Legal Medicine 62 (2023) 102243

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

The proportion of false-positives in positive Seratec® prostate-specific antigen SemiQuant test results in postmortem screening for seminal fluid

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ARTICLE INFO

Keywords:
Vaginal swabs
Autopsy
Forensic science
Prostate-specific antigen
Immunochromatographic test
False positive

ABSTRACT

Prostate-specific antigen (PSA) tests are used in forensics to conduct rapid screening for semen in vaginal swab samples from alleged victims of sexual abuse. Although PSA membrane tests have been applied to autopsy specimens, no study has evaluated predictors of false-positive test results in relation to factors such as age, cause of death, postmortem interval, drugs, and alcohol. This study describes the results obtained with the Seratec® PSA SemiQuant Kit test in 283 deceased women, with or without a history of sexual assault. Overall, 18.4% (52/283) of the vaginal swab samples tested positive for PSA. However, 63.5% (33/52) of the PSA-positive vaginal swab samples had no sperm detected. The proportion of false-positives in positive PSA results was 94.4% in those aged over 60 years. Multivariate logistic regression for PSA-positive samples showed that the proportion of false-positives in positive PSA results increased with the age of the deceased. However, the cause of death, postmortem interval, and presence of drugs or alcohol in the blood or urine of the deceased did not affect the PSA determination. These results show that PSA membrane tests are relatively unreliable and can be misleading, especially when derived from vaginal swab samples of older women, obtained at autopsy. In forensic cases, positive PSA screening test results may have an impact on subsequent legal actions and criminal charges brought against the accused. These findings are important for both forensic pathologists and the police to ensure accurate screening of older women in cases of suspected sex crimes.

1. Introduction

In sexual assault cases with alleged male perpetrators, it is important to forensically identify semen as a source on the victim's body to confirm male sexual contact. The most accepted and reliable method for identifying semen is microscopic detection of sperm cells. However, microscopic observations are time-consuming and, therefore, unsuitable for semen screening. Seratec® PSA SemiQuant is an immunochromatographic rapid test for the detection of the prostate-specific antigen (PSA) that is widely used in forensics for the detection of seminal liquid in alleged victims of sexual abuse [1–3]. In Japan, police laboratories do not perform direct analysis for the presence of semen. If the screening is negative, no further analysis is performed. Seratec® PSA SemiQuant is one of the most frequently used screening tests by police and forensics because PSA tests are more specific than the acid phosphatase tests [4]. Therefore, it is of great forensic significance to determine the accuracy of screening with this kit.

The PSA, also known as γ-seminoprotein, kallikrein-3, protein E1, or p30, is a single-chain serine protease with a molecular weight of 33 kDa [5,6]. There are three forms of PSA in the human body: free PSA (33 kDa; the predominant form in semen), PSA-α1-antichymotrypsin (100 kDa), and PSA-α2-macroglobulin (800 kDa) [5]. PSA is one of the major proteins in seminal fluid with concentrations of 0.2–3.0 mg/mL, but the fact that PSA is found only in very low concentrations in vaginal fluid (≤1.25 ng/mL) makes PSA a useful marker in forensics for the detection of even small amounts of seminal fluid [7,8]. Although PSA is produced in the prostate and secreted into the seminal fluid, it is not unique to the prostate. PSA can be found in other biological fluids in women, including serum, urine, and amniotic fluid, as well as fluids in the periurethral glands (Skene's gland), breast, ovaries, and endometrium [9–11]. Various studies have detected PSA in 45–80% of female periurethral glands [9,10,12,13].

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https://doi.org/10.1016/j.legalmed.2023.102243
Received 5 February 2023; Received in revised form 10 March 2023; Accepted 15 March 2023
Available online 21 March 2023

To the best of our knowledge, there are no studies describing the predictors of the proportion of false-positives in positive PSA results (i.e., the vaginal swab samples that were positive on the PSA test yet negative on microscopy and police investigations) in forensics. This paper describes the results obtained with Seratec® PSA SemiQuant test in vaginal swab samples collected from women with or without a history of sexual assault.

2. Materials and methods

2.1. Study material

Samples were collected from women (n = 283; mean age: 54.9 ± 25.2 years) who underwent medicolegal autopsy at a single university between 2011 and 2021. The following variables were collected for analysis: age, cause of death, postmortem interval, and toxicology (drugs and alcohol) results. This study was approved by the local ethics committee (approval number M2000-1297).

2.2. Sample collection and preparation

Vaginal swab samples were collected from each subject. Before the autopsy, the swab was inserted into the vagina until resistance was felt and was rotated five times to obtain a sample. After collection, swab was placed in screw-capped tube and air-dried at room temperature (21 to 23 °C (70 to 73°F)).

For determination of PSA, a specific membrane test, Seratec® PSA SemiQuant Cassette (Seratec Postfach 3706 D-37079; Gottingen, Germany) was used. The determination limit according to the manufacturer is 1 ng/mL or greater. The assay was performed according to the manufacturer's instructions (https://www.seratec.com). Briefly, the air-dried swab was minced and inserted into a tube prefilled with a supplied standard buffer solution with neutral pH and reacted for 10 min for extraction. After extraction, 3 drops of the extraction buffer solution (approximately 120 μL) were added to the supplied test cassette and the result was read after 10 min for PSA determination. Results, in which the control line, the internal standard line, and the test line were detected, were accepted as PSA-positive. To confirm the presence of sperm, we tested PSA-positive samples using microscopy (hematoxylin-eosin and Oppitz staining). Positive results on microscopy of both stain types were considered "true positive". Samples that were positive on the PSA test yet negative on microscopy and police investigations were considered "false positive.".

2.3. Statistical analysis

Continuous variables were summarized using means and standard deviations, and categorical variable were summarized as frequencies and percentages. The Cochran-Armitage trend test or Fisher's exact test was used to examine the associations of the PSA test results with age and postmortem interval, cause of death, drugs, and alcohol, variables that have been reported to be associated with PSA levels in previous studies [5,6,14–16]. We also performed univariate and multivariable logistic regression analyses on the PSA-positive samples to evaluate the association between each variable and the proportion of false-positives in positive PSA results. Two-sided p-values < 0.05 were considered to be statistically significant. All the analyses were performed using the SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA).

3. Results

Of the 283 samples obtained from deceased women, 52 (18.4%) were positive for PSA. Basic demographics of PSA positive cases are shown in Table 1. No sperm was observed in 63.5% (33/52) of the PSA-positive samples. The results of hematoxylin-eosin and Oppitz staining were in a perfect match. As shown in Tables 2 and 3, the results of statistical tests and univariate logistic regression analyses showed that age and the presence of alcohol in the blood or urine were significantly associated with the proportion of false-positives in positive PSA results. In the multivariable logistic regression analysis, age was the only variable associated with the proportion of false-positives in positive PSA results. The proportion of false-positives in positive PSA results increased with the age of the deceased and was 94.4% (17/18) in those aged over 60 years. Univariate logistic regression analysis showed that the proportion of false-positives in positive PSA results was higher in subjects with alcohol detected in the blood or urine, but multivariable logistic regression showed no significant difference. Collectively, the results of the multivariable logistic regression showed that false positives were more common in older women, regardless of the cause of death, time since death, medications, or alcohol consumption. In other words, the positive predictive value of the PSA test was shown to decrease with age.

Table 1
Basic demographics of PSA positive cases.

		PSA positive cases N = 52
Age	Mean ± SD	50.6 ± 23.4
	Median (range)	48 (17–95)
	≤ 20 years	4 (7.7%)
	21–40 years	15 (28.8%)
	41–60 years	15 (28.8%)
	> 60 years	18 (34.6%)
Cause of death	Disease	11 (21.2%)
	External causes	40 (76.9%)
	Undetermined	1 (1.9%)
Postmortem interval	< 2 days	22 (42.3%)
	≤ 7 days	19 (36.5%)
	> 7 days	11 (21.2%)
Drugs	Negative in blood	7 (13.5%)
	Positive in blood	45 (86.5%)
	Addictive drugs (Stimulants)	3 (5.8%)
	Addictive drugs (Suppressants)	18 (34.6%)
	Cardiovascular drugs	13 (25.0%)
	Gastrointestinal drugs	0
	Central nervous system drugs	31 (59.6%)
	Antibiotics	0
	Anticoagulants	1 (1.9%)
Alcohol	Positive in blood/urine	29 (55.8%)
	Negative in blood/urine	23 (44.2%)
	Undetermined	0

4. Discussion

The PSA test is the most-recommended immunochromagraphic membrane screening test that has been used in forensics involving suspected sexual crimes [17–19]. According to the manufacturer's brochure, this test does not cross-react with other proteins in the seminal fluid. However, this study showed that the results of the Seratec® PSA SemiQuant Kit are relatively unreliable especially in older women, and can be misleading when obtained during an autopsy, regardless of postmortem interval, therapeutic drugs, alcohol consumption, and stage of decomposition.

In Japan, police laboratories do not perform direct analysis of DNA if screening for the presence of semen is negative. Therefore, we believe that our results are important because positive results of the PSA screening test have an impact on subsequent legal actions and the criminal charges that are brought against the accused, and false-positive results can create unnecessary extra police work in the initial investigation.

To the best of our knowledge, two previous reports have reported false-positive PSA tests, one due to condom lubricant [20] and the other using samples from highly decomposed bodies [14]. However, these studies did not assess the predictors of false-positive PSA results. Our study found that the accuracy of PSA tests is not affected by the postmortem interval. Although we could not assess condom use due to lack

Table 2
Associations between each factor and true- and the proportion of false-positives in positive PSA results.

		Sample N = 52	PSA test results		p-value
			Proportion of false-positives in positive PSA results (N = 33)	True Positive (N = 19)	
Age	≤20 years	4 (7.7%)	1 (25.0%)	3 (75.0%)	<0.0001
	21–40 years	15 (28.8%)	5 (33.3%)	10 (66.7%)	
	41–60 years	15 (28.8%)	10 (66.7%)	5 (33.3%)	
	> 61 years	18 (34.6%)	17 (94.4%)	1 (5.6%)	
Cause of death	Disease	11 (21.2%)	9 (81.8%)	2 (18.2%)	0.217
	External causes	40 (76.9%)	23 (57.5%)	17 (42.5%)	
	Undetermined	1 (1.9%)	1 (100%)		
Postmortem interval	<2 days	22 (42.3%)	11 (50.0%)	11 (50.0%)	0.093
	≤7 days	19 (36.5%)	13 (68.4%)	6 (31.6%)	
	>7 days	11 (21.2%)	9 (81.8%)	2 (18.2%)	
Drugs	Negative in blood	7 (13.5%)	4 (57.1%)	3 (42.9%)	0.697
	Positive in blood	45 (86.5%)	29 (64.4%)	16 (35.6%)	
Alcohol	Negative in blood/urine	23 (44.2%)	11 (47.8%)	12 (52.2%)	0.047
	Positive in blood/urine	29 (55.8%)	22 (75.9%)	7 (24.1%)	

Table 3
Crude and adjusted odds ratios of PSA-positive samples showing risk factors for the proportion of false-positives in positive PSA results.

		Crude		Adjusted	
	Category	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	1 unit increase of age category	3.85 (1.74–8.49)	0.0009	3.07 (1.34–7.04)	0.008
Cause of death	Diseases	1 (reference)		1 (reference)	
	External causes	0.35 (0.07–1.72)	0.462	0.54 (0.09–3.13)	0.520
	Undetermined	0.79 (0.01–91.54)	0.904	1.66 (0.01–266.25)	0.743
Postmortem interval		2.03 (0.91–4.53)	0.084	1.26 (0.49–3.23)	0.636
Drugs	Negative in blood	1 (reference)		1 (reference)	
	Positive in blood	1.39 (0.28–6.98)	0.689	0.89 (0.12–6.78)	0.908
Alcohol	Positive in blood/urine	1 (reference)		1 (reference)	
	Negative in blood/urine	0.31 (0.10–0.99)	0.048	0.60 (0.15–2.47)	0.478

The proportion of false-positives in positive PSA results cases were analyzed as events. Age and postmortem interval were analyzed as continuous variables, with simple scores ranging from 1.

of information, condom use is thought to be uncommon in post-menopausal women [21].

Expression of PSA has been detected in the periurethral glands, breast, ovary, endometrium, serum, urine, and amniotic fluid of women. Additionally, PSA expression is closely related to hormone levels. The PSA gene contains an androgen response element, and its transcription is upregulated by androgen through the androgen receptor [6]. Androgen administration increases female PSA levels in women's urine [22]. Furthermore, measurable amounts of PSA can be secreted in breastmilk of women receiving oral contraceptive progestins [5].

Although PSA expression is likely to be age-dependent, because aging alters hormone production, the effect of age on PSA levels is unknown. Yu and Diamandis reported that they found more PSA positive serum in women over 50 years old (detection limit: 0.01 μg/L) based on 1064 female sera, and attributed this finding to the menopausal status of the older women [5]. According to their hypothesis, the ratio of estrogen to androgen decreases after menopause and promotes PSA production.

It might be possible for a false-positive result to occur in vaginal PSA assays if the deceased woman had detectable PSA levels in the vaginal fluid antemortem or if PSA was secreted (leaked) from the periurethral glands into the vagina postmortem. Similar to postmortem ejaculation in men, rigor mortis in certain pelvic muscles may lead to postmortem secretion of PSA from the periurethral glands in women.

Based on the above, false-positive results may occur in women with hormone levels altered by age or drugs that promote PSA expression. Alternatively, PSA expression may be affected by other unknown factors. A limitation of this study is that we were unable to examine the false-negative rate. This is because, at our institution, subsequent semen testing is not performed if the screening test is negative. It should be noted that although the possibility is low, the possibility that the women in this study with "false-positive" results had been sexually assaulted by men with aspermia or who had undergone vasectomy cannot be ruled out, and may have affected the accuracy of our findings. Statistically, it should be noted that the prevalence of semen in samples from different age groups may also affect the observed results of false-positive proportions [23].

In this study, we found that PSA testing with the Seratec® PSA SemiQuant Kit is relatively unreliable and potentially misleading when obtained from vaginal swab samples of older women at autopsy, regardless of the postmortem interval, stage of decomposition, cause of death, or presence of alcohol or drug consumption. When using PSA positivity as a screening test for male sexual contact, the interpretation of positive results should take the age of the woman into account.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement:

The authors confirm that the data supporting the findings of this study are available within the article.

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