



Research Article

## IFN- $\gamma$ AND PROSTATE SPECIFIC ANTIGEN (PAS) HOW NEW BIOMARKERS FOR IMPROVE EARLY DETECTION OF PROSTATE CANCER

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**Abstract:** Prostate cancer may be the most common cause of cancer among men. The triage by digital rectal examination (DRE) and prostate specific antigen (PSA) exam reduces the incidence of the disease. The PSA is a specific marker of the organ, but has low specificity, making it necessary to search for new biomarkers to diagnosis. Among these, highlight the cytokines. There is a correlation between pro-inflammatory cytokines and increased production of PSA. IFN- $\gamma$  can be seen as a key factor in the pathogenesis of prostatic diseases and may auxiliary in the diagnosis as a possible biomarker. The aim of this study was to investigate the relationship between IFN- $\gamma$  and PSA levels. The study was conducted with 113 men from Brazilian Legal Amazon. The concentrations of IFN- $\gamma$  and PSA were measured by Enzyme Linked Immunosorbent Assay (ELISA). The IFN- $\gamma$  concentration was higher in individuals with PSA alterations. In conclusion, measurements of serum IFN- $\gamma$  levels may be new marker provide additional for prognostic information to altered PSA in male serum and contribute to man's health.

**Keywords:** Prostate, Cytokines, Interferon-gamma (IFN- $\gamma$ ), Prostate Specific Antigen (PSA).

### Introduction

The incidence of cancer is increasing in developing countries as a result of populational aging, as well as, increasingly, an adoption of lifestyle choices associated with cancer, including smoking, sedentary lifestyle and diets. Prostate cancer may be the most common preventable type of cancer among men<sup>1</sup>. It is the second most commonly diagnosed cancer and the sixth leading cause of cancer death in men, accounting for 14% of total of new cancer cases in 2008 and 6% of cancer deaths in men<sup>2</sup> and this pathology can be traced by means early diagnostic screening<sup>3</sup>.

Some works suggest that screening by digital rectal examination and prostate specific antigen (PSA) reduces the late incidence of the pathology, interfering in mortality. The American Cancer Society recommends digital rectal examination and PSA annually from age 50 for cancer screening in asymptomatic<sup>4</sup>. In case of individuals at high risk of developing cancer, age can be anticipated to 40 years<sup>5</sup>. A younger age at diagnosis is associated with more favorable outcomes and that could potentially lead to improved survival for high-risk groups<sup>6</sup>.

Although PSA has been an advance for diagnostic, it alone still is not enough<sup>7</sup>. The PSA is a specific marker, with high sensitivity but low specificity for the detection of prostate cancer. Others situations temporarily increase the levels of PSA but not necessarily related to tumoral processes, such as manipulation of the prostate gland, digital rectal examination, biopsy, ejaculate, as well as benign tumors like the prostatic hyperplasia<sup>8,9</sup>.

Perceptions emerging on the molecular pathogenesis of prostate cancer suggest that damage to the epithelium of the prostate, potentially caused by a variety of exhibitions, trigger inflammatory processes pro-carcinogen to promote the development of the disease<sup>1</sup>. The molecular mechanisms associated with inflammation are complex and involve a variety of proinflammatory cytokines<sup>10</sup>. Studies show that there is a correlation of proinflammatory cytokines to increased production of PSA and progression of prostate cancer<sup>11</sup>.

Cytokines are inducing proteins that mediate intercellular communication in the immune system<sup>12</sup>. Cytokines such as Interleukin-1 (IL-1), Interleukin-2 (IL-2), tumor necrosis factor alpha (TNF- $\alpha$ ), Interleukin-6 (IL-6) and Interferon-gamma (IFN- $\gamma$ ) are mediators of the inflammatory response<sup>13</sup>. Studies support a crucial role of IFN- $\gamma$  as a key factor in the pathogenesis of prostatic diseases. This cytokine plays an important physiological role in the promotion of the responses of innate and adaptive immunity<sup>14</sup>. IFN- $\gamma$  assumes the function of controlling the growth of tumor cells by control cellular responses against neoplastic cells<sup>15</sup>. It can activate, recruit or increase production of cells such as "Natural Killer", macrophages and neutrophils, which can promote anti-tumor responses<sup>16</sup>. However, this cytokine may have both positive and negative effects on the development and progression of tumors<sup>17</sup>. Thus, studies addressing the role of IFN- $\gamma$  in prostate cancer are needed to prove their clinical usefulness, especially focus on the discovery of new biomarkers for the detection of this pathology. The aim of this study was to determine whether there is a correlation between IFN- $\gamma$  and PSA

levels, suggesting the hypothesis that this cytokine may serve as a potential biomarker for the detection of prostate cancer.

### Experimental Section:

This study was conducted in areas from Brazilian Legal Amazon, totaling in 107 men aged over 40 years and 6 healthy men below 40 years for control group. Individuals who agreed to participate were interviewed individually and private, with the assistance of a questionnaire with questions on the subject. All procedures were submitted for ethical evaluation and obtained Institutional approval.

#### 2.1. Blood sampling

Samples of 8 mL of blood were collected in glasses tubes. We centrifuged them at 160 G for 15 min to obtain of serum. The serum was stored at -80°C for later PSA and IFN- $\gamma$  analysis.

#### 2.2. PSA and IFN- $\gamma$ assay by ELISA (Enzyme Linked Immunosorbent Assay):

The detection of PSA was performed by an automated model ChemWell by the method of ELISA kit (Diagnostic Bioeasy S / A, Belo Horizonte, MG). The PSA was considered altered for values  $\geq 4.0$  ng/mL.

IFN- $\gamma$  concentration in serum was determined by an ELISA kit from BioLegend® Ledend Max™ (San Diego, USA). The reaction rates were measured by absorbance in a spectrophotometer with a 450 nm filter. The results were calculated using the standard curve and shown in pg/dL.

#### 2.3. Statistical analysis

Analysis of variance (ANOVA) was used to evaluate IFN- $\gamma$ . Statistical significance was considered for a p-value less than 0.05.

### Results and Discussion

Among the 107 individuals evaluated 68.2% were aged 40 to 60 years of age and 31.8% have more than 60 years. The main risk factors described for the development of prostate cancer were age, ethnicity and family predisposition. Aging is considered the most significant risk factor. The incidence of prostate cancer in men aged over 50 years is greater than 30% but increases to approximately 80% at 80 years<sup>18</sup>. Studies have shown that approximately 33% of men aged 40 to 60 years will have prostatic carcinoma histologically evident and that this value increases to 75% in men aged  $\geq 75$  years<sup>19,20,21</sup>.

The symptoms are good indicators for the diagnosis and even for going to the health service. Among the common signs and symptoms in prostate cancer, the most frequently mentioned by respondents were urinate frequently at night (32%), followed by persistent pain in the lumbar region (26%) (Table 01).

In this study, it was observed decrease in the urinary tract symptoms. The absence of symptoms related to prostate cancer is a barrier that can be taken as an indicator of lack of knowledge of preventive actions for this disease<sup>22,23</sup>. The literature showed that 56.3% of respondents

agree that the man may have prostate cancer and do not show any symptoms<sup>24</sup>. Since there are cases that may occur significant growth of the prostate and the patient remains asymptomatic or show only mild symptoms<sup>25</sup>. It's never enough to emphasize that prostate cancer at an early stage may not cause symptoms, so it is fundamental to make preventive examinations periodically.

Regarding the examinations carried out by individuals only 65% did the screening for PSA serum. Arnold-Reed et al.<sup>26</sup> reported that PSA testing was also the most performed, with 59% of individuals. This marker is the most widely used for screening and early detection of prostate cancer<sup>27</sup>. There is evidence that screening of prostate cancer by PSA results in the detection of a large number of asymptomatic tumors<sup>28</sup> including non palpable on digital rectal examination. The PSA allowed earlier diagnosis, leading to a higher cure rate or the clinical surveillance of these patients. However, its sensitivity and specificity do not allow its use as an isolated method<sup>29,30</sup>. In many cases the serum levels of PSA may be elevated, highlighting its potential for false positives<sup>20,21,28</sup>. Values  $\geq 4.0$  ng / mL are universally considered abnormal, although the American Urological Association proposes changes according to age and race. An abnormal value underlines, therefore, the suspicion of a prostatic carcinoma<sup>19,31</sup>.

**Table 01 - Signals and symptoms reported by men interviewed from Legal Amazon Region – Brazil**

Signals and symptoms	Nº	Percentagem (%)
Urinary flow weak or interrupted	05	10
Frequent urination (especially at night)	16	32
Difficulty urinating or to contain urine	01	02
Inability to urinate	01	02
Pain or burning sensation to urinate	08	16
Blood in urine or semen	04	08
Persistent pain in the lumbar region	13	26
Difficulty of erection	02	04

In this study, there was a median PSA normal value of 0.7 ng / mL. Individuals who had elevated PSA (29.8%) had a median of 7.5 ng / mL (Table 02).

PSA levels detected are similar to the findings of Pfitzenmaier et al.<sup>32</sup> who verified an average PSA for the control group of 0.89, and these levels increased in patients with prostatic carcinoma (9.67). In a study by Tang et al<sup>33</sup> in individuals with up to 50 years the PSA's average was of 0.7 ng / mL. These authors found that the initial PSA of 1.5 ng / mL or more in men with up to 50 years predicts an increased risk of prostate cancer. In men with PSA levels below 4.0 ng / mL, the risk of cancer is approximately 15% and 15% of these patients have a high-grade disease<sup>34</sup>.

However, levels below 1.0 ng / mL, the risk of high-grade disease is extremely low. The limitations of PSA as a biomarker for prostate cancer screening, characterized by a low sensitivity for acceptable rates of false positives, are well known. Therefore, new markers that distinguish prostate cancer are needed<sup>27</sup>. Currently, several studies are focusing on finding new biochemical markers capable of predicting prostate cancer and prognosis. In addition, pro-inflammatory cytokines has been linked with the production of PSA and progression of prostate cancer<sup>9,35</sup>.

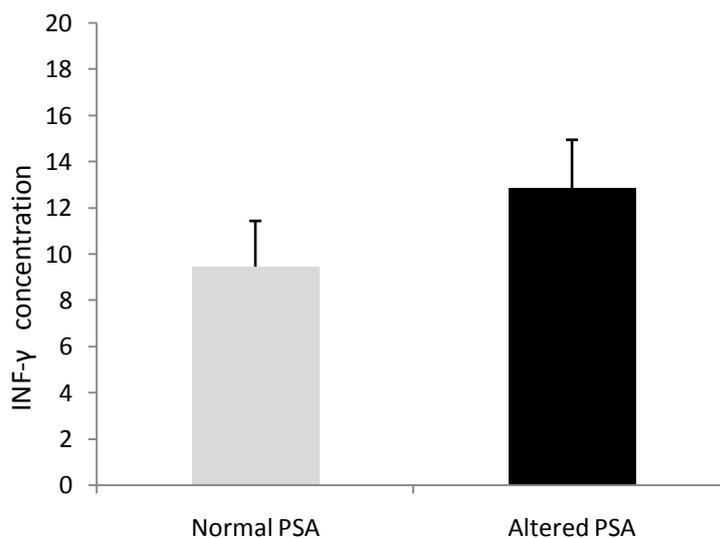
**Table 02: Average PSA concentrations in serum from individuals with altered Prostate-specific antigen (PSA) and normal prostate-specific antigen (PSA) conducted in individuals from Legal Amazon Region – Brazil.**

PSA Levels	%	Median
Normal < 4,0 ng/mL	70,2	0,7
Altered ≥ 4,0 ng/mL	29,8	7,5

Extensive efforts have focused on understanding the roles of cytokines and their interactions with effector cells to produce effective tumoral immunity<sup>17</sup>. Thus, the

cytokines represent part of the complex pattern of immune response, which may contribute to the development as well as the elimination of cancer<sup>36</sup>. A cytokine that plays a central role in the coordination of tumoral immune responses is IFN- $\gamma$ . In relation to prostate cancer, the action of IFN- $\gamma$  is not fully elucidated. The existing data on changes in the levels of cytokines that mediate inflammation for prostate cancer are limited<sup>37</sup>.

In this study was verified increase of IFN- $\gamma$  concentrations in human serum samples in individuals with altered PSA (Figure 1). The levels of IFN- $\gamma$  analyzed in prostatic secretions were also higher in subjects with prostate pathologies<sup>38</sup> suggesting that IFN- $\gamma$  may be a biomarker. In experimental study that IFN- $\gamma$  and lymphocytes collaborate for the regulation and development of tumors and IFN- $\gamma$  receptors developed tumors and with greater frequency<sup>16, 39</sup>. The main target of action of IFN- $\gamma$  is the transformed cell itself and that it possibly acts to improve the recognition of transformed cells by the immune system. Segal et al<sup>40</sup> showed that IFN- $\gamma$  is rather important for antitumor effects, but alone is not sufficient for the regression of some tumors. This cytokine also has another face, which may have an effect cytostatic, cytotoxic, and pro-carcinogenic under certain circumstances<sup>41</sup>.



**Figure 1. IFN- $\gamma$  concentrations in the human serum of male. Results were expressed as the mean and SD. \* Significant differences between serum male with altered Prostate-specific antigen (PSA) and normal serum prostate-specific antigen (PSA).**

Studies have shown changes of cytokines in prostate cancer and have demonstrated their ability to diagnosis involving neoplasias<sup>42</sup>. IFN- $\gamma$ , can exert multiple effects within the tumor microenvironment site. Many of these effects are contextual therefore since in the tumor microenvironment the target cells react differently due to different stimuli, care must be taken in creating therapeutic interventions that use compounds that may stabilize the delicate balance that exists on this medium<sup>36</sup>.

Advances are needed, especially in cases of prostate cancer, as well, with the help of other biomarkers, the number of unnecessary biopsies in men without cancer,

may decrease. As well as the detection of tumors can be increased, particularly the high grade ones, that is present in PSA levels below those of traditional cutting<sup>27</sup>. The finding that IFN- $\gamma$  plays a key role in promoting antitumor responses became the focus of renewed interest in the concept so far abandoned immunosurveillance of cancer<sup>43</sup>. However, the mechanisms by which this cytokine reaches its effects on cancer are not clear<sup>14</sup>.

**Conclusion**

In conclusion, measurements of serum IFN- $\gamma$  levels may be new marker provide additional for prognostic

information to altered PSA in male serum and contribute to man's health.

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