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PSA Quick Test in Capillary Blood

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Abstract. *The use of PSA quick testing methods with capillary blood (test strips) to screen for carcinoma of the prostate has been a controversial method. Materials and Methods: The results determined visually from whole capillary blood were compared with the PSA values obtained from serum through quantitative assay and their correspondence was evaluated. PSA values <4 ng/ml obtained through quantitative assay were regarded and as negative results and PSA ≥4 ng/ml as positive results. Results: Of 371 usable assays, 100 quantitatively obtained PSA values were positive and 271 negative. Seven test strips showed false-negative and 49 false-positive results. In comparison with the quantitative assay, this is equivalent to a sensitivity of 93% and a specificity of 82%. Comparing the distinction between PSA >4 and <4 ng/ml only, there was no significant difference between the results of the quick test and the quantitative assay (Fischer exact test, p<0.000). Considering the PSA values between 4 and 10 ng/ml, 10.3% of the results of both methods differed. Conclusion: Our series of experiments ascertained a relatively high rate of false-positive PSA test strip results. In practice this can lead to an unpredictable increase of costs, as every positive result requires a quantitative assay. Even more alarming is the loss of sensitivity in the PSA "between 4 and 10 ng/ml" range, which gives false-negative results leading to a delay of diagnosis and therapy.*

Prostate cancer is the most frequent malignant disease among men. An increased incidence of this disease is expected in the coming years (1). Only a malignant illness limited to the organ allows for a curative therapeutic approach. This requires early detection of the illness and, therefore, active participation in numerous screening examinations. The effective early detection test for prostate cancer is the quantitative PSA assay of serum (2-6). However, in Germany only 14% of men participate in such screenings (1). The acceptance of cancer screenings can be improved through the availability of sufficient information and simple and quick testing methods. The PSA quick testing method using capillary blood would meet these requirements. The quality of the method has to be examined in order not to jeopardize the effective early detection of prostate cancer (7-10).

The accuracy of the PSA quick test Uralen® (Hoyer-Madaus) was evaluated in a prospective monocentric study.

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Materials and Methods

In the period between November 2001 and February 2002, venous blood as well as capillary blood was simultanuously extracted from our patients with various urologic illnesses, who received in-patient or out-patient treatment. Venous blood was used for the extraction of serum for quantitative PSA assay using a chemiluminescence immunoassay. Capillary blood was extracted from the tactile elevation for the PSA quick test. The PSA assays were carried out in parallel on a total of 407 samples, of which 371 could be analysed. The PSA quick test Uralen® (Hoyer-Madaus, Germany) functions on the principle of solid phase immunochromatography (11).

The execution of the tests, from taking of the capillary blood to the reading of the results, was carried out by our laboratory staff according to the following strict conditions: 2 drops of capillary blood from the tactile elevation were mixed with 5 drops of dilution liquid in the designated field of the test strip. The result then was read from the indicator field after an incubation time of exactly 10 minutes. Any band visible on this field was taken as a positive result, if the integrated control band showed the correct functioning of the test strip. The analysis of the test strips was carried out independently by two persons who had, as in a blind study, no information about the PSA value obtained through the quantitative assay. In the event that the test results of the two persons differed, a third person was called in for a decision. A pre-trial with approx. 20 PSA test strips guaranteed correct handling and evaluation of the quick test. The results of this pre-trial series were not included in the study. Defective test strip results were also not included in the final analysis. All experiments were carried out with test strips belonging to the same series (Serial number 03091).

The quantitative PSA assay from serum blood was done in parallel with the quick test in the laboratory using Immulite-PSA®, a solid phase sandwich chemiluminiscence assay for the measurement of total PSA, with the automatic immunoassay system "Immulite" (DPC Biermann, Bad Nauheim, Germany) (12).

Quality control with two control materials, one from Biorad (correctness) and a homemade serum pool (precision) in various concentrations, showed an interassay coefficient of variation (CV) from 4.7% to 5.8% for the quantitative assay at the time of the study.

Table I. *Results of the comparison of quick test PSA assay in age group 35-75 years (male patients); p<0.000.*

	PSA Immulite (ng/ml)	
	<4 ng/ml	≥4 ng/ml
Test strips <4 ng/ml		
number	157	6
% PSA Immulite	79.7	7.1
Test strips ≥4 ng/ml		
number	40	78
% PSA Immulite	20.3	92.9
Sensitivity		92.9
Specifity	79.70%	

Table II. *Correspondence of results of quick test and quantitative PSA assay in various areas of concentration.*

PSA quantitative (ng/ml)	n	Test strips		Correspondence %
		negative	positive	
0 - <2	238	203	35	85.3
2 - <3	21	15	6	71
3 - <4	12	4	8	33
4 - <5	8	2	6	75
5 - <6	16	1	15	94
6 - <8	17	2	15	88
8 - <10	17	1	16	94
≥ 10	42	1	41	98
4 - <10	58	6	52	89.7

The results determined visually from whole blood were compared with the PSA values obtained from serum blood through quantitative assay and their correspondence was evaluated. PSA values <4 ng/ml obtained through quantitative assay were considered as negative results, PSA ≥4 ng/ml as positive results. The sensitivity, specificity, negative and positive predictive values were calculated for the PSA quick test referring to the results of the quantitative assay.

The statistical calculations were carried out using the Fischer exact test (the χ^2- and the *t*-test) (13).

Results

The median patient age was 66.6 years (range: 22-91 years). The 407 blood samples analysed showed PSA values from <0.04 to 1602 ng/ml. Sixteen of these samples originated from female patients serving as a control group and were negative. The results of the corresponding quantitative assays were <0.04-0.16 ng/ml PSA.

Of 100 blood samples with PSA ≥4.0 ng/ml, 93 showed true-positive test strip results, which is equivalent to a sensitivity of 93%. Of 271 blood samples with PSA values <4 ng/ml, the test strips showed a true-negative result in 222 cases, which means a specificity of 82%. In total, a correspondence rate of 84.9% was found between the two methods and the results are significantly comparable (Fischer exact test: $p<0.000$; *t*-test: $p<0.0031$)

Two hundred and eighty-one (79.2%) blood samples out of 355 came from men 35 to 75 years old. In this group of patients, there were 6 false-negative (sensitivity: 92.9%) and 40 false-positive test strip results (specificity: 79.7%). In this group, the test strip overlooked 7.1% of the PSA values ≥4 ng/ml obtained through the quantitative assay (see Table I). The statistical analysis (Fischer exact test: $p<0.000$; *t*-test: $p<0.0023$) showed no difference between the results of the PSA quick test and the quantitative assay.

Division of the PSA levels. Most of the discrepant results between the quick test and the quantitative assay occurred at the PSA level between 3 and 5 ng/ml (Table II). At a PSA-level of 3 through <4 ng/ml, only approx. one-third of the results were true-negatives. Between PSA 4 and <5 ng/ml, approx. one quarter of the results were false-negative, not detecting increased PSA values. Taking PSA between 4 and 10 ng/ml, 10.3% of the results of both methods differed (Table II). With the grouping of the PSA levels, the results were not significantly comparable (χ^2- after Pearson: n.s.; *t*-test: n.s.)

The positive predictive value for the PSA quick test with reference to the quantitative PSA Immulite method was 65.5% in our selected group of patients (27%), with a relatively high share of PSA values ≥4.0 ng/ml. The negative predictive value was 96.9%.

Discussion

The new generation of PSA test strips uses whole blood and thus complies with some important demands made on screening tests: (a) The tests can be carried out without any additional equipment and laboratory kits (no centrifuge needed for the preparation of serum); (b) The tests are only slightly invasive and therefore easily accepted by the patients. Additionally, the PSA quick test Uralen® (Hoyer-Madaus) is easy to learn and to administer and thus can also be carried out by persons without specific laboratory training, provided they adhere to the indicated procedure. However, it was found that a pre-trial employing the test strips was necessary because the band in the indicator field is often hardly visible. The question of whether an untrained person is able to apply the two necessary drops of capillary blood to the test strip without air bubbles remains unanswered because the capillary blood extraction in our

series of experiments was carried out exclusively by laboratory staff. Our experience shows that it would be impractical for a patient to use the strip test.

The criteria of validity for the PSA quick test obtained in our study confirm the results (Table I) of Berg *et al.* (14) (sensitivity: 90.5%; specificity: 83.8%). The identification of the groups PSA <4.0 ng/ml and PSA >4.0 ng/ml with the two methods was statistically comparable.

Regarding the question of whether the PSA quick test represents a valuable contribution to the early detection of prostate cancer, one must consider the composition of the group of patients examined. Differences in the level and distribution of PSA values in different groups must be taken into account when interpreting the results.

Programs for the early detection of prostate cancer aim at detecting as many cases as possible at a stage when curative treatment is possible. At a PSA value of ≥10 ng/ml, approx. 67% prostate carcinomas discovered are already in an advanced stage (15). It is therefore critical in a screening examination that the test strip discovers a PSA value below 10 ng/ml with certainty. The results (Table II) of this monocentric prospective study showed that the correspondence rate of quantitative and qualitative PSA test depends on the PSA values evaluated. Especially at the level which is important for the early detection of prostate cancer, PSA 4-10 ng/ml, the obtained sensitivity of the quick test is clearly lower than at PSA >10 ng/ml (89.7% *versus* 97.6%). In this study, the PSA quick test did not discover approx. 10% of the PSA values within the "grey area".

An increased PSA level above the commonly used threshold value of ≥4 ng/ml served as reference point for the evaluation of sensitivity and specificity in our study (16, 17). The histologically confirmed diagnosis of prostate illness was not a criterion for the assessment of the validity of the PSA test strip. Thus, it can only be supposed that within the target group of the early detection program for prostate cancer (patients with carcinoma limited to the organ), more carcinomas remain undetected because of this low specificity of the test strip at PSA between 4 to 10 ng/ml in comparison with the use of a validated quantitative PSA assay. Twenty % of patients with diagnosed carcinoma of the prostate have a PSA value <4 ng/ml anyway (18, 19), so that the use of a less sensitive method narrows the validity of a good diagnostic tool unnecessarily.

When discussing the validity of qualitative and semi-quantitative quick tests, it should be considered that quantitative methods also show an analytic deviation (dispersion) which finds its mathematical expression in the coefficient of variation (20). In the case of the quantitative PSA immunoassay applied here, the maximum interassay coefficient of variation was 5.8% (calculated on the basis of the daily quality control data). This means the "true" value for a measured value of, for example, 4 ng/ml lies with 95% reliability between 3.54 and 4.46 ng/ml ("interval of confidence"). That also means in the area around the established cut-off of 4 ng/ml, there is an uncertainty in assigning a PSA value into the group < or ≥4 ng/ml. This is where the advantages of a quantitatively measured value as opposed to a qualitative analysis become apparent. Considering all accompanying circumstances and risk factors, *i.e.* age of patient, family anamnesis, a PSA value of 3.5 ng/ml should dictate a repeat examination within less than one year.

Another important advantage of quantitative PSA assays over the test strip method is accumulation of a PSA value history. Provided that similar testing systems are used and the usual quality criteria are observed, the speed of PSA increase can be recorded (7). This procedure allows the early detection of high-risk patients even before the commonly used cut-off at 4 ng/ml is reached (21, 22).

An important factor in early detection of cancer is the individually optimized screening interval depending on the age and PSA level (23-26). The PSA test strip, in its present form, does not allow for such an optimization of the screening examination. As the analysis of several studies shows, a two-yearly screening examination for prostate cancer is sufficient for men with an initial PSA value below 2 ng/ml and whose digital rectal examination gave no suspect results, whereas men with an initial PSA value above 2 ng/ml should be screened annually in the future (23-27).

The high rate of false-positive results of the PSA quick test (specificity: 82%) should be mentioned. Only two-thirds of all positive results of the test strips in our series of experiments were correct. Since every positive test result should be checked with the help of a quantitative PSA assay before further diagnostic measures are applied, a higher cost is involved in comparison with the use of the quick test. Furthermore a false-positive test result may lead to a considerable anxienty among the affected men. However, the relatively low specificity is not an obstacle in detecting as many patients with locally limited prostate cancer as possible.

Conclusion

The PSA quick test Uralen® examined in the present study works on the basis of capillary blood, is easy to carry out and barely invasive. However, it should only be carried out by trained staff. Regarding the classification according to increased or "normal" PSA value (cut-off 4 ng/ml), there was no statistical difference between the quick test and the quantitative PSA assay concerning male patients between 35 to 75 years old in our group. However, the correspondence rate depended on the PSA range examined. With PSA values between 4 and 10 ng/ml – which is the relevant area for the early detection of prostate cancer – the PSA quick test overlooks approx. 10% of the increased PSA values

(with reference to the quantitative PSA assay). It can only be speculated whether the number of increased PSA values which remain potentially undetected by the PSA quick test, and accordingly of undetected carcinoma of the prostate, can be balanced by an increased number of detected carcinoma as a result of the wider acceptance of this kind of screening examination. Only a quantitative assay can provide the information necessary for the identification of high-risk patients whose PSA value is still below the fixed cut-off at 4 ng/ml, as for example consideration of PSA age-specificity and the speed of PSA increase. In addition to the loss of sensitivity in the PSA range 4 to 10 ng/ml, this is another argument against the use of a qualitative PSA assay as a screening method. However, in view of the desired progress towards early detection of prostate cancer and the high acceptance of this form of examination by the men concerned, the further development of PSA quick testing methods is desirable and useful, although quantitative methods should be given priority.

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