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Accuracy assessment of the immunochromatographic method (rapid test) for the qualitative determination of prostate-specific antigen (PSA) in patients from the "Novembro Azul" campaign

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Running tittle: Accuracy of the qualitative rapid test for PSA determination

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Abstract

Prostate cancer is the most common non-cutaneous tumor and the second cause of death due to tumor among men. The high incidence of clinically diagnosed prostate cancer in the last twenty years reflects the result of an effective screening using prostate-specific antigen (PSA) tests. Electrochemiluminescence (ECLIA) still remains the gold standard method for determining serum PSA. However, faster and more straightforward methods are available on the market, which are usually based on the qualitative immunochromatographic me-

thod that informs whether serum PSA levels are above 4 ng / mL, whose concentrations are present in 70 to 80% of the patients with malignant prostate tumors. The aim of this work is to demonstrate the accuracy of the qualitative immunochromatographic method in comparison with the results from the quantitative ELISA tests. 212 men aged between 39 and 101 were used in this study, where three blood aliquots were withdrawn, one for the ECLIA test and the other two for the immunochromatographic tests with kits from two different manufacturers. 3.3% of the individuals had PSA content within the range of 4 to 7 ng / mL (values with prediction of malignancy of 30%). In this range, 42.9% of the immunochromatographic tests of brand A and 71.4% of brand B showed positive results. In the range above 7 ng / mL (2.8% of the samples), 100% of both tests were positive. Immunochromatographic tests had an average sensitivity of 77.5% at those PSA contents near their detection limits and 100% in samples with PSA contents at an upper range, where 95% of prostate tumors can be detected in association with other tests. Therefore, the immunochromatographic tests seem to be satisfactory when used in screening patients with prostatic neoplasia, especially if we take into account the simplicity and the low cost of this method.

Introduction

Prostate cancer is the most common non-cutaneous malignancy and the second cause of death by tumor among men, with 61,200 new reports in Brazil, 14,290 in the Northeast region and only second to lung cancer in lethality among male cancer patients (BRASIL, 2019). The likelihood of prostate cancer increases with age. In fact, hyperplastic alterations are present in more than 90% of individuals older than 80 and malignant alterations are observed in more than 70% of this population (DAN L, 2013). The average biannual incidence is 66.12 new cases per 100,000 men. About 1 in 9 men will be diagnosed with prostate cancer during their lifetime (BRASIL, 2018).

The risk of prostate cancer

increases by a factor of two when a first degree relative is affected and by a degree of four if two or more are affected. Within the same age group, African-American men have a higher number of multifocal and highly unstable prostatic intraepithelial neoplasia (NIP) lesions, which are precursors of cancer, and higher volume tumors, possibly due to higher levels of testosterone (WEIN, 2012).

The high incidence of clinically diagnosed prostate cancer reflects the effectiveness of screening by using the prostate-specific antigen (PSA) test. Before the PSA test was available, about 19,000 new cases of prostate cancer were reported each year in the United States. This number reached 84,000 in 1993, with a peak of around 300,000 in 1996 (GOLDMAN, 2014).

PSA is a kallikrein-related serine protease that causes seminal clot liquefaction. It is produced by non-malignant and malignant epithelial cells and therefore is prostate-specific, even though it is not specific to prostate cancer as its serum levels might also increase due to prostatitis and benign prostatic hyperplasia (BPH) (CARTER *et al*., 2013). Regarding the serum PSA level, the cutoff point for normal male is 4.0 ng / mL. Values above this threshold should be investigated and may typically represent BPH, prostate cancer or acute prostatitis. Values greater than 10 ng / mL are more frequently associated with prostate cancer, although other causes might be involved such as prostatitis (RODDAM et. al., 2006).

In Brazil, a government-sponsored screening campaign named "Novembro Azul" offers free PSA tests for population with the purpose of finding new cases of prostate cancer in early stages so that treatment can be initiated as soon as possible. The Brazilian Ministry of Health accepts a PSA level up to 4 ng / mL as normal, although it does rule out the existence of tumors with PSA below this value. When the PSA level is above 10 ng / mL, there is a formal recommendation for biopsy (BRASIL, 2018). For values between 4-10 ng / mL, one should also consider the rate of increase in the level of PSA and the free/total PSA ratio (ZERATI *et al*.,

2010).

Among the analytical methods available to determine the serum PSA levels, the electrochemiluminescence assay (ECLIA) is considered the gold standard, however, it requires sophisticated kits and equipment that is usually available only in reference laboratories. On the other hand, there are the rapid tests that are based on the immunochromatographic method, which are able to predict whether PSA levels are above or below 4 ng / mL. Such tests are easier, faster and cheaper than ECLIA. In addition, it can be done regularly in the site of attendance without the need of specific equipment (ROBLES, 2006) Although the immunochromatographic test (also known as rapid test) is frequently performed, studies that investigate its accuracy is still scarce.

The aim of this work is to investigate the accuracy of the immunochromatographic method (rapid test) for qualitative determination of PSA by using the quantitative data obtained from ECLIA as the standard values. The outcome of this study might give more confidence to the results given by the rapid tests, which are routinely used in the screening protocols for prostate cancer, especially during the Blue September campaign.

Material and Methods

Patients

212 men between the ages of 39 and 101 were selected from the Health Basic Units of six districts of the Mata Norte region of the State of Pernambuco during the "Novembro Azul" campaign (November 1st to 30th). As inclusion criteria, men aged 50 or older with no risk factors for prostate cancer were selected. Under the age of 50, only those with risk factors (afro-descendants and positive family history) were able to participate. The exclusion criteria were patients who: 1- ejaculated in the last 48 hours; 2- did exercise on a bicycle (ergometric or not) in the last two days; 3- have been riding a motorcycle for the past two days; 4- have practiced riding a horse in the past two days; 5- have used suppository for the last three days; 6- have received urethral catheter

or have had rectal examination in the past three days; 7- have been submitted to cystoscopy in the last five days; 8- have performed transrectal ultrasonography in the last seven days; 9- have submitted to colonoscopy or rectosigmoidoscopy in the last 15 days; 10- have performed a urodynamic study in the last 21 days; 11- have submitted to prostate biopsy in the last 30 days. This study was submitted and approved by the Research and Ethics Committee of Universidade Potiguar (UnP), according to protocol number 2.837.029. All participants received a clear explanation about the study and had to signed an informed consent.

Sampling

Blood samples were collected from each individual by venipuncture. After centrifugation and serum separation, three aliquots were separated from each sample: one for the ECLIA test, which was performed in a reference laboratory, and the other 2 for the immunochromatographic tests, which were performed with kits from two different brands, both with a detection limit of 4 ng / mL.

Electrochemiluminescence (ECLIA) test

The ECLIA test consists of first subjecting the sample to a medium composed of biotinylated PSA-specific monoclonal antibody and anti-Ruthenium-labeled antibody, allowing the formation of a complex (sandwich). After the addition of the streptavidin-coated microparticles, this complex binds to the solid phase through a biotin-streptavidin interaction. In the measurement cell, the microparticles are magnetically captured on the surface of the electrode, where the application of a voltage induces chemiluminescent emission, whose intensity is directly proportional to the concentration of PSA in the sample, which is measured by a photomultiplier (DEW et. al., 1999).

Immunochromatographic test (rapid test)

The rapid test uses the solid phase immunochromatographic

method for the qualitative detection of serum PSA levels. This test essentially predicts whether these levels are below or above the detection threshold of 4 ng / mL. The kits consist of tapes made of a nitrocellulose membrane matrix with anti-PSA antibody and a cushion impregnated with the color-anti-PSA antibody conjugated in a matrix protein containing 0.1% sodium azide and a desiccant. One drop of sample and one drop of buffer are used in the assay. If two parallel red lines appear (positive result) it means serum PSA level is above 4 ng / mL. On the other hand, PSA levels are below this threshold if only one red line appears (negative result) (CAPLAN & KRATZ, 2002).

Data analysis

Data analysis was performed using statistical software R version 3.5.0, where mean, median and standard deviations of the data were calculated. In addition, sensitivity, specificity as well as positive predictive values and negative predictive values of both immunochromatographic kits were evaluated. Graphing was performed using GraphPadPrism software (version 7.0).

Results

This current study analyzed the concentrations of total PSA by electrochemiluminescence and immunochromatography methods in 212 patients aged between 39 and 101 years old. Table 1 shows the distribution of PSA levels determined by ECLIA according the age. The mean PSA concentration was 2.43 ng / mL and half of the patients had levels lower than 0.8 ng / mL. The minimum PSA level was 0.04 and the maximum was 134 ng / mL (Table 1).

By analyzing the PSA ranges according to the age groups it can be observed that the PSA levels increase as the age progresses, as none of the 50 analyzed individuals between 39 and 49 years old presented levels above 4 ng/mL, whereas 25% of the individuals above 70 years old presented PSA levels above this cutoff value. Benign and malignant changes in the prostate gland increase with age and hence the amount of PSA

produced by the gland. In fact, previous reports with necropsies of men in their eighties revealed hyperplasic alterations in more than 90% of the individuals and malignant alterations in more than 70% (ANDRIOLE, 2009). A similar trend can be observed in this current study (Figure 1), where the PSA average increases with the age group with a significant increase in this average with the group aged between 70 and 101 years old.

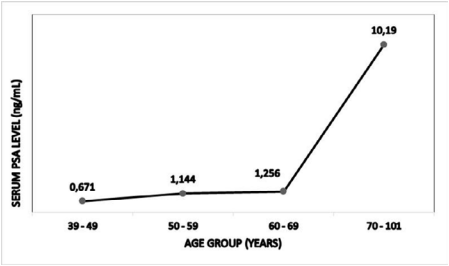

Figure 7. Immunoreactive photomicrographs for β-tubulina III (7A) and NF-200 (7B) demonstrate cells treated with SNCM + MP.

Table 2 presents the results of the immunochromatographic method (rapid test), determined with 2 kits from 2 different brands. The qualitative results (either positive or negative) was compared with the quantitative results previously determined by ECLIA and grouped in three ranges of PSA concentrations. The results show that at PSA concentrations below 4 ng/mL and above 10 ng/mL, the immunochromatographic tests were accurate in classifying the results at either positive or negative. On the other hand, at PSA levels between 4.1 and 10 ng/mL, the rapid tests were not as accurate. The immunochromatographic kits from brand A displayed a negative result in 36.4% of the samples whose PSA levels fell within the 4.1 – 10 ng/mL range, which means that 63.6% showed real positive results and 36.4% showed a false negative. A better accuracy was achieved with brand B, where only 18.2% of the results were false negative within the 4.1 – 10 ng/mL range.

Table 3 shows the determined accuracy parameters: sensitivity, specificity and negative likelihood ratios as well as positive and negative predictive values, calculated for the rapid PSA tests performed using kits from two different brands. Both brands used

in the study showed positive likelihood ratios that tended to infinity as the specificities of the tests were 1.

Discussion

The recruitment of patients during the Novembro Azul campaign occurs through the Primary Care of the Basic Health Units throughout Brazil, where peripheral blood samples are collected from each patient for screening purposes. The blood samples are then sent to a third party laboratory for PSA determination. One of the drawback that prevents the campaign from reaching a larger contingent of people is that there is no "filter" in the initial approach that would separate the healthy population from those with potential chance of being positively diagnosed with prostate cancer.

All samples from the participants of the Novembro Azul campaign are sent for PSA quantification by the most sophisticated method available (electrochemiluminescence – ECLIA), which is generally carried out by laboratories located in other cities, to which the samples are routed. In addition to this logistic, more time are taken to deliver the results to the patient, which makes the whole process more expensive and time-consuming, especially when we consider that 95% of the general population is healthy and does not have altered PSA levels (BRASIL, 2018). Therefore, one way to overcome this problem is to use a rapid qualitative method that would show either a positive or negative result. The immunochromatographic test fits into this category, as it is a fast and low cost method that can show whether the PSA level is below or above the cutoff value of 4 ng/mL and therefore, could be used as way to initially screen which patients must go forward for a more precise examination trough quantitative analysis by ECLIA. However, in order to use the rapid test during the initial screening, one should know about the accuracy of this method.

Among the 212 patients analyzed in this current study, 6.1% presented total PSA levels above 4 ng / mL, which is considered high risk for prostate cancer

according to the Consensus of the Ministry of Health. Thus, only this percentage would be eligible for further investigation by more sophisticated and quantitative analysis, such as total and quantitative free PSA dosage (using the ECLIA method), as well as imaging and pathology examinations. It is worth mentioning that PSA screening alone is not enough to confirm whether the patient has prostate cancer, as there is a possibility of neoplasia with normal levels of PSA which makes the rectal examination essential for men over 45 years with risk factors or over 50 years without risk factors (SADI, 2017).

Serum levels of PSA between 4 and 7 ng / mL (3.3% of the patients) have predicted values for malignancy of 30% (HUGOSSON et. al., 2010), and may be more commonly related to benign processes such as benign prostatic hyperplasia (BPH). In the range of 4.1 to 10 ng/mL, 36.4% and 18.2% of the results were false negative by using the kits from brand A and B, respectively (Table 2). In fact, 70 to 80% of prostate cancer patients have blood PSA levels above 7 ng / mL (GOLDMAN, 2014). On the other hand, neither false negative nor false positive was observed in the PSA levels below 4 ng/mL and above 10 ng/mL, respectively, regardless of the manufacturer of the kit. This finding was reflected in the specificity equal to 1 for both brands (Table 3).

The 2 immunochromatographic kits analyzed in this study had an average sensitivity of 77.5% near their detection limits and 100% in samples with PSA levels in an upper range, where in association with other examination techniques can detect 95% of prostate tumors (GOLDMAN, 2014). Such accuracy is satisfactory when used in initial screening, especially when the simplicity of the method is taken into account in addition to the fact that it can be performed by any professional of the multidisciplinary team.

The PSA level is considered abnormal depending on the men's age, where PSA greater than 2.5 and 3.5 are considered abnormal for man aged between 40 and 49 years and between 50 and 59, respectively. On the other hand, le-

vels higher than 4.5 in men aged between 60 and 69 mean that a more in-depth assessment should be requested, whereas for patients between 70 and 79 years old, PSA level should be 6.5 or less (HAYES *et al*., 2010). The positive predictive value for cancer at a PSA level greater than 10 ng / mL is 60%, while positive predictive value for a PSA level between 4 and 10 ng / mL is only about 30% (KRUMHOLTZ *et al*., 2002). Therefore, even with a low sensitivity to PSA levels close to the detection threshold of 4 ng / mL, the rapid PSA test is effective in screening the general population as its sensitivity was 100% when serum PSA levels exceed 7ng / mL.

Age group (years)	PSA concentration by ECLIA (ng/mL)		
	≤4	4.1 – 9.9	≥ 10
39 – 49 (n=50)	100%	-	-
50 – 59 (n=69)	97.1%	2.9%	-
60 – 69 (n=61)	95.1%	4.9%	-
70 – 101 (n=32)	75%	18.8%	6.2%

Table 1: Distribution of PSA levels, as determined by ECLIA, according to the age of the patients.

Rapid test	RESULT	Concentration range of PSA - ECLIA (ng/mL)		
		0–4	4.1 – 10	≥ 10
Brand A	POSITIVE	0%	63.6%	100%
	NEGATIVE	100%	36.4%	0%
Brand B	POSITIVE	0%	81.8%	100%
	NEGATIVE	100%	18.2%	0%

Table 2: Comparison between the qualitative results of the immunochromatographic method and the quantitative results of the ECLIA method.

Brand	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Ratio of negative verisimilitude
A	0.846 (±0.196)	1	1	0.99 (±0.014)	0.154 (0.043; 0.55)
B	0.692 (±0.251)	1	1	0.98 (±0.019)	0.308 (0.136; 0.695)

Table 3: Accuracy parameters of the immunochromatographic test for qualitative PSA determination

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