Research Article

Serum Epcam And BSG As Detectable Circulating Tumor Cell Associated Biomarkers For Diagnosis And Chemotherapy Monitoring In Lung Cancer Patients By Using ELISA

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Abstract

Background: Lung cancer continues to be one of the major causes of cancer-related deaths globally and requires novel minimally invasive biomarkers for diagnosis and during systemic therapy. Circulating tumor cell-associated proteins could be used as dynamic markers of tumor burden and biological changes associated with chemotherapy. **Objective**: This study aimed to assess the potential of serum epithelial cell adhesion molecule (EpCAM) and Basigin (BSG) as Protein expression detectable circulating tumor cell-associated biomarkers in lung cancer patients with focus on diagnostic performance, modulation by chemotherapy and correlation with the tumor aggressiveness. **Methods**: This was an analytical case-control study that incorporated lung cancer patients and healthy controls. Enzyme-linked immunosorbent assay (ELISA) was used for measuring the levels of serum EpCAM and BSG (Protein expression). The distribution of the biomarkers was mostly non-normal, therefore Kruskal-Wallis and Dunn post-hoc testing, Mann-Whitney U test, Spearman correlation, receiver operating characteristic (ROC) analysis, and DeLong comparison of AUCs were performed as non-parametric analysis of these values. **Conclusion**: EpCAM and BSG was significantly higher in the serum of lung cancer patients than in the controls. Median EpCAM was higher in patients than controls (7.65 vs. 5.75 ng/mL, $p < 0.001$), while BSG showed a more pronounced increase (624.53 vs. 117.77 ng/mL, $p < 0.001$). BSG showed the highest effect size ($r = 0.67$) and the highest diagnostic accuracy (AUC = 0.939; DeLong $p = 0.004$) compared with EpCAM (AUC = 0.768; DeLong $p = 0.139$). The relationship of both BSG and EpCAM with the number of chemotherapy doses was found to be moderately negative (rho = -0.42, $p<0.001$) and moderately positive (rho = 0.22, $p=0.09$), respectively, with higher stage and grade, while stage and grade were not significantly associated with EpCAM. EpCAM and BSG are valuable Protein expression detectable markers in cancer of the lung. BSG seems to be a better option for discrimination of the cancer for diagnosis, monitoring treatment with chemotherapy, and representing the general aggressiveness of the cancer. The authors recommend larger longitudinal studies with a standardized clinical response endpoint prior to routine clinical use.

Keywords: Lung cancer, EpCAM, CTC, Chemotherapy response.

Introduction

Lung cancer is a significant health problem in the world and a leading cause of cancer deaths. According to the Global cancer statistics, lung cancer is the most common cancer globally, accounting for 2.5 million new cases and 1.8 million deaths in 2022 (Bray et al., 2024). Improvements in imaging, histopathology, targeted therapy, immunotherapy, and supportive care have not stopped many patients from being diagnosed with advanced disease, or progressed to advanced disease, and this has resulted in a range of responses to systemic therapy and continued monitoring of patients remains a clinical challenge.

While conventional tissue biopsy is crucial for histological characterization and molecular characterization, it is invasive, can lack representation of intratumor heterogeneity, and is only a snapshot of tumor biology. Liquid biopsy has thus become a complementary approach in the non-invasive monitoring. Circulating tumor cells (CTCs) are especially interesting because they are indicative of the dissemination of the tumour and can yield real-time data on tumor evolution, immune escape, treatment resistance and metastatic potential (Gu, Wei and Lv, 2024; Sugai et al., 2024). EpCAM is one of the most widely used epithelial markers in CTC detection because many carcinomas express EpCAM on the cell surface, making it useful for label-dependent enrichment and immunological detection. Meta-analytic evidence has suggested that EpCAM overexpression has diagnostic value in lung cancer, although its prognostic value appears less consistent (Zhu et al., 2021). A key limitation of EpCAM-based detection is that CTCs may undergo epithelial-mesenchymal transition (EMT), during which epithelial markers such as EpCAM are downregulated, potentially leading to underestimation of the total CTC-associated tumor burden (Gu, Wei and Lv, 2024; Sugai et al., 2024).

BSG or extracellular matrix metalloproteinase inducer, is a transmembrane glycoprotein implicated in tumor progression, extracellular matrix remodeling, glycolytic adaptation, invasion, metastasis, and drug resistance. Experimental evidence in lung adenocarcinoma has demonstrated that BSG is upregulated and that silencing BSG suppresses tumor cell proliferation,

migration, invasion, and lipid-metabolic dysregulation while promoting apoptosis (Zhang et al., 2023). These attributes render BSG as a potential, biologically relevant circulating biomarker for tumor aggressiveness and treatment monitoring.

Considering the above mentioned, the present manuscript emphasizes only the measurement of EpCAM and BSG in serum of lung cancer patients by Protein expression. The aim was to assess their diagnostic utility, test their correlations with chemotherapy exposure, and verify if either of the markers is associated with the stage and grade of the tumor.

Materials and Methods

Study design and population

This study was a analytical case-control study in which the serum EpCAM and BSG levels of the patients were compared with healthy controls. Sixty-eight lung cancer patients and 20 healthy controls were included in the major pooled patient-control analysis. Patients were classified as newly diagnosed untreated patients or chemotherapy-treated group. Subgroup analyses were conducted based on the available Protein expression data for each comparison.

Sample collection

Lung cancer patients and healthy people provided venous blood samples. This serum was then stored in the laboratory under appropriate conditions until the time of protein expression analysis. Clinical/ pathological parameters such as treatment status, chemotherapy regimen, number of chemotherapy doses, tumor stage and histological grade were documented for statistical analysis of correlation with biomarker levels.

To assess the expression of EpCAM and BSG in protein level.

The levels of serum EpCAM and BSG were determined by commercially available enzyme-linked immunosorbent assay kits based on the manufacturer's instructions. The concentrations were expressed as ng/mL. This manuscript focused on only those protein-level results for EpCAM and BSG that were obtained through Protein expression, and excluded gene expression markers and other protein markers from the scope of the manuscript because of the desire to maintain a focused scope of the manuscript.

Statistical analysis

The Shapiro-Wilk test and Kolmogorov-Smirnov test were used to assess normality. Since the majority of the EpCAM and BSG data showed non-normal distribution, they were analysed using non-parametric statistical techniques. Multiple group comparisons were performed using the Kruskal-Wallis test and Dunn post-hoc test with Bonferroni correction as appropriate. Two group comparisons were made using the Mann Whitney U test. Spearman correlation was applied to test their association with the number of chemotherapy doses and age. The ROC curve analysis was used for assessing the accuracy of diagnosis and the DeLong test for comparing AUC between EpCAM and BSG. A p-value <0.05 was considered statistically significant.

Results

Data is assessed for normality and the reasons for using non-parametric procedures are explained. Data are checked and discussed for their normality and why non-parametric analyses are used.

The protein levels data for EpCAM and BSG were mostly non-normal distributed, especially in patient groups that were treated and controls. This pattern was the reason why non-parametric analyses were used throughout the manuscript. The apparent normality in the untreated group was treated with caution due to the small sample size in each group.

Table 1. Serum EpCAM and BSG were normally distributed based on the Shapiro Wilk test.

Marker	Group	N	W	p-value	Interpretation
EpCAM	Diagnosis	11	0.912	0.262	Normal
EpCAM	C1	24	0.886	0.011	Non-normal
EpCAM	C2	33	0.818	<0.001	Non-normal
EpCAM	Control	20	0.735	<0.001	Non-normal
BSG	Diagnosis	11	0.861	0.064	Borderline normal
BSG	C1	24	0.760	<0.001	Non-normal
BSG	C2	33	0.734	<0.001	Non-normal
BSG	Control	20	0.682	<0.001	Non-normal

C1: Early chemotherapy exposure; C2: later or more intense chemotherapy exposure. The distribution of genes among different groups. Distribution of genes between different groups. A Kruskal-Wallis test showed that there were significant differences between the groups of patients and controls in serum EpCAM and BSG levels. For BSG this difference was greater than for EpCAM.

Table 2. Serum EpCAM and BSG were compared between the study groups using the Kruskal-Wallis comparison.

Marker	Kruskal-Wallis H	Df	p-value	Interpretation
EpCAM	8.52	3	0.036	Significant
BSG	12.10	3	0.007	Significant

Patient group differences when compared to healthy controls, at the pair level EpCAM was significantly higher in newly diagnosed patients than in controls (Dunn post-hoc testing), and lower in the late-treatment group than in newly diagnosed patients. The disease-associated elevation was higher than BSG in all patient groups compared to controls and remained so throughout the treatment strata.

Table 3. Large Dunn post hoc pairwise comparisons for EpCAM and BSG.

Marker	Comparison	Adjusted p-value	Direction
EpCAM	Diagnosis vs. Control	<0.05	Diagnosis > Control
EpCAM	Diagnosis vs. C2	<0.05	Diagnosis > C2
EpCAM	C1 vs. Control	0.067	Trend; not significant
BSG	Diagnosis vs. Control	<0.01	Diagnosis > Control
BSG	C1 vs. Control	<0.01	C1 > Control
BSG	C2 vs. Control	<0.01	C2 > Control

Patients that are grouped together compared to healthy control patients The pooled analysis showed significant differences between the lung cancer patients and controls in both theEpCAM and BSG. BSG had the greatest difference and effect size.

Table 4. The serum descriptive statistics of EpCAM and BSG from pooled patients and controls.

Marker	Group	N	Median	Q1	Q3
EpCAM (ng/mL)	Patients	68	7.65	5.73	10.02
EpCAM (ng/mL)	Controls	20	5.75	4.11	7.61
BSG (ng/mL)	Patients	68	624.53	375.36	1000.88
BSG (ng/mL)	Controls	20	117.77	27.41	323.03

Table 5. The serum EpCAM levels were compared to BSG levels in pooled patients versus controls using the Mann Whitney U test.

Marker	Mann-Whitney U	Z-score	p-value	Effect size (r)	Interpretation
EpCAM	316.0	-3.47	<0.001	0.38	Medium to large
BSG	83.5	-6.15	<0.001	0.67	Large

Effect size r: 0.1 = small, 0.3 = medium, 0.5 = large.

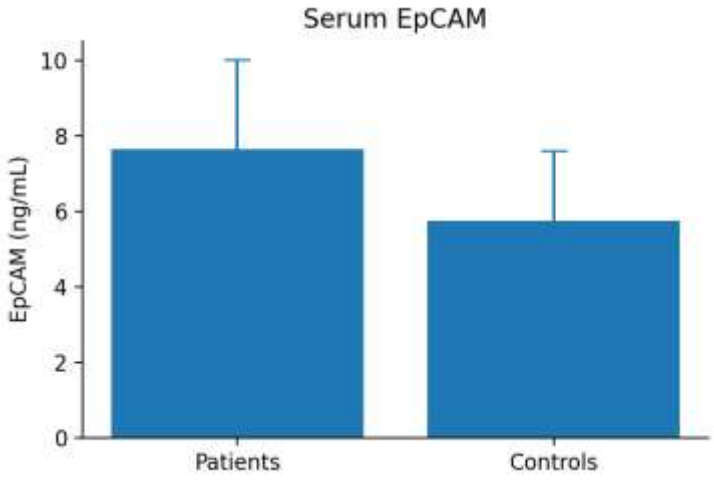

Figure 1. Median serum EpCAM concentration with interquartile range in lung cancer patients and controls.

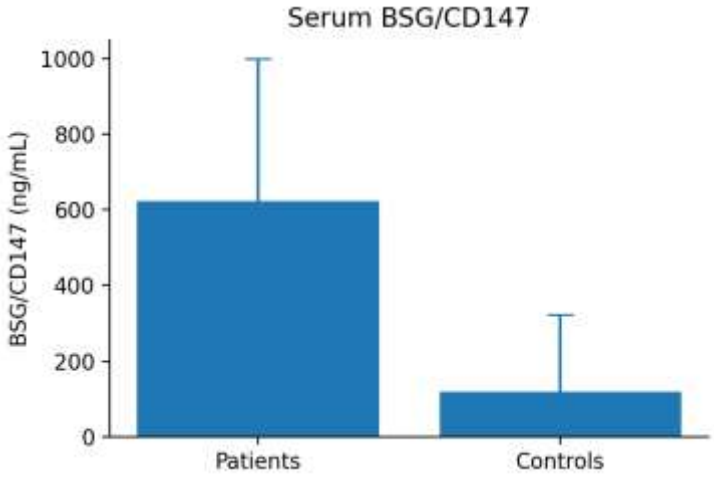

Figure 2. BSG median values and interquartile range (IQR) in serum among lung cancer patients and controls.

Patients with newly diagnosed untreated disease vs controls Newly diagnosed untreated patients were directly compared with healthy controls to minimize the confounding effect of chemotherapy. Prior to treatment, both markers were significantly increased, thus demonstrating baseline elevation of the marker due to disease. The effect size for BSG was very high when comparing to this.

Table 6. Mann-Whitney U comparison of newly diagnosed untreated patients versus controls.

Marker	U	Z	p-value	Effect size (r)
EpCAM	47.0	-2.57	0.010	0.50
BSG	7.0	-4.61	<0.001	0.90

Effect of chemotherapy regimen on serum biomarkers

Chemotherapy regimen type did not significantly influence EpCAM levels. In contrast, BSG levels were significantly affected by chemotherapy backbone, with higher values in patients receiving Platinum + Gemzar compared with Platinum + Alimta.

Table 7. Effect of chemotherapy regimen on serum EpCAM and BSG.

Marker	Kruskal-Wallis H	df	p-value	Significant pairwise difference
EpCAM	2.87	4	0.580	None
BSG	10.62	4	0.031	Platinum + Gemzar > Platinum + Alimta

Correlation with number of chemotherapy doses

BSG showed a significant moderate negative correlation with the number of chemotherapy doses, indicating that circulating BSG tends to decrease with increasing chemotherapy exposure. EpCAM showed no significant correlation with dose number.

Table 8. Spearman correlation between chemotherapy dose number and serum biomarkers.

Marker	Spearman rho	p-value	Interpretation
EpCAM	-0.19	0.131	Not significant
BSG	-0.42	<0.001	Moderate negative correlation

Diagnostic performance by ROC analysis

ROC analysis demonstrated fair-to-good diagnostic performance for EpCAM and excellent diagnostic performance for BSG. DeLong comparison confirmed that BSG significantly outperformed EpCAM.

Table 9. Diagnostic performance of serum EpCAM and BSG in differentiating lung cancer patients from controls.

Marker	AUC	95% CI	Cut-off	Sensitivity	Specificity	Youden's index
EpCAM	0.768	0.662-0.873	>6.50 ng/mL	65%	70%	0.35
BSG	0.939	0.891-0.987	>350 ng/mL	85%	80%	0.65

Table 10. DeLong comparison of AUC values.

Comparison	Difference in AUC	Z-statistic	p-value	Interpretation
BSG vs. EpCAM	0.171	2.91	0.004	BSG significantly superior

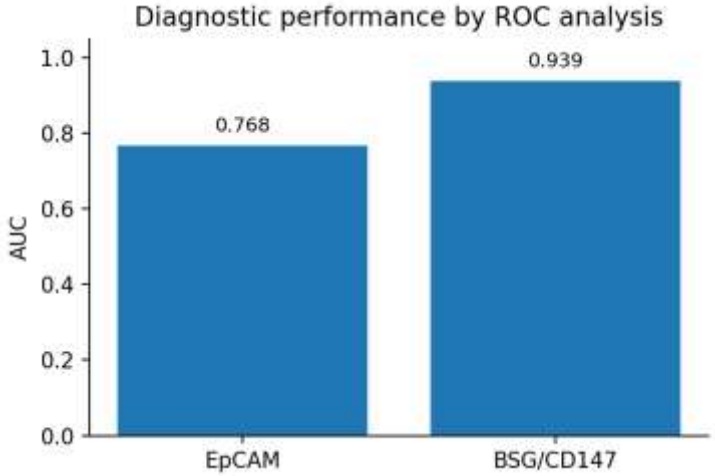

Figure 3. AUC comparison for EpCAM and BSG based on ROC analysis.

Association with stage and grade

BSG increased significantly with advancing stage and higher grade. The median BSG level in Stage IV patients was 752.8 ng/mL, whereas it was 375.4 ng/mL in Stage I-II patients. No significant differences were observed between the stages or grades of EpCAM.

Table 11. EpCAM and BSG were correlated with the stage and grade of tumor.

Marker	Stage association	Trend p-value	Grade association	Trend p-value
EpCAM	Not significant	0.845	Not significant	>0.05
BSG	Significant increase with advancing stage	0.004	Significant increase with higher grade	0.011

Discussion

The present study shows the significant elevation of serum EpCAM and BSG expression in lung cancer patients than the healthy controls suggesting the potential of use as Protein Expression-detectable circulating tumor cell-associated biomarkers. But there were different clinical patterns between the two. EpCAM was moderately discriminating in terms of diagnosis, while BSG was found to be better discriminating in terms of patient-control separation, better discriminating in terms of ROC, significantly related to chemotherapy exposure and stage and grade of the tumour. The increased expression of EpCAM on lung cancer patients is in accordance with its well known function as an epithelial marker and is often utilized in CTC detection approaches. Zhu et al. (2021) reported that the expression of EpCAM was significantly increased in lung cancer compared to controls and that it was diagnostic. However the present results do suggest that EpCAM is not a standalone marker for comprehensive monitoring as it exhibited equivalent effect size as BSG, no correlation with chemotherapy dose and no stage or grade association within the cohort. One of the most important biological causes of the poor performance of EpCAM is CTC heterogeneity.

EpCAM-based approaches are very specific for epithelial CTC populations but could fail to detect tumor cells that have undergone EMT or reduced expression of epithelial markers. Recent research in the field of CTCs highlights that the loss of EpCAM during EMT can make the detection of this type of CTC more challenging and underestimate the biologically relevant pool of CTCs (Gu, Wei and Lv, 2024; Sugai et al., 2024). The role of EpCAM as a diagnostic marker has been its most prominent one in the current study, which may be the reason. By comparison, BSG exhibited a significantly better biomarker profile. The difference between the lung cancer patients and the controls was large, as was the diagnostic accuracy as determined by ROC diagnostics.

DeLong testing was used to statistically support the superiority of BSG compared to EpCAM. BSG's biological role in tumor invasion, extracellular matrix remodeling, metabolic reprogramming, angiogenesis and treatment resistance. The study by Zhang et al. (2023) demonstrated that silencing of BSG inhibited the proliferation, migration and invasion of lung adenocarcinoma cells, inhibited lipid metabolic dysregulation, and induced apoptosis, indicating that BSG plays a direct role in lung adenocarcinoma progression. One of the most important findings of this study is the high negative correlation between the number of chemotherapy doses and BSG. This indicates that BSG may not only be used as a diagnostic tool but can be dynamically manipulated when receiving chemotherapy.

The decline of BSG with treatment exposure could be due to a reduction in tumor burden, the suppression of aggressive BSG expressing tumor populations, and/or changes in protein release induced by treatment. For example, unlike radiological assessment, serum biomarker monitoring is minimally invasive and can be performed throughout treatment cycles, making BSG desirable for monitoring over time. Another added advantage of the regimen specific difference in BSG levels is its potential relevance for the treatment monitoring. The BSG levels were significantly higher in the Platinum + Gemzar group compared to the Platinum + Alimta group. This could be due to differential sensitivity of the BSG expressing tumor cell population, tumor biology influencing the choice of the backbone for the chemotherapy or chemotherapy backbone influencing BSG. This was not the case for EpCAM, for which there was no such regimen-specific variation, further solidifying its use as a treatment unresponsive serum protein in this dataset. The presence of BSG in the advanced stage and increased tumor grade suggests that it may be linked with the aggressiveness of the tumour.

The median BSG levels were higher for Stage IV patients than Stage I-II patients and a significant grade related trend was also identified. These results are consistent with known roles of BSG in invasion and tumor progression, and indicate that BSG could be a biomarker for diagnosis and prognosis. However, in order to provide definitive prognostic interpretation, survival endpoints like progression-free survival and overall survival are required. In summary, the data provide a loud and clear publication message that EpCAM is a useful biomarker for the identification of lung cancer patients, detectable by Protein expression, but BSG is more informative, combining with better diagnostic discrimination, with change associated with therapy, and with association with the aggressiveness of the tumor. Thus, BSG must be included in future, longitudinal studies for validation and could be included in Protein expression-based lung cancer monitoring panels.

Conclusion

The serum EpCAM levels and BSG levels in the lung cancer patients are much higher than the healthy controls. BSG exhibited the highest diagnostic accuracy, greatest effect size, significant negative correlation with the chemotherapy dose number, and significant association with advanced stage and high tumor grade. Moderate diagnostic utility but less clinical relationship was observed for EpCAM. The results suggest that BSG could be useful as a clinically relevant biomarker for the diagnosis of lung

cancer, monitoring chemotherapy treatment and evaluating the aggressiveness of the disease based on the protein expression levels.

Strengths of the Study The specificity of the approach (Protein expression-) to this study, which is a clinically accessible and reproducible technique and can be used in routine laboratory settings, is an added advantage. Biomarkers were analyzed under various clinically important aspects such as patient-control discrimination, untreated baseline comparators, chemotherapy regimen, cumulative treatment exposure, ROC performance, and tumor stage/grade association. Limitations There are a number of caveats that should be noted here. In the first place, the number of patients in each subgroup was rather small, particularly for newly diagnosed untreated patients. Second, response to chemotherapy was not assessed directly using RECIST but rather by trends of the biomarkers and exposure to chemotherapy. Third, survival endpoints like progression-free survival or overall survival wasn't included. Fourth, this manuscript only included serum protein levels, which could be further enhanced by including the inclusion of CTC enumeration, ctDNA, extracellular vesicles or tissue immunohistochemistry.

Lastly, an independent, outside validation is needed prior to clinical use. Conflict of Interest The authors have no conflict of interest. Funding This study did not receive external funding. Ethical Approval [Type the name of the institutional ethics committee, its approval number and date]. All participants gave written informed consent prior to enrolment. Data Availability The datasets obtained and analysed during the present study will be made available by the corresponding author on reasonable request.

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