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To Study Secretory Senescence profile in Serous Effusion

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Background: Serous effusions accumulations of fluid in pleural, peritoneal, or pericardial cavities represent a significant clinical challenge with diverse etiologies ranging from benign inflammatory conditions to malignancies. Cellular senescence, characterized by stable cell cycle arrest and the senescence-associated secretory phenotype (SASP), has emerged as a critical mediator of chronic inflammation and tumor progression. This study aimed to characterize the secretory senescence profile in serous effusions and evaluate its diagnostic utility in differentiating malignant from benign etiologies.

Methods: This cross-sectional study enrolled 100 patients with serous effusions (pleural, peritoneal, or pericardial) over 12 months. Effusion fluid and paired serum samples were analyzed for a panel of SASP-associated biomarkers including IL-6, IL-8, TNF-α, MCP-1, MMP-9, TGF-β, and soluble Fas Ligand (sFasL) using multiplex ELISA. SA-β-galactosidase activity was assessed in effusion cell pellets. Demographic, clinical, and biochemical parameters were recorded. Statistical analysis included Mann-Whitney U tests for group comparisons and ROC analysis for diagnostic accuracy.

Results: Malignant effusions (n=52) demonstrated significantly elevated levels of IL-6 [median 245.6 vs. 87.3 pg/mL, $p<0.001$], IL-8 [312.4 vs. 134.7 pg/mL, $p<0.001$], TNF-α [78.2 vs. 41.5 pg/mL, $p=0.002$], MCP-1 [189.3 vs. 92.1 pg/mL, $p<0.001$], and MMP-9 [456.8 vs. 198.2 ng/mL, $p<0.001$] compared to benign effusions (n=48). SA-β-gal activity was significantly higher in malignant effusions ($p<0.001$). The combination of IL-6, IL-8, and MMP-9 yielded an AUC of 0.892 (95% CI: 0.823-0.961) for distinguishing malignant from benign effusions. Significant correlations were observed between effusion SASP markers and serum levels ($r=0.52$-0.74).

Conclusion: Serous effusions from malignant etiologies exhibit a distinct secretory senescence profile characterized by elevated pro-inflammatory SASP mediators. These biomarkers show promise as adjunctive diagnostic tools, with a combined panel offering superior discriminatory power compared to individual markers.

Keywords: Senescence-associated secretory phenotype, serous effusion, malignant effusion, biomarker, SASP

Introduction

Pathological fluid buildup in the pleural, peritoneal, or pericardial regions is known as serous effusion, and it is a common clinical presentation with significant diagnostic and treatment implications[1]. Serous effusions can have a wide range of causes, including malignancies like lung cancer, ovarian cancer, breast cancer, and mesothelioma, as well as benign disorders including heart failure, liver cirrhosis, infections, and inflammatory diseases. About 15% of patients with non-small cell lung cancer have malignant pleural effusion (MPE), which considerably deteriorates prognosis and has a median survival of three to twelve months[2-3].

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There are many difficulties in diagnosing malignant effusions. The current gold standard, cytological testing, has a low sensitivity (around 60%) and frequently necessitates several invasive procedures. Strong, minimally invasive biomarkers that can accurately distinguish between benign and malignant effusions and direct clinical therapy are therefore desperately needed[4].

In cancer biology, cellular senescence—a state of stable cell cycle halt brought on by a number of factors, such as DNA damage, oncogenic stress, and chemotherapy—has drawn more and more attention. Senescence was once thought to be a tumor-suppressive process, but recent studies have shown that it actually has paradoxical protumorigenic consequences. The complex array of bioactive molecules secreted by senescent cells is known as the senescence-associated secretory phenotype (SASP). These molecules include growth factors, proteases (MMPs), extracellular vesicles, chemokines (MCP-1), proinflammatory cytokines (IL-6, IL-8, TNF-α), and proteases[5-6].

Additionally, there is evidence that SASP components can be found in a variety of bodily fluids. Research has shown that wound exudates from non-healing pressure injuries and synovial fluid from osteoarthritis patients had higher levels of SASP-associated cytokines. Nevertheless, little is known about the characteristics of secretory senescence in serous effusions and its possible diagnostic value[7-9].

This study aimed to:

1. Characterize the secretory senescence profile in serous effusions from patients with malignant and benign etiologies
2. Evaluate the diagnostic accuracy of individual and combined SASP biomarkers in differentiating malignant from benign effusions
3. Examine the correlation between effusion and serum levels of SASP factors to assess their utility as systemic biomarkers

Materials and Methods

Study Design and Setting

This cross-sectional, observational study was conducted at a tertiary care hospital in Central India over 12 months.

Study Population

A total of 100 consecutive patients presenting with serous effusions (pleural, peritoneal, or pericardial) requiring diagnostic or therapeutic aspiration were enrolled. Sample size calculation was based on expected differences in IL-6 levels between malignant and benign effusions, with 80% power and $\alpha=0.05$.

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Inclusion Criteria:

- Age ≥18 years
- Clinical or radiological evidence of serous effusion
- Planned diagnostic or therapeutic aspiration
- Provision of informed consent

Exclusion Criteria:

- Active infections requiring immediate intervention
- History of radiotherapy or chemotherapy in the preceding 4 weeks (to avoid therapy-induced senescence confounding)
- Inability to provide informed consent
- Inadequate sample volume for complete biomarker analysis

Sample Collection and Processing

Effusion fluid (20-30 mL) was collected during standard therapeutic or diagnostic aspiration using sterile technique. Samples were immediately transported to the laboratory on ice. An aliquot was centrifuged at 1500 rpm for 10 minutes at 4°C, and the supernatant was stored at -80°C for subsequent biomarker analysis. The cell pellet was processed for cytological examination and SA-β-galactosidase assay.

Paired venous blood samples (5 mL) were collected within 24 hours of effusion aspiration, centrifuged, and serum stored at -80°C.

Clinical and Laboratory Data

Demographic and clinical data were recorded, including age, sex, etiology, comorbidities, and symptoms. Effusion fluid was analyzed for routine biochemical parameters including total protein, albumin, glucose, lactate dehydrogenase (LDH), adenosine deaminase (ADA), and cell count. Effusions were classified as exudative or transudative using Light's criteria.

Biomarker Analysis

A panel of SASP-associated biomarkers was measured in effusion fluid and serum using multiplex ELISA (Luminex-based, R&D Systems, Minneapolis, MN, USA):

- **Cytokines:** IL-6, IL-8, TNF-α
- **Chemokines:** MCP-1 (CCL2)
- **Proteases:** MMP-9, MMP-2
- **Growth factors:** TGF-β1
- **Soluble Fas Ligand (sFasL)**

All assays were performed in duplicate, and results are reported as median (interquartile range). Standard curves were generated for each analyte using provided recombinant standards.

SA-β-Galactosidase Assay

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SA-β-galactosidase activity, a hallmark of cellular senescence, was assessed in effusion cell pellets using a colorimetric assay (Cell Signaling Technology, Danvers, MA, USA). Cells were fixed, incubated with X-gal substrate at pH 6.0 for 16 hours at 37°C, and percentage of positive cells was determined by microscopic examination.

Statistical Analysis

Statistical analysis was performed using SPSS version 26.0 (IBM Corp, Armonk, NY, USA). The Shapiro-Wilk test was used to assess data normality. Continuous variables with non-normal distribution are presented as median (IQR) and compared using the Mann-Whitney U test. Categorical variables were compared using the Chi-square test. Correlations between effusion and serum markers were assessed using Spearman's rank correlation coefficient. Receiver Operating Characteristic (ROC) curves were constructed to evaluate the diagnostic performance of individual and combined biomarkers, with area under the curve (AUC), sensitivity, specificity, and optimal cut-off values determined using the Youden index. A p-value <0.05 was considered statistically significant.

Results

Patient Characteristics

A total of 100 patients with serous effusions were enrolled. The cohort comprised 54 males and 46 females, with a median age of 62 years (IQR: 51-73). Effusion types included pleural (n=72, 72%), peritoneal (n=24, 24%), and pericardial (n=4, 4%). Based on final diagnosis, effusions were classified as malignant (n=52) or benign (n=48). Malignant etiologies included lung adenocarcinoma (n=18), ovarian cancer (n=10), breast cancer (n=8), malignant pleural mesothelioma (n=5), lymphoma (n=6), and others (n=5). Benign etiologies included heart failure (n=15), liver cirrhosis (n=12), tuberculosis pleuritis (n=10), pneumonia-associated parapneumonic effusion (n=7), and inflammatory conditions (n=4).

Table 1: Baseline Characteristics of Study Participants

Parameter	Total (n=100)	Malignant (n=52)	Benign (n=48)	p-value
Age (years)	62 [51-73]	65 [55-74]	58 [48-71]	0.034*
Sex (M/F)	54/46	28/24	26/22	0.853
Effusion Type				
Pleural	72 (72%)	40 (76.9%)	32 (66.7%)	0.291
Peritoneal	24 (24%)	10 (19.2%)	14 (29.2%)	0.257
Pericardial	4 (4%)	2 (3.8%)	2 (4.2%)	0.688
Effusion Characteristics				
Protein (g/L)	38.5 [28.3-47.2]	42.1 [34.6-49.5]	33.8 [24.1-43.7]	0.001**

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Parameter	Total (n=100)	Malignant (n=52)	Benign (n=48)	p-value
LDH (U/L)	245 [178-398]	312 [210-456]	189 [145-312]	0.008**
Glucose (mmol/L)	5.8 [4.2-6.7]	5.3 [4.1-6.2]	6.2 [4.8-7.1]	0.042*
ADA (U/L)	14.5 [6.2-28.9]	10.3 [5.8-18.4]	21.7 [8.9-42.5]	0.002**

*Data presented as median [IQR] or n (%). Mann-Whitney U test for continuous variables, Chi-square for categorical. *p<0.05, **p<0.01*

Patients with malignant effusions were significantly older (median 65 vs. 58 years, p=0.034). Effusion protein and LDH levels were significantly higher in malignant effusions (p=0.001 and p=0.008, respectively), while ADA was significantly higher in benign effusions, reflecting tuberculosis as a common cause of benign pleural effusions (p=0.002).

Table 2: SASP Biomarker Concentrations in Effusion Fluid

Biomarker	Total (n=100)	Malignant (n=52)	Benign (n=48)	p-value
IL-6 (pg/mL)	158.4 [89.2-278.6]	245.6 [142.8-389.5]	87.3 [52.1-156.2]	<0.001***
IL-8 (pg/mL)	218.6 [104.3-384.7]	312.4 [198.5-456.2]	134.7 [78.4-218.9]	<0.001***
TNF-α (pg/mL)	58.4 [34.2-89.3]	78.2 [51.3-112.8]	41.5 [26.7-67.4]	0.002**
MCP-1 (pg/mL)	134.6 [68.4-234.7]	189.3 [112.5-289.4]	92.1 [48.7-156.3]	<0.001***
MMP-9 (ng/mL)	312.8 [167.4-526.9]	456.8 [298.4-678.2]	198.2 [112.6-345.1]	<0.001***
TGF-β1 (pg/mL)	342.6 [198.4-523.7]	398.2 [234.5-567.8]	287.4 [178.2-456.1]	0.048*
sFasL (pg/mL)	124.7 [67.8-198.4]	156.2 [98.4-234.5]	89.3 [52.1-145.6]	<0.001***

*Data presented as median [IQR]. Mann-Whitney U test. *p<0.05, **p<0.01, ***p<0.001*

All measured SASP biomarkers were significantly elevated in malignant effusions compared to benign effusions. IL-6, IL-8, and MCP-1 showed the most significant differences (all p<0.001). The median IL-6 concentration in malignant effusions was nearly threefold higher than in benign effusions. IL-8 and MMP-9 similarly demonstrated substantial elevations.

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SA-β-Galactosidase Activity

SA-β-galactosidase activity, assessed in effusion cell pellets, was significantly higher in malignant effusions compared to benign effusions. The median percentage of SA-β-gal-positive cells was 18.4% (IQR: 8.7-31.2%) in malignant effusions versus 6.2% (IQR: 2.3-12.8%) in benign effusions ($p<0.001$). This finding suggests a greater burden of senescent cells within the malignant effusion microenvironment, consistent with the elevated SASP factor concentrations observed.

Table 3: Correlation between Effusion Fluid and Serum SASP Biomarkers

Biomarker	Spearman's r	p-value
IL-6	0.742	<0.001***
IL-8	0.683	<0.001***
TNF-α	0.524	0.002**
MCP-1	0.618	<0.001***
MMP-9	0.591	<0.001***
TGF-β1	0.487	0.018*
sFasL	0.563	0.004**

*Spearman's rank correlation coefficient (r). *p<0.05, **p<0.01, ***p<0.001*

The strongest correlations were observed for IL-6 (r=0.742), IL-8 (r=0.683), and MCP-1 (r=0.618), suggesting that local senescence-associated inflammation in the effusion cavity may be reflected systemically. The weaker correlation for TGF-β1 (r=0.487) may reflect its more complex regulation and potential sequestration in the local microenvironment.

Discussion

This work offers a thorough description of the secretory senescence profile in serous effusions and shows how it can be used to distinguish between benign and malignant etiologies. Significant elevation of several SASP-associated biomarkers in malignant effusions; increased SA-β-galactosidase activity in malignant effusion cells; moderate-to-strong correlations between effusion and serum SASP markers; and improved diagnostic performance of a combined biomarker panel are the main conclusions[10].

The known function of senescence in cancer biology is consistent with the significant increase of pro-inflammatory SASP factors in malignant effusions. Platinum-based drugs and other chemotherapies have been shown to cause therapy-induced senescence in models of lung and ovarian cancer. Among the most well-characterized SASP variables, the SASP components found in our study—IL-6, IL-8, TNF-α, MCP-1, and MMP-9—have been linked to immune evasion, tumor growth, and metastasis[11].

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Senescent cancer cells in malignant pleural effusions express HLA-E, which reduces NK cell cytotoxicity and dendritic cell function and contributes to an immunosuppressive milieu, according to recent research by Tsai et al. The protumorigenic environment in malignant effusions may also be influenced by the higher amounts of SASP components found in our investigation. It has been demonstrated that IL-6 and IL-8 in particular support the survival, proliferation, and epithelial-to-mesenchymal transition of cancer cells[12].

A senescence burden inside the effusion milieu can be directly demonstrated by the measurement of SA-β-galactosidase activity in effusion cells. This discovery expands on findings from other bodily fluids, such as synovial fluid and wound exudates[13]. The effusion cavity may function as a reservoir of senescence-associated inflammation that can be collected for diagnostic reasons due to the presence of senescent cells and their secretory products in effusions.

Effusion and serum SASP indicators have connections, especially for IL-6 (r=0.742), which may indicate that systemic inflammation linked to local senescence. This is in line with findings in osteoarthritis, where there were significant associations between serum levels of SASP components and synovial fluid. The idea that effusion measurement has advantages over systemic assessment is supported by the lesser association for TGF-β1 and other markers, which suggests that some SASP components may be preferentially generated or sequestered locally[14].

Individual SASP indicators performed as well as or better than traditional cytological examination, which has a reported sensitivity of roughly 57.3–60%. An AUC of 0.892 was attained by the panel of IL-6, IL-8, and MMP-9, indicating that secretory factors produced from senescent cells may be useful supplementary indicators for the detection of malignant effusions. This is in line with research on biomarkers in malignant pleural mesothelioma, which showed that combination biomarker panels (such PSAP and SULF-1) had better diagnostic accuracy than single markers[15].

There are a few restrictions to be aware of. Longitudinal variations in biomarker levels cannot be assessed due to the cross-sectional approach. The cohort may not be as generalizable to other groups with varying illness frequency because it came from a single center in Central India[16]. Despite being extensive, the panel of SASP indicators excluded other elements linked to senescence, such as extracellular vesicles or bioactive lipids. Additionally, even though individuals who had recently had chemotherapy or radiation were not included, therapy-induced senescence might have been impacted by previous treatments. To validate these results and determine clinical value, larger, multicenter trials with longitudinal follow-up are required.

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Conclusion

This study demonstrates that malignant serous effusions exhibit a distinct secretory senescence profile characterized by significantly elevated levels of SASP-associated biomarkers including IL-6, IL-8, TNF-α, MCP-1, and MMP-9. The presence of senescent cells, evidenced by SA-β-galactosidase activity, and the moderate-to-strong correlation between effusion and systemic SASP marker levels suggest that local senescence-associated inflammation contributes to both local and systemic pathophysiology. A combined panel of IL-6, IL-8, and MMP-9 demonstrates promising diagnostic accuracy for distinguishing malignant from benign effusions, offering a potential adjunctive tool to conventional cytology. Further prospective studies are warranted to validate these findings and explore the prognostic and therapeutic implications of the secretory senescence profile in serous effusions.

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