ORIGINAL RESEARCH

# Optimizing Diagnostic Strategies for Malignant Serous Cavity Effusion: The Role of Cytomorphological Examination Combined with High Fluorescent Cells and Total Protein

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## Abstract

### Objective

This study evaluated the diagnostic value of cytomorphological examination in malignant serous cavity effusion (MSCE) and optimized clinical strategies by integrating routine cytological and biochemical analysis.

### Methods

A retrospective analysis was conducted on 3,998 patients with serous cavity effusion at the First Affiliated Hospital of Xi'an Jiaotong University. Based on cytopathological results, patients were classified into MSCE (1,078 cases) and benign serous cavity effusion (BSCE, 2,920 cases) groups. Diagnostic performance of cytomorphological examination was assessed, and routine cytological and biochemical parameters were compared. Receiver operating characteristic (ROC) curves were used to evaluate diagnostic efficacy.

### Results

Cytomorphological examination showed a sensitivity of 82.9%, specificity of 86.3%, and accuracy of 85.0%, with high concordance with cytopathological diagnosis ($\varkappa = 0.687$, $P < 0.001$). High fluorescence cell count (HFC) and total protein (TP) were significantly elevated in the MSCE group and positively correlated with MSCE ($P < 0.001$). HFC (AUC: 0.765, 95% CI: 0.748–0.782; cutoff: $24.5\times10^6$/L) and TP (AUC: 0.735, 95% CI: 0.719–0.750; cutoff: 29.75 g/L) combined with cytomorphological examination provided supplementary diagnostic value. Two combinatorial diagnostic strategies based on cytomorphological examination, HFC, and TP were developed, with Strategy I achieving 93.4% sensitivity and 72.6% specificity, and Strategy II achieving 92.3% specificity and 78.9% sensitivity. Both strategies showed substantial concordance with cytopathological diagnosis ($\varkappa$=0.622/0.724, $P < 0.001$).

### Conclusion

Cytomorphological examination showed high sensitivity and high specificity in the diagnosis of MSCE. Its combination with HFC and TP effectively enhances diagnostic performance by achieving a balance between reducing missed diagnoses and minimizing misdiagnoses.

**Key Words**: cytomorphological examination; malignant serous cavity effusion; high fluorescent cell; total protein; diagnostic strategy

## Introduction

Serous cavity effusion (SCE) refers to the pathological accumulation of fluid in body serous cavities such as the pleural, peritoneal, and pericardial cavities. According to statistics from the British Thoracic Society, approximately 70 individuals per 100,000 people worldwide are affected by malignant pleural effusion[1]. The presence of malignant serous cavity effusion (MSCE) significantly reduces the patient's quality of life and is usually associated with poor prognosis, with median survival ranging from 3 to 12 months[2-4].

The detection of malignant cells in serous cavity fluid or tissue specimens through pathological examination is regarded as the gold standard for diagnosing MSCE. MSCE is a common complication in advanced cancer patients, and early differentiation is crucial for clinical staging and treatment decisions[5,6]. Imaging tests such as computed tomography (CT) and ultrasound-guided pleura or peritoneum biopsy, along with thoracoscopy or laparoscopy, offer high diagnostic accuracy for MSCE. However, these methods still face challenges in early differentiation between benign and malignant effusions[7], and they are invasive, carrying the risk of procedure-related complications[8]. In recent years, extensive research has explored various diagnostic



https://dx.doi.org/10.4314/mmj.v37i2.5

approaches for MSCE, including cell-free DNA[9,10], immune cells[11], microbiomes[3], and commonly used tumor markers[12] leading to certain clinical advancements[13]. Although these emerging methods show potential in some clinical trials, most of them rely on costly equipment and consumables, and require specialized laboratory and operational personnel. As such, their widespread application in primary healthcare settings faces significant challenges. Therefore, developing a cost-effective and convenient approach for diagnosing MSCE using existing resources could offer substantial clinical benefits to patients.

In recent years, the value of cytomorphological examination in the diagnosis of MSCE has gained increasing attention. Compared to the diagnostic gold standard—cytopathological analysis of SCE, cytomorphological examination is technically straightforward, requires no specialized instrumentation, and offers significant advantages in processing speed and cost-effectiveness. Nevertheless, current clinical guidelines caution against establishing a definitive diagnosis of malignancy based exclusively on cytomorphological findings. This study aimed to assess the diagnostic performance of cytomorphological examination for MSCE and to optimize diagnostic strategies by integrating routine cytological and biochemical parameters. We retrospectively analyzed SCE cytomorphological examination results alongside routine cytological and biochemical test data to address the two key issues: (1) Does cytomorphological examination demonstrate sufficient diagnostic efficacy to serve as an effective screening tool for MSCE? (2) Can a combined multi-parameter strategy optimize the application of cytomorphological examination in the early screening of MSCE?

## Method

This retrospective cross-sectional study was conducted at the First Affiliated Hospital of Xi'an Jiaotong University from January 2020 to December 2023. Located in northwest China, the hospital is one of the largest general hospital under the direct administration of the National Health Commission of the People's Republic of China. It ranks among the top institutions nationwide in both medical care and research. The Its Department of Clinical Laboratory is regionally renowned for its advanced facilities, extensive diagnostic capabilities, and strong performance in scientific research and quality management, ensuring high reliability in clinical diagnostics.

### *Study Population*

A total of 3,998 patients with SCE who underwent routine cytological, biochemical, and exfoliative cytopathological examinations were retrospectively enrolled in this study. Among them, 193 patients also underwent cytomorphological evaluation to assess its consistency with cytopathological results.Based on the cytopathological results, patients were classified into the malignant SCE (MSCE) group (n = 1,078) and the benign SCE (BSCE) group (n = 2,920). The enrolled cases included 63.5% (n = 2,537) with pleural effusion, 32.4% (n = 1,297) with peritoneal effusion, and 4.1% (n = 164) with pericardial effusion.

### *Inclusion and Exclusion Criteria*

Patients who underwent routine cytological testing, biochemical analysis, and cytopathological examination were eligible for inclusion. Patients were excluded if their cytopathological findings showed atypical or suspicious neoplastic cells without definitive classification, or if their clinical or laboratory data were incomplete.

### *Sample Analysis*

### *Cytomorphological Analysis of SCE*

Fresh SCE samples were centrifuged, and the resulting cell pellets were used to prepare smears, which were air-dried and preserved. Smears were subsequently stained using Wright's stain (BASO Diagnostics Inc., Zhuhai, China) following the manufacturer's instructions. Microscopic evaluation was performed to identify neoplastic cells. Each slide was independently assessed by two experienced cytology technicians. In cases of discrepant interpretations, a third senior cytology technicians adjudicated the final diagnosis.

### *Routine Cytological Parameters of SCE*

Routine cytological parameters of SCE included total nucleated cells (TC-BF), high fluorescent cells (HFC), white blood cells (WBC-BF), mononuclear cells (MN) and their percentage (MN%), polymorphonuclear cells (PMN) and their percentage (PMN%), and red blood cells (RBC). All measurements were performed using the SYSMEX XN9000 fully automated hematology analyzer (Sysmex Corporation, Kobe, Japan) in body fluid mode.

### *Biochemical Analysis of SCE*

Biochemical analysis included total protein (TP), lactate dehydrogenase (LDH), glucose (GLU), chloride (Cl), and adenosine deaminase (ADA). These were conducted using the Johnson & Johnson Vitros 5600 Fully Automated Biochemical and Immunoassay Analyzer (New Brunswick, NJ, USA) and its accompanying reagents, with the ADA reagent kit provided by Ningbo Ruiyuan Co., Ltd.

The experimental results were derived under optimal instrument conditions, standardized operational procedures, and meticulous data recording.

### *Data Collection*

Patient data, including age, gender, effusion site, primary disease diagnosis, laboratory results, and pathological findings, were retrieved from the Hospital Information System (HIS) and the Laboratory Information System (LIS).

## Ethical Approval

All procedures performed in our studies involving human participants followed all the ethical standards of the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (approval number: XJTU1AF2018LSK-228). Due to the retrospective nature of the study, the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University waived the need of obtaining informed consent.

## Statistical Analysis

IBM SPSS 25.0 software (IBM Corp., Armonk, NY) was employed for statistical analyses. As all continuous variables exhibited non-normal distributions through Shapiro-Wilk testing, they were expressed as median with interquartile range (IQR). Inter-method agreement was evaluated using Cohen's kappa coefficient with Landis & Koch interpretation criteria. Non-parametric comparisons between groups were performed using the Mann-Whitney U test, whereas categorical variables were analyzed through $\chi^2$ tests with Yates' continuity correction where appropriate. Point-biserial correlation analysis was used to assess the correlation between categorical and continuous variables. A receiver operating characteristic (ROC) curve analysis was conducted

using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA). A two-sided P-value threshold of <0.05 was set for statistical significance throughout the study.

## Results

### *Baseline Information*

The median age of patients in the MSCE group was 63 (55, 71) years, with 511 males and 567 females. The primary underlying diseases in this group were malignant tumors, including but not limited to lung cancer, ovarian cancer, gastric cancer, breast cancer, colon cancer, and lymphoma. The median age of patients in the BSCE group was 63 (52, 72) years, with 1,340 males and 1,580 females. The etiological spectrum of the BSCE group included both malignant neoplasms—overlapping with those observed in the MSCE group—and a range of non-malignant conditions, including but not limited to pneumonia, chronic obstructive pulmonary disease, hepatic cirrhosis, chronic renal insufficiency, and congestive heart failure. There were no statistically significant differences between the two groups in terms of age ($Z = -1.648$, $P = 0.099$) and gender ($\chi^2 = 0.724$, $P = 0.395$) distribution.

### *Diagnostic Consistency between Cytomorphology and Cytopathological Analysis in SCE*

Among the patients included in this study, 193 underwent cytomorphological analysis of serous cavity effusion, and the results were compared with cytopathological findings to assess diagnostic concordance. The results showed that cytomorphological examination demonstrated high sensitivity (82.9%) and specificity (86.3%) in diagnosing MSCE, with a positive predictive value (PPV) of 79.7%, a negative predictive value (NPV) of 88.6%, and an accuracy of 85.0%. It showed substantial diagnostic consistency between cytomorphology and cytopathological analysis ($\varkappa = 0.687$, $P < 0.001$). (Table 1)

### *Comparative Analysis of Routine Cytological and Biochemical Markers between BSCE and MSCE*

To identify potential diagnostic biomarkers for distinguishing MSCE from BSCE, we performed a comparative analysis of routine cytological and biochemical parameters between the two groups. Significant elevations in MSCE group were observed for cytological biomarkers (TC-BF, BF-WBC, HFC, MN, PMN, RBC-BF) and biochemical markers (TP, LDH, ADA) compared to BSCE group ($P < 0.001$).

**Table 1 Diagnostic Consistency between Cytomorphology and Cytopathological Analysis in SCE**

| Cytopathological Examination | Cytomorphology | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 63 | 13 | 76 |
| Negative | 16 | 101 | 117 |
| Total | 79 | 114 | 193 |

**Table 2 Comparative Analysis of Routine Cytological and Biochemical Markers between BSCE and MSCE**

| | BSCE Group （n=2920） | MSCE Group （n=1078） | Z | P Value |
|---|---|---|---|---|
| TC-BF ($\times10^6$/L) | 492(180,1591) | 1173(587,2218) | -15.479 | <0.001 |
| BF-WBC($\times10^6$/L) | 466(171,1531) | 1019(508,1928) | -13.609 | <0.001 |
| HFC ($\times10^6$/L) | 10(4,29) | 61(19,180) | -25.729 | <0.001 |
| MN ($\times10^6$/L) | 330(124,965) | 759(390,1433) | -15.719 | <0.001 |
| MN% (%) | 84(65.7,93.1) | 85.4(69.2,92.6) | -1.194 | 0.232 |
| PMN ($\times10^6$/L) | 56(20,225) | 123(49,360) | -11.542 | <0.001 |
| PMN% (%) | 16(6.9,34.3) | 14.6(7.4,30.8) | -1.158 | 0.247 |
| RBC-BF ($\times10^6$/L) | 3000(1000,12000) | 5000(2000,28000) | -9.723 | <0.001 |
| TP (g/L) | 27.5(20.6,39.9) | 41(33.8,46.5) | -22.837 | <0.001 |
| LDH (U/L) | 156(100,316) | 298(178,645) | -16.988 | <0.001 |
| GLU (mmol/L) | 6.37(5.34,7.9) | 5.84(4.74,7.1) | -8.55 | <0.001 |
| Cl (mmol/L) | 104.3(100.1,107.9) | 103.9(99.9,106.9) | -3.145 | 0.002 |
| ADA (U/L) | 6(3,12) | 8(5,12) | -6.874 | <0.001 |

https://dx.doi.org/10.4314/mmj.v37i2.5

**Table 3 Diagnostic Efficacy of HFC and TP for MSCE**

| | AUC (95% CI) | Cut-off value | Sensitivity | Specificity |
|---|---|---|---|---|
| HFC ($\times 10^6$/L) | 0.765(0.748-0.782) | 24.5 | 69.8% | 71.1% |
| TP (g/L) | 0.735(0.719-0.751) | 29.75 | 85.3% | 55.1% |

**Table 4 Diagnostic Performance of Cytomorphology Combined with HFC and TP in the Diagnosis of MSCE.**

| Diagnostic Pathway | Sensitivity | Specificity | Accuracy | PPV | NPV |
|---|---|---|---|---|---|
| Cytomorphology | 82.9% | 86.3% | 85.0% | 79.7% | 88.6% |
| Strategy I | 93.4% | 72.6% | 80.8% | 68.9% | 94.4% |
| Strategy II | 78.9% | 92.3% | 87.0% | 87.0% | 87.1% |

**Strategy I: Diagnostic criteria for MSCE: a) cytomorphology confirmed MSCE, OR b) cytomorphology-negative specimens meeting both biomarker thresholds (HFC > 24.5×10$^6$/L and TP > 29.75 g/L). Otherwise, it will be classified as BSCE. Strategy II: Diagnostic criteria for BSCE: a) cytomorphology confirmed BSCE, OR b) cytomorphology-positive specimens showing both biomarkers below thresholds (HFC < 24.5×10$^6$/L and TP < 29.75 g/L). Otherwise, it will be classified as MSCE. PPV: positive predictive value. NPV: negative predictive value.**

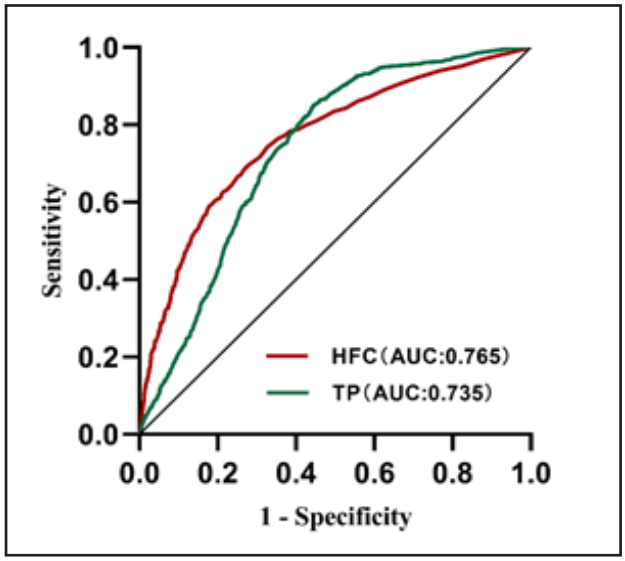


**Figure 1 ROC Curves of HFC and TP for Discriminating MSCE from BSCE. HFC: High fluorescent cell counts, TP: Total Protein, AUC: Area Under the Curve**

Conversely, GLU ($P < 0.001$) and Cl ($P = 0.002$) concentrations were markedly reduced in MSCE. However, the MN% and PMN% showed no intergroup differences ($P > 0.05$). (Table 2). We performed point-biserial correlation analysis between the indicators showing significant statistical differences in intergroup comparisons and MSCE. The results revealed significant positive correlations between MSCE and HFC ($r = 0.058$, $P < 0.001$), TP ($r = 0.350$, $P < 0.001$), and LDH ($r = 0.039$, $P = 0.013$). In contrast, GLU ($r = -0.087$, $P < 0.001$) and Cl ($r = -0.054$, $P = 0.001$) showed significant negative correlations with MSCE. No significant correlation was found between TC-BF, BF-WBC, MN, PMN, and RBC-BF and MSCE ($P > 0.05$).

## *Diagnostic Efficacy Evaluation of Indicators Significantly Associated with MSCE*

After identifying the parameters that significantly associated with MSCE in routine cytological and biochemical examinations, we conducted ROC curve analysis on these parameters. The results revealed that the AUCs for LDH, GLU, and Cl were all below 0.70, while the AUCs for HFC and TP were 0.765 (0.748-0.782) and 0.735 (0.719-0.751), respectively, indicating better diagnostic performance for MSCE. When the cut-off value for HFC was set at 24.5×10$^6$/L, the sensitivity and specificity were 69.8% and 71.1%, respectively. For TP, when the cut-off value was set at 29.75 g/L, the sensitivity and specificity were 85.3% and 55.1%, respectively (Figure 1, Table 3).

## *Diagnostic Performance of Cytomorphology Examination Combined with HFC and TP in the Diagnosis of MSCE*

To optimize the diagnostic efficacy of cytomorphological examination alone for MSCE, we developed two novel combinatorial diagnostic strategies(Strategy I and II) based on the established cut-off values for HFC (24.5×10$^6$/L) and TP (29.75 g/L) in our regional clinical context.

Strategy II employed an inclusive diagnostic criterion: specimens were classified as MSCE if they were either cytomorphology-positive or exhibited concurrent elevation of both HFC and TP beyond their respective cut-offs, even in the absence of cytomorphological positivity. Otherwise, it will be classified as BSCE. Compared to cytomorphology alone, this approach exhibited superior diagnostic sensitivity (93.4% vs. 82.9%), with a specificity of 72.6% and an overall accuracy of 80.8%. Furthermore, it showed substantial concordance with cytopathological diagnosis ($\kappa = 0.622$, $P < 0.001$).

Strategy I utilized a more stringent confirmation rule: cases were considered BSCE if they were either cytomorphology-negative or exhibited dual negativity of both HFC and TP (below their cut-offs) in cytomorphology-positive specimens. Otherwise, it will be classified as MSCE. Compared with cytomorphology alone, this strategy achieved enhanced specificity (92.3% vs. 86.3%) and accuracy (87.0% vs. 85.0%), while maintaining a sensitivity of 78.9%. It also demonstrated substantial concordance with cytopathological diagnosis ($\kappa = 0.724$, $P < 0.001$). (Table 4).

https://dx.doi.org/10.4314/mmj.v37i2.5

## Discussion

This retrospective study evaluated the diagnostic performance of cytomorphological examination in diagnosing MSCE. The diagnostic efficacy of cytomorphological analysis was evidenced by its balanced performance profile (sensitivity: 82.9%; specificity: 86.3%) coupled with an overall accuracy of 85.0%. It showed substantial consistency with cytopathological analysis ($\kappa$=0.687, P <0.001), establishing its clinical utility in effusion characterization. Accumulating evidence from clinical investigations has substantiated the pivotal role of cytomorphological examination in the diagnosis of MSCE, positioning this technique as the minimally invasive modality for early detection in current clinical practice[14,15]. Another study evaluated commonly used tumor markers in SCE, such as CEA, CA-15-3, CYFRA 21-1, CA-19-9, and CA-125. However, even CEA, which demonstrated the best diagnostic value, showed sensitivity (77%), specificity (81%), and accuracy (79%) that did not surpass those of cytomorphological examination in diagnosing malignant pleural effusion[16], or malignant peritoneal effusion[17,18], as found in this study. Additionally, the examination time for cytomorphology was notably shorter compared to cytopathology. Furthermore, cytomorphological examination was significantly less expensive than both cytopathology and tumor marker analyses, making it a cost-effective and efficient option for patients with SCE.

For the routine cytological examination of SCE, although MN and PMN showed statistically significant differences between BSCE and MSCE, correlation analysis did not find a significant association between either of them and MSCE. It is important to note that, although MSCE is often characterized by a predominance of MN cells, viral infections or tuberculous effusions can also present similar features. Additionally, clinical studies have shown that the proportion of PMN cells can significantly increase in malignant effusions associated with acute infection[19, 20], suggesting that relying solely on the MN/PMN ratio is not a reliable method for differentiating between BSCE and MSCE.

HFC refers to abnormal cell populations that, after being labeled with fluorescent dyes, exhibit significantly higher fluorescence signal intensities compared to normal cells. The underlying mechanisms primarily involve the following pathological processes: tumor cells may have increased nuclear nucleic acid (DNA/RNA) content or altered cell membrane permeability, which allows fluorescent dyes to more easily penetrate and bind with nucleic acids. Activated or functionally abnormal white blood cells (such as activated lymphocytes or macrophages that phagocytize nucleic acids) may exhibit enhanced fluorescence signals due to changes in metabolic activity. Additionally, degenerative cells in infected or inflammatory microenvironments may present increased fluorescence because of structural damage that exposes intracellular materials. HFC testing provides an effective method for screening pathological cells in body fluid samples, and several studies have confirmed its diagnostic value for MSCE[21,22]. In this study, HFC was found to have a significant positive correlation with MSCE. The area under the receiver operating characteristic curve (AUC) for HFC was 0.765 (95% CI: 0.748–0.782), suggesting that this marker holds considerable potential for differentiating between BSCE and MSCE. The optimal cut-off value was set at $24.5 \times 10^6$/L.

Although significant statistical differences were observed in LDH, CL, GLU, and ADA levels between the MSCE and BSCE groups, these markers did not demonstrate high diagnostic value for MSCE. However, LDH holds significant clinical value in assessing the prognosis and therapeutic efficacy of MSCE[23-25]. Recent studies have indicated that the TP-to-Cl ratio in pleural effusion can serve as an independent predictor of overall survival in patients with newly diagnosed malignant pleural effusion[26]. This study found that TP demonstrated superior diagnostic performance for distinguishing MSCE, with an AUC of 0.735 (95% CI: 0.719-0.751), and the optimal cut-off value was confirmed at 29.75 g/L.

It is important to note that, despite the high diagnostic sensitivity and specificity of cytomorphological examination, its clinical application remains limited. In cases where cell counts are low or morphological features are atypical, relying solely on cytomorphological characteristics may result in misdiagnosis. Based on the findings of this study, the diagnostic approaches combining cytomorphology with HFC and TP were recommended. To address clinical needs, our team developed two different diagnostic strategies. Strategy I improves diagnostic sensitivity to 93.4%, while Strategy II increases diagnostic specificity from 86.3% to 92.3%. Compared with Strategy I, Strategy II achieved the optimal balance between PPV and NPV (87.0% and 87.1%, respectively). These two strategies serve as complementary pathways: Strategy I is suitable for initial screening, whereas Strategy II is intended for diagnostic confirmation. Together, they provide strong evidence-based support for the diagnosis of MSCE.

## Limitations

This study has two key limitations. First, the diagnostic sensitivity of cytomorphological examination may vary across different tumor types[27]. The inclusion of patients with heterogeneous malignancies in our study may have affected the overall diagnostic performance. Second, the interpretation of cytomorphological features is inherently subjective, which could introduce variability in diagnostic outcomes.

## Recommendations

To address these limitations, future studies should consider enrolling tumor-specific patient cohorts to more precisely assess the diagnostic performance of cytomorphological examination in different cancer subtypes. Additionally, we recommend establishing a comprehensive three-tier quality assurance framework: (1) regular staff training and proficiency assessments, (2) periodic evaluations of inter-observer agreement, and (3) participation in external quality assessment (EQA) programs. These measures would enhance standardization and ensure the consistency and reliability of diagnostic interpretations.

## Conclusion

Cytomorphological examination of SCE has proven effective in differentiating benign from malignant effusions. It demonstrated sensitivity and specificity comparable to those of commonly used tumor markers, enabling timely clinical decision-making with high diagnostic accuracy. The method is cost-effective, technically straightforward, and does not require specialized equipment, offering significant socioeconomic and diagnostic advantages—particularly in resource-limited primary healthcare settings. Furthermore, its strategic combination with HFC and TP diagnostic

https://dx.doi.org/10.4314/mmj.v37i2.5

approaches markedly enhances its clinical utility in the evaluation of MSCE.

## Statements and Declarations

## Author Contributions

Ning Zhang and Xiaoqin Wang contributed to the conception and design of the study. Ning Zhang and Thomas Stuart Mughogho contributed to the statistical analyses and manuscript preparation. Yingfei Duan and Rashid Kaseka participated in data collection. Dokani Michael Ndovi,Jie Zheng and Jian Hu reviewed and edited the manuscript. All authors read and approved the final manuscript.

## Funding Sources

This research was funded by grants from the Science and Technology Program of Xi'an (grant number: 24LLRHZDZX0028).

## Competing Interests

The authors declare no conflict of interest.

## Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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