

## TECHNICAL NOTE

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# Expression of Seminal Vesicle–Specific Antigen in Serum of Lung Tumor Patients

**ABSTRACT:** Protein markers are commonly used in forensic medicine to establish the origin of human fluids detected in crime scenes. Semenogelins, the major protein constituents of semen coagulum, are the most effective markers for semen detection. Recently, it has been demonstrated that semenogelins are also ectopically expressed in small cell lung carcinomas (SCLC) and in a minority of non-small cell lung carcinomas (NSCLC). This finding prompted us to look for semenogelin expression in the serum of lung cancer patients. A set of 13 serum samples (3 from SCLC and 10 from NSCLC patients) was screened by enzyme–linked immunosorbent assay (ELISA), using a commercially available kit. Four of the NSCLC cases showed positive results. Ectopic expression of marker proteins in individuals affected by cancer could represent a potential source of forensic pitfalls.

**KEYWORDS:** forensic sciences, semen, semenogelins, immunoassay, seminal vesicle specific antigen

Detection and identification of seminal traces is one of the main forensic tools in cases of sexual assault. At present, many laboratory tests, based either on sperm microscopic identification or acid phosphatase activity detection (1,2), are available to forensic scientists in order to detect the presence of semen. However, while acid phosphatase can also be found in other tissues, microscopic sperm identification would exclude azoospermic or vasectomized males.

In recent years two semen proteins have been thoroughly investigated: prostate–specific antigen (PSA), and seminal vesicle specific antigen (SVSA), also known as semenogelins (Sg). Semenogelins are the major protein constituents of the semen coagulum (3). In human semen, two major proteins have been described, namely semenogelin I (SgI) ( $M_r$  50 KD), and semenogelin II (SgII). The latter presents two isoforms with an apparent mass difference of 5 KD due to glycosylation process (71 KD and 76 KD, respectively) (3). For a long time Sg expression was considered restricted to the seminal vesicles epithelium and epididymis (4,5). Their site specific expression has allowed the use of semenogelin specific antibodies for forensic detection of seminal fluid in sexual assaults (6). A commercial kit based on ELISA immunoassay has also been developed for this purpose.

Recently, ectopic expression of Sg was detected in cell lines derived from small cell lung carcinoma (SCLC), from both male and female patients, as well as in cell lines from a minority of non small cell lung carcinoma (NSCLC) tumors (7). This finding prompted us to investigate the serum of patients affected by lung carcinoma for the presence of Sg.

## Materials and Methods

Serum samples from 13 lung cancer patients (3 SCLC and 10 NSCLC) and 10 healthy donors were collected.

Tumor classification (Table 1) was performed according to the World Health Organization (WHO) classification (8). Serum samples were diluted to 0.02 mg/mL with saline solution. ELISA tests for Sg were performed on a 96 well plate by SEMA assay kit (Humagen Fertility Diagnostic, Inc., Charlottesville, VA), following the manufacturer instructions (9). In order to identify false positives due to blood contamination, mock samples (no antibody added) were assayed in parallel, as recommended by manufacturer.

After colorimetric detection, samples were diluted by adding 1 mL of distilled water to wells, and color intensity was measured spectrophotometrically at 415 nm. Each test was performed in duplicate.

## Results and Discussion

Four out of 13 serum samples from lung tumor patients showed positive results for Sg, as detected by the ELISA test (Table 1). Control samples from healthy donors, negative controls provided in the kit, and mock controls all yielded negative results.

Lung cancer is the most common cancer in the world (10), either in term of incidence of mortality, and is still increasing in particular in the developing world and among women (11). Histologically, lung cancers are commonly classified in two major categories, namely non small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC). The former category represents the overwhelming majority of cases, including squamous cell carcinoma and adenocarcinoma. In the present series, we found positive expression in the serum samples of patients affected by adenocarcinoma or squamous cell carcinoma of lung.

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TABLE 1—Case population and results.

#Patients	Diagnosis	Sg ELISA Test Results
1	Small cell carcinoma	—
2	Small cell carcinoma	—
3	Small cell carcinoma	—
4	Adenocarcinoma	—
5	Adenocarcinoma	—
6	Adenocarcinoma	+++
7	Adenocarcinoma	—
8	Adenocarcinoma	—
9	Adenocarcinoma	—
10	Squamous cell carcinoma	+
11	Squamous cell carcinoma	+
12	Large cell Carcinoma	+
13	Pleomorphic Carcinoma	—

— Negative.

+ Positive.

+++ Highly positive.

In a previously reported series (7) Sg expression was detected by immunohistochemical analysis in 12 out of 13 tissue samples of small cell carcinoma and in 4 out of 21 and 2 out of 7 squamous cell carcinoma and adenocarcinoma samples respectively. In our series we could not demonstrate the presence of Sg in the serum of our 3 samples of small cell carcinoma of the lung, whereas Sg was detectable in 4 out 13 non-small cell carcinomas (2 squamous carcinoma, 1 adenocarcinoma and 1 large cell carcinoma of the lung). This discrepancy in the relative frequency of Sg positivity in lung carcinomas could be explained either by the small size of our series either by the different techniques used in the two studies. In fact the presence of Sg in tissue samples not necessarily correlate with its secretion in the serum.

The origin of this ectopic secretion of semen specific proteins remains largely unknown. Recently, Sg gene transcripts and gene product expression have been documented in several non-genital tissues (12) including breast, prostate, trachea and bronchi. In the trachea and bronchi Sg expression was limited to the basal layer of the respiratory epithelium. It is intriguing to note that squamous cell carcinoma and adenocarcinoma of the lung are supposed to have origin from these latter cells and therefore the serum presence of Sg in patients affected by the aforementioned tumors could be explained by the clonal expansion of the basal layer.

Our findings appear to confirm a broader distribution of Sg expression than previously supposed, including also serum of patients affected by lung carcinoma.

This must be taken into account when the presence of semen in vaginal blood samples is assessed by Sg ELISA test. Nowadays, the issue becomes significant due to a raising incidence of lung

cancer in women. Particular attention will have to be made towards vaginal blood samples from women aged over 40 and having a smoking history. A possible source of error may derive from the unsuspected presence of lung tumor in the presumed victim. A thoracic computerized tomography (CT) scan should exclude this possibility. A negative CT scan in conjunction with supplementary tests (such as PSA evaluation in the forensic sample) will confirm the seminal origin of the detected Sg.

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