral) seems to be crucial in cancer pathogenesis. Although Sotosec et al. did not find a percentage difference between NK cells and their subsets in peripheral blood from patients with localized, locally advanced and metastatic PCa, [3] Koo et al. demonstrated that the $CD56^{dim}$-to-$CD56^{bright}$ cell ratio was significantly higher in PCa patients than controls along with a significant gradual increase in the ratio according to cancer stage progression [15].

There are also some contradictory statements regarding the impact and clinical significance of immune cell infiltration, including that of different NK subpopulations into tumor tissue. Mononuclear peri-prostatic lymph node cells from PCa patients (localized, locally advanced and metastatic) were reported to be significantly less reactivated by beta-interferon stimulation than those of healthy subjects and the simultaneously tested autologous peripheral mononuclear cells. These results may confirm that the tumor microenvironment of PCa affects NKA due to a distorted subpopulation [34]. Additionally, the primary impact of NK cells on tumor infiltration was argued to be the physical disruption of the tumor capsule possibly leading to tumor invasion in patients with localized and advanced PCa despite showing an activatory subpopulation [35]. In 2016 Pasero et al. reported that NK cell infiltrates into localized and metastatic PCa tissue were mainly by the CD56/ Neural Cell Adhesion Molecule 1 (NCAM1)-positive phenotype, which has low or no cytotoxic potential [20].

Defective Recognition of tumor: The mechanisms of immune escape of tumor cells in PCa can be attributed to low killing capability of NK cells, e.g. due to Hypoxia-Inducible Factor 1 (HIF-1α) induced NCR1/NKp46 pathway inhibition through miR-224 [36].

Down regulation of NKG2D on peripheral NK cells and in intra tumoral regions is argued for in PCa. Gallazi et al. proposed that down-regulation of NKG2D in tumor associated circulating NK cells in patients with localized or locally advanced PCa can increase the level of markers of NK cell exhaustion, including PD-1 and T cell immunoglobulin mucin domain molecule *3* (*Tim-3*) [37]. A significant down regulation of NKG2D induced by tumor-derived exosomes on circulating NK cells in CRPC patients compared to controls was demonstrated in a study by Lundholm et al. [38]. In de-

Table 1: NK cells and Pathogenesis.

Year	Author	No.	Population	Sample/ Method	Main findings
2021	[37]	35 27	Local, locally advanced PCa Control	Whole Blood / Flow cytometry	Compared to the control group, tumor-associated NK cells derived from PCa patients showed a significantly higher mRNA expression for pro-inflammatory and pro-angiogenic factors. Tumor-associated NK cells in PCa patients showed a significant down-regulation of NKG2D compared to control group.
2018	[36]	10 10	PCa Control	Tumor tisuue isolated lymphocyte / Flow cytometry	Compared to normal tissue, lymphocytes isolatefrom PCa showed clearly higher miR-224,which can protect cancer cells from NK cells.
2016	[20]	16 5 20	Localized PCa mPCa Normal tissue from PCa	PBMC/Tumor and normal tissue/ Flow cytometry and / 51-Cr release	Compared to normal tissue, the activating receptors were significantly decreased, while the inhibitory receptors were significantly increased in NK cells infiltrating tumor with prominent alteration after metastatic progression.
2015	[39]	8 10	mPCa No treatment LCR SCR	PBMC/ Flow cytometry	Compared to mPCa patients with shorter survival, NK cells from LCR mPCa patients had stronger cytotoxicity (degranulation).
2014	[38]	18 8	CRPC with relapse after ADT, before chemotherapy Control	PBMC, isolated lymphocte/ Flow cytometry	Prostate tumor-derived exosomes down regulated NKGD-mediated response.
2013	[15]	51 54	Treatment naïve PCa Control	PBMC/ NK Vue	Higher CD56dim to CD56bright ratio in patients with PCa compared to control.
2013	[35]	100 150	Localized and advanced PCa BC	Tummor tissue/ Immunohistochemistry	NK cell infiltration in early stage is mostly distributed in pre-invasive compared to invasive cancer tissue and is associated with focal disruption of the tumor capsule.
2012	[45]	200 185	Untreated Localized PCa Control	Blood sample (DNA)/ PCR	Compared to control group, no significant difference in KIR was seen in patients with PCa.
2011	[3]	20 20 20	BPH Local, locally advanced Control	PBMC / Flow cytometry	Compared to patients with PCa, no difference was seen among other groups regarding the percentage of NK cells and their subsets.
2004	[42]	23 10	Localized and advanced PCa Control	Serum/ Immunohistochemical staining	Compared to localized PCa, the patients with advanced PCa showed significantly higher level of sMIC ($p<0.05$). No significant levels of sMIC were detected in serum from healthy controls.
1989	[41]	49 15	Localized and advanced PCa Control	PBMC/ Flowcytometry	Compared to control group and localized PCa, the patients with locally advanced and metastatic PCa demonstrated a significantly lower NKA.
1989	[40]	7 6 25	Localized PCa Advanced PCa Control	PBMC/ 51-Cr release	Compared to a control group and localized PCa, MRC was significantly lower in patients with advanced PCa.
1987	[22]	49 10 10 20	Advanced PCa Localized PCa BPH Control	PBMC/ 51-Cr release	Compared to other groups, the patients with advanced PCa had significantly lower NKA, which was not due to decrease of the NK cell population.
1985	[34]	8 8 7	PCa T2N0M0 PCa T3-T4NxM0-1 Control	PBMC/51-Cr release	Compared to a control group, beta-interferon induced NKA augmentation was reduced significantly in peri-prostatic lymph nodes in PCa.

Maximal Killing Potential (Vmax); Maximal Recycling Capacity (MRC); Long Castration Response (LCR); Short Castration Response (SCR); Peripheral Blood Mononuclear Cell (PBMC); Prostate Cancer (PCa); Metastatic Prostate Cancer (mPCa); Castration-resistant Prostate Cancer (CRPC); Breast Cancer (BC); Benign Prostatic Hyperplasia (BPH); Natural Killer Activity (NKA); Peripheral Blood Mononuclear Cell (PBMC); Time to Castration Resistance (TCR); Overall Survival (OS); Killer Cell Immunoglobulin-like Receptor (KIR); MHC class I c molecules (MICs).

novo metastatic PCa, the process of tumor recognition by NK cells was mediated by NKp46, NKG2D and also NKp30 [39]. The maximal killing potential and recycling capacity has also shown to be reduced in patients with advanced PCa, which leads to suppressed NKA [40]. Lahat's group hypothesized cytokine regulation of NK cells to occur in patients with advanced PCa, who had the lowest NKA and IL-2 secretion compared to those with localized PCa and healthy controls [41]. Also, the association of a high level of sMICA with reduced circulating NKG2D expression and shedding of MICA, inducing NK cell deficiency, was observed in the patients with advanced PCa [42].

Inhibitory receptors involved in the regulation of NK cell tolerance may also favor tumor escape from NK cell cytotoxicity. Some studies argued for an association between KIR genes and HLA in relation to cancer, including hepatocellular carcinoma [43] and bladder tumor [44]. To clarify this relationship in PCa, Portela et al. [45] compared the distribution of KIR and HLA gene frequencies in healthy controls with that of untreated localized PCa patients who had a PSA level<2.5 ng mL and normal digital rectal examination. No difference was found between the groups.

Some of the mechanisms behind immune escape of tumor cells and the NK cell dysfunction observed in the PCa microenvironment were reviewed by means of 14 studies mentioned in Table 1. Despite the use of different methods in different studies there were consistent results of reduced NKA in PCa patients and all studies agreed that low NKA leads to high levels of tumor occurrence or metastasis. This may suggest NKA augmentation as single agent adjuvant therapy to prevent relapse or in combination with other cancer therapies to prevent progression.

A molecular understanding of the function of the NK cells in immune surveillance is essential for the discovery of novel approaches such as MIC shedding and HIF-1α inhibitors to combat PCa. The role, however, of KIR genes in tumorigenes in untreated locally advanced PCa was not confirmed, which may suggest their role to

Table 2: NK cells and diagnosis.

Year	Author	No.	Population	Sample	Method	Main finding
2020	[48]	41 31	PCa (asymptomatic, PSA<20ng ml) Benign	Purified PBMC	Flowcytometric profiling	NK cell profiling identified 8 features of NK cells, which distinguished between PCa and benign disease.
2020	[49]	18 8 24 52	PCa, GS >7 PCa, GS =7 PCa GS=6 Benign	Whole blood	NK Vue	NKA sensitivity 68%, specificity 73% for detecting of PCa, NKA cut-off value 500 pg/ml.
2019	[50]	25 37 32	Localized PCa Local advanced, advanced Not detected	Whole blood	NK Vue	Low NKA as a predictive tool for a positive biopsy of PCa (NKA cut-off value 200 pg/ml).
2018	[52]	18 25 49 43 86	PCa GS 9-10 PCa GS 8 PCa GS 7 PCa GS 6 Not detected	Whole blood	NK Vue	NKA was not useful for detection and prediction of Gleason grade in PCa.
2017	[51]	21 22	PCa Not PCa	Whole blood Biopsy	NK Vue (IVDD)	Subjects with low levels of NKA had more positive biopsies.
2013	[15]	8 8 25 10 54	PCa GS 9 PCa GS 8 PCA GS 7 PCa GS 6 Control	Whole blood	NK Vue/ Immunohistochemistry	NKA sensitivity 72%, specificity 74% in detection of PCa.
2004	[42]	23 10	Localized, advanced PCa Control	Serum	ELISA	sMIC as a novel biomarker for detection of high grade PCa.
1993	[47]	(11, 13) 14 3 7 6 10	Untreated PCa Localized, advanced Well-differentiated Moderately differentiated Poorly differentiated Autoimmune disease Healthy Control	Purified PBMC	51-Cr release	NKA in untreated PCa was not useful in detection of grades, but was reliable to differentiate localized and advanced disease significantly.
1987	[22]	49 10 10 20	Advanced PCa Localized PCa BPH Control	PBMC	51-Cr release	Compared to other groups, the patients with advanced PCa had significantly lower NKA, which was not due to decrease of the NK cell population.
1985	[34]	8 8 7	PCa T2N0M0 PCa T3-T4NxM0-1 Control	PBMC	51-Cr release	Compared to a control group, beta-interferon induced NKA augmentation was reduced significantly in peri-prostatic lymph nodes in PCa.

Prostate Cancer (PCa); Metastatic Prostate Cancer (mPCa); Metastatic Breast Cancer (mBC); Metastatic Colorectal Cancer (mCRC); Castration-Resistant Prostate Cancer (CRPC); Benign Prostatic Hyperplasia (BPH); Gleason Score (GS); Natural Killer Activity (NKA); Circulating Tumor Cells (CTCs); Solubale MHC class I chain–related molecules (sMIC); Invitro Diagnostic Device (IVDD); Peripheral Blood Mononuclear Cells (PBMCs)

differ in cancers with different origins. Further studies of advanced PCa are required to clarify the association of KIR with this subgroup of patients.

Interestingly, one preclinical study [46] identified an unrecognized role of NK cells in the prostate tumor micro environment. It showed that NK cells migrate to the CRPC cells better than to the normal prostate cells and may suppress androgen receptor splicing variant 7 (ARv7) expressions, which results in suppression of enzalutamide resistant CRPC cell growth and invasion. NK cell *adoptive transfer* therapy may therefore be an effective approach to treating enzalutamide resistant CRPC.

A better understanding of the detailed mechanisms of NK cell dysfunction in the PCa microenvironment may be instrumental in designing PCa specific NK cell-based therapy or prophylaxis.

NK Cells and Screening/Diagnosis

One of the many clinical challenges in the administration of PCa is risk prediction, which is essential for the treatment approach. Men with low-risk disease do not necessarily need active treatment, but just having a PCa diagnosis may have social, financial, and psychological consequences. The PSA test alone lacks accuracy and reliability as a screening tool, which may lead to a higher risk of unnecessary biopsies. It is therefore required to introduce alternative and non-invasive approaches such as circulating biomarkers with both higher specificity and sensitivity. In this section, we present 10 studies (Table 2) with four different types of measuring of NKA.

Radionuclide labeling method: Two of the three studies using 51-cr release suggested that NK cells can be applied as a biomarker for diagnosis [35] and staging [47]. Another study from 1993 assessed the ability of NKA to predict PCa stage and tumor grade. A sharp drop in NKA was reported in patients with tumor lesions in lymph nodes, bone or soft tissues, while tumor differentiation had no effect on the results. The study suggested that an assay of the lytic capacity of NK cells may be used as a supplement to routine clinical staging [47].

Flow cytometry and machine learning: A computerized model was suggested to detect PCa by profiling NK cell subsets in the blood from 72 asymptomatic men with PSA levels < 20 ng/ml and a panel of 8 out of 32 phenotypic features of which CD56dim CD16 high, CD56+NKp30+, and CD56+NKp46+ are assumed to identify the presence of PCa. Moreover, the potential of NK cell profiling to discriminate accurately between low, intermediate, and high-risk PCa by using all of the 32 phenotypic features was demonstrated [48].

NK Vue cytokine release method: One study from 2013 demonstrated NKA as a potential diagnostic marker with a sensitivity and specificity of 72% and 74%, respectively, whereas the $CD56^{dim}$-

Table 3: NK cells and Prognosis/Prediction.

Year	Author	No.	Population	Sample/ Method	Main findings
2020	[57]	3 24 19 5	PCa Stage I Stage II Satge III Stage IV	PBMC/ NK Vue	Compared to the patients with lower NKA after RARP, the risk of post-operative margin positivity was significantly lower in those with high NKA.
2019	[61]	19 51 23	mPCa mCRC mOCa	Whole blood/ NK Vue	Lower NKA during the first 2 months after treatment may indicate a poor prognosis.
2017	[62]	22 16	Non metastatic PCa Non-castrate Castration-resistant	PBMC/ Flow cytometry	NKA could not predict the treatment response of DNA vaccination in PCa. No difference between responders and non-responders was shown regarding NKA.
2015	[39]	8 10	Untreated mPCa LCR SCR	PBMC/ Flow cytometry	NKp30 and NKp46 were the most significant predictive markers of OS and TCR.
2014	[60]	30	mCRPC	PBMC/ Flow cytometry	Tim-3+ NK cells elevation post- versus pre-vaccination indicates longer OS.
2014	[56]	72 50	mPCa before ADT or no longer than 3 months mPCa ADT+/- Residronate	Serum/ Multiplex electrochemiluminescence	No association between IL-2 (NK cell activator) and TCR nor OS was shown.
2014	[59]	8 36 30	mPCa mBC mCRC	Whole blood/ CellSearch CTC test	High CTCs represents low NKA and high risk of poor prognosis.
2013	[15]	8 8 25 10 54	PCa GS 9 PCa GS 8 PCa GS 7 PCa GS 6 Control	Whole blood/NK Vue/ Immunohistochemistry	NKA and the proportion of $CD56^{bright}$ cells decreased gradually according to cancer progression and may be used as a prognostic biomarker.
2013	[35]	100 150	Localized, advanced PCa BC	Tummor tissue/ Immunohistochemistry	NK infiltration in early stages may indicate poor prognosis due to the possibility of induction of tumor invasion.
2011	[55]	116 81	PCa, 3DRT T1-T4 WP T1-T4 PO	PBMC/ Flowcytometry	After radiotherapy completion, NK cells became more sensitive to higher doses and during radiotherapy NK cell function increased due to toxicity induced by Hsp70. More toxicity better prognosis.
2009	[29]	40 35	Control PCa RP, PCa neaoadj. ADT+RP)	Tumor tissue/ Immunohistochemistry	Higher levels of CD56+NK cells resulted in lower risk of PCa progression.
2004	[42]	23 10	Localized, advanced PCa Control	Serum/ ELISA	Higher levels of sMIC represented poor prognosis.
1995	[30]	11 41 10	Localized PCa, RP, TURP Advanced, Castration (medical or surgical) BPH	Tumor tissue/ Immunohistochemistry	Higher HNK-1 antigen indicated better prognosis.
1992	[64]	(46&15) 6 11 9 7 12 6 7	mPCa&nonmetastatic PCa, treated with DES Treated with Estracyt Treated with CPA Orchiectomy Orchiectomy+CPA Orchiectomy+ Flutamide Healthy control	Purified PBMC/51-cr release	Compared to patients with remission or stable disease, NKA was significantly lower in the patients with progression under treatment. Dynamic of NKA under treatment may be used to monitor response to the treatment.
1989	[40]	13 25	Localized, advanced PCa Control	PBMC/ 51-Cr release	Compared to the control group and localized PCa, NKA was significantly lower in patients with poor prognosis.
1980	[58]	24 6 7	BC PCa UBC	PBMC/ 51-Cr release	NKA after RT fluctuated due to affection of mature NK cells or their progenitors.

Prostate Cancer (PCa); Metastatic Prostate Cancer (mPCa); Breast Cancer (BC); Metastatic Colorectal Cancer (mCRC); Metastatic Ovarian Cancer (mOCa); Urinary Bladder Cancer (UBC); Peripheral Blood Mononuclear Cells (PBMCs); Diethylstilbestrol (DES); Cyproterone Acetate (CPA); Benign Prostatic Hyperplasia (BPH); Gleason Score (GS); Castration-Resistant Prostate Cancer (CRPC); Natural Killer Activity (NKA); Human Natural kille-1 (HNK-1); Circulating Tumor Cells (CTCs); Soluble MHC class I Chain–related molecules (sMIC); Genitourinary (GU); Gastrointestinal (GI); NKA ratio (postoperative NKA/preoperative NKA); Radiotherapy (RT); Robot-Associated Radical Prostatectomy(RARP); Whole pelvic (WP); Prostate-only (PO); 3-Dimenshional Conformal Radiotherapy (3DRT); Radical prostatectomy(RP); Transurethral Resection of the Prostate (TURP); Time to castration resistance (TCR); Overall survival (OS)

to-$CD56^{bright}$ cell ratio showed a sensitivity of 66% and a specificity of 71% [15]. These results were supported by Tae et al. finding the sensitivity and specificity of NKA in detecting PCa to be 68-72% and around 74%, respectively [49]. Additionally, it was found that patients with a low NKA value had five times the risk of biopsy verified PCa and NKA was therefore suggested as a predictor of a positive biopsy [50]. A pilot study demonstrated that patients with NKA lower than 200 pg/mL had an absolute risk of around 86% of having PCa [51]. In contrast, the results of an observational, cross-sectional study indicated that NKA cannot be used in the detection of PCa and prediction of Gleason score. The serial changes of NKA, however, were not available for assessment of the cut-off value, as the measurements were performed only prior to the biopsy, i.e. not after treatment [52].

MICA ELISA: One paper observed a significant amount of sMICA (21 ng/ml) in the serum of nearly all PCa patients with a Gleason score ≥6 compared to healthy controls and proposed sMIC as a novel biomarker of PCa (Gleason score ≥6) [42].

Natural killer cell assays can be used for screening and staging purposes and may supplement routine clinical management of PCa. Assays using 51Cr-release to measure the NKA by means of a radioisotope reagent may be difficult to manage. The adenosine triphosphate chemiluminescence assay (ATP assay) is another method, which does not require a radioisotope reagent. The results of some studies proposed that the ATP assay would be more suitable than the 51Cr-release assay as it can measure not only NKA but also other cytotoxic tests. Flow cytometry-based assays are also effective. The NK Vue test measures the concentration of IFNγ easily compared to the ATP assay. Detecting sMICA by ELISA is another way to measure NKA indirectly, as soluble MICA molecules impair NKA.

NK Cells and Prognosis/Prediction

Both the prognostic and predictive significance of NK cells in many types of cancer, including PCa, have been widely addressed [53,54]. To improve individualized treatment, e.g. adjuvant therapy, it is important to investigate whether NK cell assays may be used both as prognostic and predictive biomarkers in PCa. In this section, we reviewed 15 papers (Table 3) investigating the prognostic and predictive significance of NK cells in PCa.

Prognostic significance: The literature seems to support a prognostic benefit from high levels of NK cell infiltration in PCa tissue. Gannon et al. investigated immune cell infiltration in localized PCa and demonstrated that a high number of CD56+ NK cells were significantly associated with a lower risk of PCa progression [29]. These results were supported by Pasero et al. showing an inverse correlation between the quantity of CD56+ NK cells in prostate tumor and seminal vesicle tumor invasion. This was translated into a prognostic benefit with a 3-year survival in patients with NKp30 high of 85% versus 38% in those with NKp30low [39].

Results from 1995 argued for an association between highly expressed HNK-1 antigen, well-differentiated PCa, and better prognosis [30]. The patients with radiotherapy-induced gastrointestinal side effes had a better prognosis, which may attribute to the fact that radiotherapy can stimulate release of Heat Shock Proteins (HSPs), induce the augmentation of immune function, and up regulate NK cell-mediated cytotoxicity [55].

Similar relationships have been documented when focusing on the impact of NKA on localized PCa. Marumo et al. analyzed NKA in patients with localized and advanced PCa as well as healthy controls and found that NKA was significantly lower in advanced PCa compared to the two other groups [56]. These results were supported by another study addressing the impact of the local intervention. Lu et al. demonstrated that postoperative NKA was significantly higher in the lower stage than in higher-stage PCa and patients with negative surgical margins had significantly higher postoperative NKA than those with positive margins [57]. Post-radiotherapy NKA was assessed by Blomgren et al. and found to be lower than NKA before radiotherapy, which may induce further progression of the disease [58]. In the blood samples of more advanced stages of PCa, lower levels of $CD56^{bright}$ and higher levels of $CD56^{dim}$-to $CD56^{bright}$ NK cell ratio have been demonstrated. The gradual increase of $CD56^{dim}$-to-$CD56^{bright}$ ratio with PCa stage progression was confirmed [15].

A significant reduction in NK cell cytotoxicity in metastatic cancer patients was observed as an indicator of a poor prognosis in patients with Circulating Tumor Cells (CTCs) greater than the threshold level (breast cancer=5, PCa=5, colorectal cancer=3) compared to patients with CTCs below the threshold level. The intensity of Toll-Like Receptor (TLR2 and TLR4) expression was also reduced in the patients with a relatively high number of CTCs [59].

Therapeutic interventions may affect the activity of NK cells. A Phase I trial of combination therapy with Ipilimumab and PROST-VAC showed the NK-cell immature subset that expresses Tim-3+ was significantly increased in the patients with increased survival [60]. On the other hand, one study could not confirm an association between the level of selected cytokines, including IL-2 (NK cell activator cytokine), shortly after starting ADT with neither time to CRPC nor OS in metastatic PCa [56]. Another paper reported that ADT can increase the number of CD56+ NK cells in PCa, which was associated with a good prognosis [29]. The expression level of NKp46 was found to be predictive of OS (94% and 39%) and *Time to Castration Resistance (*TCR) [39]. Also, a paper from 2019 monitored serial serum NKA before and during treatment in patients with disseminated disease, including mCRPC, until disease progression and showed a significantly lower response rate and median progression-free survival in patients with IFNγ<200 pg/mL [61].

Predictive significance: The efficacy of NKA as a marker of response prediction, including immunotherapy response in cancer, is discussed in many studies, but the amount of NK cells alone is not assumed to be a suitable biomarker. Johnson et al. measured antigen-specific or antigen non-specific immunity prior to anti-tumor DNA vaccination in patients with non-metastatic PCa who had biochemical recurrence. The amount of NK cells was not statistically different between immune responding and non-responding subjects after treatment [62]. A retrospective observational study reported that patients with untreated mPCa with high versus low expression of NKp46 showed response rates of 39% and 8%, respectively, to castration treatment at 3 years [39].

All the above-mentioned studies initially proposed NKA as a marker of prognosis and response in PCa. Four studies analyzed the relationship between NKA and TCR or OS and all argued that low levels of NKA at diagnosis correlated to a shorter TCR or OS. One of the studies showed that radiotherapy-induced GI or GU toxicity upregulated NKA, which may result in a better prognosis.

Natural killer cell assays in PCa may be used as supplements in the detection of microscopic residual disease after prostatectomy in order to design a better personalized adjuvant treatment for patients with high risk of relapse. The low level of IFNγ and CD56+bright NK cells, HNK-1 antigen, and the high level of CD56dim-to-CD56 bright NK cell ratio and sMIC may indicate a high risk of microscopic disease. In patients with no radiotherapy-induced side effects, the monitoring of NKA during radiotherapy can help to make sure that NKA is not decreasing.

Three studies investigated NKA as a predictive biomarker. One

study found NKA as a potential predictive biomarker to stratify patients likely to have longer castration responses arguing for therapies aimed at NKA augmentation in mPCa patients. Although a small study could not confirm an association between the amount of NK cells and treatment response in PCa patients after DNA vaccination, it was suggested that different measures of antigen-specific tolerance, or regulation, might help predict the immunological outcome of DNA vaccination. Only one paper focused on the activating receptors NKp30 and NKp46 and confirmed them as some of the most obvious predictive markers of TCR of mPCa patients.

The sample size of these studies was between three and 116 patients, but only seven studies had a sample size of 25 or more. Four studies used the statistical analysis mentioned by Riley at al. [63], but only two of them had an appropriate sample size. One paper was from 1995 and the only one to assess HNK1 in tumor tissue. In the fourth paper no association between IL-2 and time to CRPC or OS was shown, although NKA was not investigated directly.

An evidence-based approach with larger prospective cohort studies seems to be essential to confirm the importance of NK cell assays as prognostic and predictive biomarkers in PCa. The reporting of such studies needs to be based on guidelines [63] in order to be useful clinically.

Strength and Limitations

This review provides an up-to-date overview of the role of NK cells in the management of PCa patients. The search query used for the retrieval of studies for this review resulted in 348 papers, which were evaluated by only one reviewer. This may have caused bias in the selection and screening of search results considering the topic of the review. Although, when in doubt, the reviewer involved all other authors for a consensus decision.

Conclusion

Natural killer cells may be used to supplement personalized approaches in the management of PCa. In vivo experiments indicate NK cell assays may be applied clinically as biomarkers for screening, diagnosis, prognosis and prediction of PCa. Further validation in larger, prospective cohort studies is required in order to implement NK cells for the management of PCa.

Conflicts of Interests

The authors declare that they have no conflicts of interest.

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