

MINISTRY OF EDUCATION MINISTRY OF DEFENSE
AND TRAINING
VIETNAM MILITARY MEDICAL UNIVERSITY

DUONG THUY LINH

**RESEARCH ON THE VALUE OF METHYLATION
OF THE *SEPT9* GENE IN PLASMA FOR THE
DIAGNOSIS OF COLORECTAL CANCER**

Specialization: Oncology

Item No.: 9 72 01 08

SUMMARY OF DOCTOR OF MEDICINE THESIS

HÀ NỘI - 2025

THE PROJECT WAS COMPLETED AT THE
VIETNAM MILITARY MEDICAL UNIVERSITY

Scientific supervisor:

1. Prof. PhD Nguyen Van Ba
2. PhD. Ho Huu Tho

Reviewer 1: Prof. PhD Pham Van Binh
Reviewer 2: Prof. PhD Nguyen Dang Ton
Reviewer 3: Prof. PhD Tran Ngoc Dung

The thesis was defended before the national thesis grading council
At time: hour day month year 2025

The thesis can be found at:

1. National Library
2. VietNam Military Medical University Library

INTRODUCTION

Colorectal cancer (CRC) is a disease with a high incidence and mortality rate worldwide. According to GLOBOCAN 2022, CRC ranks third in incidence and second in mortality among current cancers. In Vietnam, it is the fourth most common cancer. The overall five-year survival rate for CRC patients in stages I and II can reach 90%, but it drops to 15% in stage IV. To date, 25% of CRC cases are metastatic at diagnosis, and 25% to 50% of patients develop metastases after curative treatment, particularly in stage III, which is the group most likely to be treated curatively but has a high risk of metastatic recurrence due to prior lymph node metastasis and depends on the level of response to adjuvant therapy. Therefore, tests that can detect the disease at an early stage and have the potential to monitor for metastatic recurrence are essential.

Currently, the first biomarker based on DNA methylation in blood approved by the U.S. Food and Drug Administration for the diagnosis of CRC is the screening test for CRC using the methylated *SEPT9* gene (m*SEPT9*). Globally, there have been numerous studies on the diagnostic value as well as the prognostic and monitoring capabilities of the m*SEPT9* plasma test in CRC patients. However, to date, research on the value of m*SEPT9* in CRC in Vietnam is limited. Therefore, we conducted a study titled: “Research on the value of methylation of the *SEPT9* gene in plasma for the diagnosis of colorectal cancer,” with two objectives:

- 1. To determine the diagnostic value and correlation of plasma mSEPT9 with the clinical and paraclinical characteristics of colorectal cancer patients using PCR with extendable blocking probes.*
- 2. Research on the characteristics of SEPT9 gen methylation in serum before and after surgery in stage III colorectal cancer patients.*

NEW CONTRIBUTIONS OF THE THESIS

Clinical Aspects: This study is the first to investigate the value of the mSEPT9 plasma test in diagnosing CRC, establishing a correlation between the clinical and paraclinical characteristics of CRC patients in Vietnam and their plasma mSEPT9 expression status. The research highlights changes in plasma mSEPT9 levels after curative surgery and provides initial insights into the prognostic value of mSEPT9 in monitoring metastatic recurrence in stage III CRC patients.

Molecular Biology Aspects: A testing protocol for detecting mSEPT9 in CRC patients was developed using semi-nested PCR with a extendable blocking probes

THESIS STRUCTURE

The thesis consists of 127 pages (excluding references and appendices), divided into 4 chapters, including 28 tables, 07 charts, 09 figures, and 01 diagram. There are 115 references, comprising 13 Vietnamese sources and 102 English sources. The structure is as follows: Introduction: 2 pages, Literature Review: 35 pages, Research Subjects and Methods: 20 pages, Research Results: 38 pages, Discussion: 29 pages, Conclusion: 2 pages, Limitations and Recommendations: 1 page.

CHAPTER 1 – LITERATURE REVIEW

1.1. Overview of colorectal cancer

1.1.1 Epidemiology

Globally, the incidence rate of CRC ranks third, accounting for 9.6% of new cases, following lung and breast cancer. The mortality rate ranks second at 9.3%, only behind lung cancer (18.7%) for both genders. In Vietnam, according to statistics from the WHO and the National Cancer Institute in 2022, there were 16,835 new cases of CRC, making it the fourth most common cancer, with a mortality rate ranking fifth, comprising 7% of all cancer cases. Recent reports indicate a rising incidence of CRC among younger individuals (under 50) in many high-income countries, such as the United States, Canada, and Australia, with annual increases in disease rate.

1.1.2 Pathogenesis mechanism

CRC is the result of the long-term accumulation of genetic

changes and cellular epigenetic alterations. There are three main pathways related to the pathogenesis of CRC: Chromosomal Instability (CIN): The most common pathway, characterized by the accumulation of mutations in oncogenes and tumor suppressor genes.

Microsatellite Instability (MSI): Caused by dysfunction of DNA repair genes, leading to a high mutation rate in microsatellite regions.

CpG Island Methylator Phenotype (CIMP): Distinguished by hypermethylation processes, which silence tumor suppressor genes.

Thus, these three main pathways highlight the significant role of the accumulation of genetic mutations and gene methylation in the pathogenesis of CRC.

1.1.3 Diagnosis of Colorectal Cancer

1.1.3.1 Definitive Diagnosis

The definitive diagnosis of CRC is based on clinical symptoms, paraclinical tests, and the gold standard, which is histopathological examination of the tumor or biopsy specimens obtained through endoscopy.

1.1.3.2 Staging Diagnosis

Staging is classified according to the TNM system of the AJCC, 8th Edition, published in 2017.

1.1.4 Treatment Decision-Making, Prediction, and Prognosis

For decades, the treatment strategy for colorectal cancer (CRC) patients has been determined based on TNM staging and tumor location. This helps decide whether the patient is suitable for surgical intervention, requires neoadjuvant chemotherapy or radiation therapy, or needs adjuvant therapy post-surgery to achieve improved progression - free survival (PFS) and overall survival (OS). Historically, most stage II patients after curative surgery do not require adjuvant chemotherapy. However, studies show that 15% of patients in this group experience recurrence, metastatic progression, and mortality. For stage III patients, adjuvant chemotherapy is routinely indicated after surgery. Yet, scientific evidence indicates that the response to adjuvant chemotherapy varies; some patients do not benefit from it and may suffer from side effects, extended treatment duration, and increased costs.

This highlights the limitations of treatment strategies based on clinical stratification factors in terms of efficacy. Therefore, ongoing

advancements in recent years demonstrate the potential to improve treatment outcomes by combining therapeutic approaches with patient stratification systems based on tumor biology, genetic characteristics, and epigenetic profiles.

1.2 Challenges and Difficulties in Early Screening for Colorectal Cancer

Current screening methods for CRC primarily include fecal occult blood tests, stool DNA tests, colonoscopy, and various imaging techniques (CT, ultrasound, etc.). Fecal Occult Blood Test: This test is simple and less expensive than other methods, but it has limited sensitivity (33% - 75%) and a high rate of false positives. Colonoscopy: Typically performed as a follow-up step for positive initial screening tests in most programs. However, the drawbacks of colonoscopy include its invasive nature, risks of complications (such as perforation and bleeding), the need for bowel preparation, and the burden of resources and costs. These factors contribute to lower compliance rates compared to fecal occult blood testing. Given these limitations of current detection and diagnosis methods for CRC, there is a pressing need for a new screening method that offers higher sensitivity and specificity, along with more convenient clinical application. In 2016, the mSEPT9 test in peripheral blood was approved by the FDA for CRC screening with the commercial kit Epi proColon® 2.0.

1.3 Methylation of the *SEPT9* Gene in Colorectal Cancer

1.3.1 Structure and Function of the SEPT9 Gene

The *Septin* gene family consists of 13 genes encoding over 30 protein isoforms, categorized into four groups: *SEPT2*: (*SEPT1*, *SEPT2*, *SEPT4*, *SEPT5*); *SEPT6*: (*SEPT6*, *SEPT8*, *SEPT10*, *SEPT11*, *SEPT14*); *SEPT7*; *SEPT9*: (*SEPT9*, *SEPT3*, *SEPT12*)

The *Septin* genes play a crucial role in molecular and cellular structures, contributing to the formation of tissues and organs in the human body. They are involved in various cellular processes, including cytokinesis, cell division, and maintaining cell shape, which are essential for proper cellular function and organization.

The *SEPT9* gene is located on chromosome 17q25.3 and is expressed throughout human cells with 18 transcripts. *SEPT9* stabilizes polymers and promotes the synthesis of subunits, playing a crucial role in the final separation of daughter cells during cell

division. Therefore, when the *SEPT9* gene is deleted or abnormally expressed, it severely impacts the cell division process. Abnormal expression of the *SEPT9* gene has been observed in various malignant tumors.

1.3.2 Pathogenesis Mechanism of the SEPT9 Gene in Tumor Formation and Development

The pathogenesis of the *SEPT9* gene in the formation and development of malignant tumors occurs through five main pathways: HIF-1 Signaling Pathway: The Hypoxia Inducible Factor-1 (HIF-1) is a transcription factor that regulates tumor cells in hypoxic environments, consisting of HIF-1-alpha and HIF-1-beta subunits.

JNK Signaling Pathway: The Jun N-terminal kinase (JNK) pathway is essential for the development, division, and transformation of cancer cells, regulating the cell cycle. Rho Signaling Pathway: Rho proteins are small GTPase proteins that can modulate the expression of downstream effector molecules, affecting cytoskeletal formation, mediating cell adhesion to the extracellular matrix, and influencing tumor cell metastasis and invasion. Mitosis: During mitosis, *SEPT9* polymerizes to form fibers that are dispersed in the cytoplasm during prophase and then assemble at the cleavage furrow during telophase. High levels of *SEPT9* expression promote genomic instability, causing spindle defects, hindering cytokinesis, and leading to chromosomal segregation disorders. *SEPT9* DNA Methylation: DNA methylation is a crucial epigenetic modification that involves transferring a methyl group to bases by DNA methyltransferase, with S-adenosylmethionine as the methyl donor. The main manifestations of these abnormalities are low overall DNA methylation levels in non-promoter regions and high methylation levels in promoter regions. *SEPT9* acts as a tumor suppressor gene and has been shown to be methylated in various tumor types.

1.3.3 Mechanism of SEPT9 Methylation in Colorectal Cancer

High methylation of the CpG loci in the *SEPT9* gene inhibits gene expression and impairs its tumor-suppressing function. This DNA methylation process suppresses gene expression in two primary ways, promoting tumor formation. Structural Changes: DNA methylation alters the structure, making it unfavorable for transcription factor binding, ultimately inhibiting gene expression. Binding of Methylated CpG-Binding Proteins: Methylated DNA interacts with methyl-CpG

binding domain proteins. These proteins subsequently bind to other proteins (such as histone deacetylases), leading to the formation of compact and inactive chromatin, which further inhibits gene expression. Autophagy is a cellular regulatory mechanism that prevents malignant transformation by reducing oxidative stress, eliminating oncogenic proteins, and maintaining genomic stability, thereby enhancing tumor-suppressing capabilities. Studies have shown that autophagy inhibits the onset of colorectal cancer. SEPTin proteins are involved in the autophagy process.

Thus, *SEPT9* plays a crucial role in cell cycle regulation, and methylation of the CpG region in its promoter results in the loss of its tumor suppressor function. Therefore, the m*SEPT9* test can detect the potential for colorectal cancer even before clinical symptoms manifest and can be identified in paraclinical tests.

1.4 Methods for Analyzing mSEPT9 Detection

In recent years, three main approaches for single-gene DNA methylation testing have been developed:

1.4.1 Restriction Enzyme Methylation Analysis

1.4.2 Affinity Enrichment Methylation Analysis

1.4.3 Bisulfite Treatment Methylation Analysis

1.5 Global Research on the Value of mSEPT9 Plasma Biomarker in Colorectal Cancer Patients

1.5.1 Value of mSEPT9 Plasma Biomarker in Early Screening for Colorectal Cancer

1.5.2 Value of mSEPT9 Plasma Biomarker in Prognosis and Post-Treatment Monitoring of Colorectal Cancer

1.6. Research Status in Vietnam on Biomarkers in Colorectal Cancer

In Vietnam, numerous studies on CRC have been conducted with the aim of identifying specific biomarkers for diagnosis and treatment. The majority of research focuses on the correlation between gene mutations and prognosis as well as treatment outcomes for CRC. However, there is a noticeable gap in studies aimed at discovering specific biomarkers that possess high sensitivity for screening and early detection of CRC. This highlights a need for more research in this area to enhance early diagnosis and improve patient outcomes in colorectal cancer management.

CHAPTER 2 – MATERIAL AND METHODS

2.1 Research design

Prospective, cross-sectional, analytical study

2.2 Study Population

The study involves a total of 152 colorectal cancer patients undergoing inpatient treatment at Military Hospital 103; K Hospital, Tan Trieu Facility. These patients were enrolled from January 2021 to December 2024. Additionally, 92 individuals undergoing routine health check-ups and testing at the Genetic Technology and Genetics Department of the Military Medical Research Institute, Military Medical Academy were included in the research.

2.2.1 Sample Selection Criteria

+ Control Group: Individuals undergoing routine health check-ups with overall evaluations showing no acute or chronic diseases. Colonoscopy results indicating no polyps, tumors, or inflammatory bowel conditions. mSEPT9 testing conducted prior to colonoscopy. No family history of cancer. Voluntary participation in the study

+ Patient Group: Patients diagnosed with CRC, including stage diagnosis and treatment according to the Ministry of Health's protocols. Complete mSEPT9 and CEA testing conducted prior to treatment. Stage III CRC patients underwent mSEPT9 testing 7 days post curative surgery. Voluntary participation in the study.

2.2.2 Exclusion Criteria

+ Coexisting cancer or other life-threatening conditions.

+ Patients who have undergone chemotherapy, radiation therapy, or surgery prior to mSEPT9 testing.

+ Stage III colorectal cancer patients who experience severe complications post-curative surgery and require intensive care for more than 7 days.

+ Individuals who do not consent to participate in the study or have incomplete data in their records.

2.3 Sample Size Calculation

The sample size was determined using the WHO formula for cross-sectional descriptive studies. In this study, we set $\alpha = 0.05$, with an expected positive rate of mSEPT9 in CRC at 90.8%, thus $p = 0.9$, and an absolute error of $d = 0.05$. Based on this calculation, the minimum sample size required for the study is 128.

The final study sample comprises 244 participants, including:

152 CRC; 92 healthy individuals

- Objective 1: To determine the diagnostic value of the *mSEPT9* test, including sensitivity, specificity, positive predictive value, and negative predictive value, using PCR techniques in diagnosing CRC. This analysis is based on the 152 CRC patients distributed across all four disease stages and the 92 healthy individuals. Additionally, it will assess the correlation between *mSEPT9* characteristics and the clinical and paraclinical features of CRC patients.

- Objective 2: To evaluate the variability of plasma *mSEPT9* levels before and after treatment in stage III CRC patients. This includes monitoring 50 stage III CRC patients selected from the group of 152. Stage III is chosen due to its potential for curative treatment while also carrying a significant risk of recurrence and metastasis within a 5-year timeframe, aligning with the duration of this research project.

2.4 Equipment and Tools Used in the Study

2.5 Research content

The following equipment and tools were utilized for the *mSEPT9* testing process using PCR techniques in the Genetic Technology and Genetics Department of the Military Medical Research Institute, Military Medical University.

2.5.1 Clinical Research

Patients are thoroughly examined and diagnosed to confirm the presence of CRC and to determine the disease stage according to the guidelines set forth by the Ministry of Health. Information is systematically collected regarding medical history, disease history, and clinical examinations using a standardized patient record form.

+ Collection of Clinical Characteristics

Age, gender, time to diagnosis, clinical symptoms.

+ Collection of Paraclinical Characteristics

Imaging Studies: Endoscopy, CT Scan, MRI, PET/CT

Histopathological: Tumor classification through biopsy samples according to the 2019 WHO classification of tumors.

Staging Assessment: theo hệ thống phân loại Disease staging at the time of initial diagnosis using the TNM classification system (AJCC, 8th edition, 2017).

CEA Testing: Normal range: 0 – 5 ng/ml; $CEA \leq 5$ ng/ml: Negative result; $CEA > 5$ ng/ml: Positive result.

mSEPT9 testing: Determination of positive or negative results based on PCR analysis and melt curve analysis, repeated three times for accuracy.

+ Collection of Treatment Information and Sample Timing

Control Group: Blood samples for mSEPT9 testing will be collected prior to colonoscopy for examination.

Patient Group: All colorectal cancer patients will have blood samples collected for mSEPT9 testing before the initiation of any treatment. For stage III colorectal cancer patients undergoing curative surgery, blood samples for mSEPT9 testing will be collected 7 days after the surgical procedure.

+ Follow-Up Information Collection

Pathological Assessment: Evaluate the results of tumor and lymph node pathology post-surgery according to the WHO 2019 classification.

Patient Status Tracking: Monitor patient status through medical records from the time of diagnosis until the completion of curative treatment and adjuvant therapy. Follow-up schedule: Every 3 months during the first 2 years post-treatment. Every 6 months in subsequent years. This structured follow-up will facilitate the identification of disease progression, recurrence, metastasis, and mortality, contributing to the overall understanding of treatment outcomes and patient prognosis.

2.5.2 Principles and Technical Process of the Study

2.5.2.1 Methods for Sample Collection, Storage, and Extraction

+ Procedure for Peripheral Venous Blood Sampling:

+ Procedure for DNA Extraction from plasma

2.5.2.2 Bisulfite Treatment of Extracted DNA

2.5.2.3 Semi-Nested PCR Method for Detecting Methylated SEPT9 DNA with Extendable blocking probe - ExBP

a. Design of Primers for Real-Time PCR with ExBP

b. Semi-Nested PCR Method and Melt Curve Analysis for mSEPT9 Detection

The PCR reaction was conducted on a Rotor-Gene Q system with a reaction volume of 20 μ L and 5 μ L of DNA after bisulfite treatment was used as the template. Realtime PCR data are collected and saved on a computer and then analyzed using Rotor-Gene Q Software (Qiagen). Each sample was tested in triplicate, and a sample

was considered positive for mSEPT9 if at least one of the three replicates was positive, with a positive result for each PCR reaction when a melting peak with a peak characteristic for methylated SEPT9 sequences occurred at 78,0–78,7 degrees Celsius.

2.6 Data processing method

- Input and process research data using SPSS 20.0 software.
- $p < 0,05$ represents a statistically significant difference

2.7 Research ethics

The research has been approved by the Ethics Council of Military Hospital 103 (Decision No. 98/CNChT - HDĐĐ dated July 23, 2020) and approved for research at Tan Trieu K Hospital and at Military Hospital 103.

CHAPTER 3 - RESEARCH RESULTS

3.1 Some clinical and paraclinical characteristics of study subjects

3.1.1. Distribution by age and sex

The median age of all study subjects was 59,0 years old, with no difference in age or gender between the colorectal cancer group and the control group ($p > 0,05$).

3.1.2. Distribution according to location of colorectal cancer

Left colon cancer has the highest rate of 40,0% (61/152), rectal cancer is second at 37% (56/152), right colon cancer is the lowest at 23% (35/152).

3.1.3. Time from symptoms until diagnosis is confirmed

The average time from symptoms to confirmed diagnosis is 2 months.

3.1.4. Clinical characteristics of the group of CRC

The most common sign of abdominal pain is 78,95% (120/152), bloody stool is second at 55,92% (85/120).

3.1.5. Paraclinical characteristics of the group of patients with colorectal cancer

- Tumor characteristics on endoscopy: The group of patients with tumor size occupying $>3/4$ of the colorectal circumference accounts for the largest proportion of 40,13% (61/152); The main type of injury is the "Wart" type, accounting for the highest proportion (73/152, accounting for 48,03%); The "Ulcer" type of injury accounts for the lowest rate (4/152, accounting for 2,63%),

only in the rectum.

- Plasma CEA concentration is highest in stage 4 patients. The median CEA concentration in stage 4 cancer patients is 43,37 ng/ml, the lowest in early stage is stage 1 with 1,89 ng/ml and the difference is statistically significant ($p < 0,0001$).

3.1.6 Treatment characteristics and post-treatment evaluation

- The difference in tumor size in length with a cutoff point of 5cm and diameter of 4cm, between subgroups of tumor location, has a statistically significant difference with $p < 0,05$.

- Adenocarcinoma accounts for the majority (82,9%), medullary cancer has only 1 patient accounting for 0,7%, the rest are mucinous and ring adenocarcinoma. Moderate histology is the most common, accounting for 84,2%, poorly differentiated 12,5%.

- The patient group is fairly evenly distributed in all 4 stages of the disease, of which stage III accounts for the highest rate of 32,9%; Tumor stage T1 accounts for only 7,2% while stage T4 accounts for 38,2%; Lymph node stage has 48% stage N0, 32,9% stage N1 and a lower proportion is stage N2 accounting for 19,1%. There are 21,7% of distant metastases, corresponding to 21,7% of stage 4 patients.

3.2 Determining the diagnostic value and relationship of plasma mSEPT9 with clinical and paraclinical characteristics of CRC using PCR technique with Extendable blocking probe

3.2.1. Diagnostic value of plasma mSEPT9 test in diagnosing colorectal cancer

Table 3.1 Sensitivity, specificity, PPV, NPV values of mSEPT9 in diagnosing colorectal cancer

mSEPT9 plasma	CRC n (%)	Non-CRC n (%)	Total
Negative	36 (30,5)	82 (69,5)	118
Positive	116 (92,1)	10 (7,9)	126
Diagnostic value			CI (95%)
Sensitivity (%)	76,31		68,75-82,82
Specificity (%)	89,13		80,91-94,66
PPV (%)	92,06		86,52-95,44
NPV (%)	69,49		62,92-75,35
OR	26,42 ($p = 0,0001$)		12,41-56,24

Analysis of the mSEPT9 test achieved a sensitivity of 76,3% with CI95%: 68,75-82,82; Specificity 89,1% with CI95%: 80,91-94,66. Positive predictive value (PPV) reached 92,06% with CI: 86,52-95,44; Negative predictive value (NPV) reached 69,49% with CI: 62,92-75,35. The mSEPT9 positive group has a 26,42 times higher risk of colorectal cancer than the mSEPT9 negative group (OR = 26,42, $p < 0,0001$)

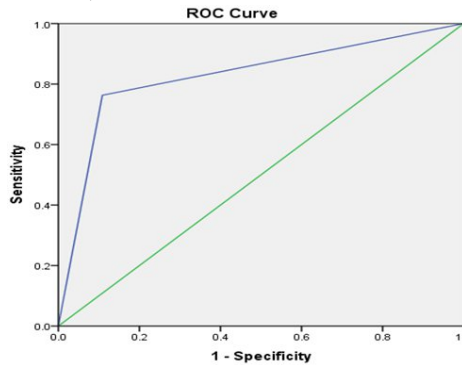


Figure 3.1 ROC curve of mSEPT9 in diagnosing CRC to differentiate it from healthy people

The diagnostic value of colorectal cancer distinguished from healthy people group, mSEPT9 has good diagnostic value with AUC reaching 0,827 (95%CI: 77,3 – 88,2) $p < 0,0001$.

Table 3.2 The level of plasma mSEPT9 expression distinguishes early-stage CRC from late-stage CRC

Variable	mSEPT9 (-) n (%)	mSEPT9(+) n (%)	n
CRC	36 (23,7)	116 (76,3)	152 (100%)
Stage			
Early - stage	25 (36,2)	44 (63,8)	69 (100%)
Late - stage	11 (13,3)	72 (86,7)	83 (100%)
OR	3,719		
<i>p</i>	0,001		

95% CI	1,667 – 8,295	
--------	---------------	--

In the group of late-stage CRC (stages III, IV), 86,7% of mSEPT9 are positive, the rate of positive mSEPT9 in early stages (stages I, II) is 63,8%. mSEPT9-positive patients have a 3,719 times higher risk of late-stage cancer than the mSEPT9-negative group (OR = 3,719, $p < 0,001$).

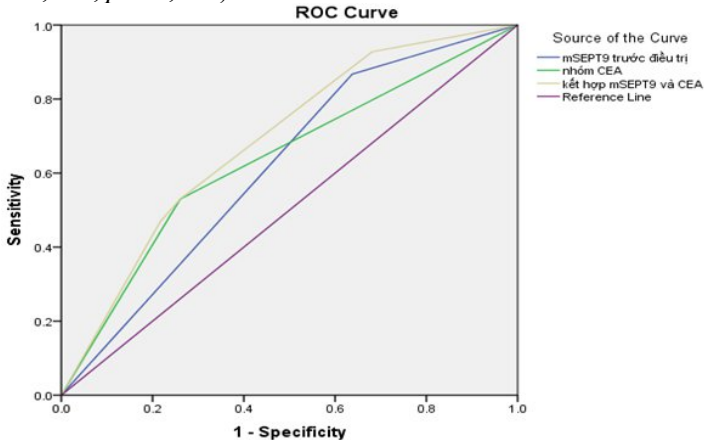


Figure 3.2 ROC curve combining mSEPT9 and CEA in diagnosing CRC distinguishes early stages from late stages.

The diagnostic value of early stage CRC (stages I and II) versus late stages (stages III and IV) of mSEPT9 combined with CEA is average with AUC 0,686, $p < 0,05$, sensitivity 86,7%, specificity 36,2%.

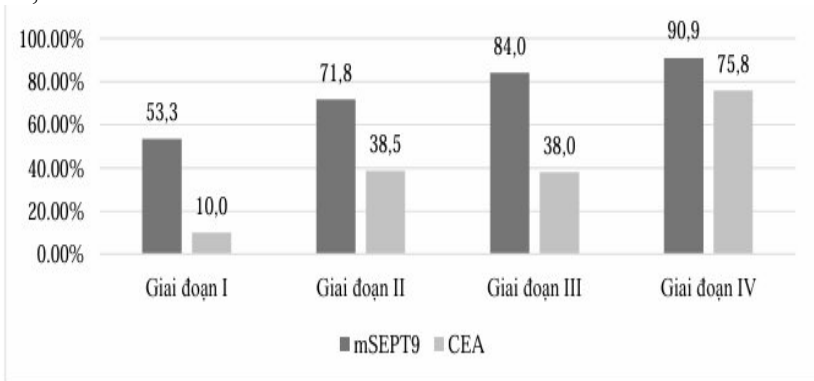


Chart 3.1 Diagnostic value of mSEPT9 and CEA according to

disease stage

mSEPT9 showed higher sensitivity at all colorectal cancer stages compared to CEA. Specifically, in stage I, 53,3% of patients had positive mSEPT9 while only 10%. The patient has a positive CEA; In stage II, mSEPT9 can detect nearly twice as many patients (71,8%) as CEA (38,5%); In stage III, mSEPT9 was 2,2 times more detectable (84%) than CEA (38,0%).

3.2.2 Determining the relationship between plasma mSEPT9 status and clinical and paraclinical characteristics of the group of CRC

- Note the statistically significant difference in mSEPT9 status when grouped by tumor length with a threshold of 5cm; classify tumor diameter according to the threshold of 4cm; Tumor stage grouping, lymph node metastasis stage grouping, colorectal tumor distant metastasis stage grouping, TNM stage grouping, CEA level grouping before treatment with a threshold of 5 ng/ml with $p < 0,05$.

- No statistically significant differences were recorded in mSEPT9 status when classified by age group, gender, chronic disease, clinical symptoms, tumor location, macroscopic features on endoscopy, histopathological features, and microsatellite instability features with $p > 0,05$.

3.3 Characteristics of mSEPT9 fluctuations before and after radical treatment in the group of stage III colorectal cancer patients

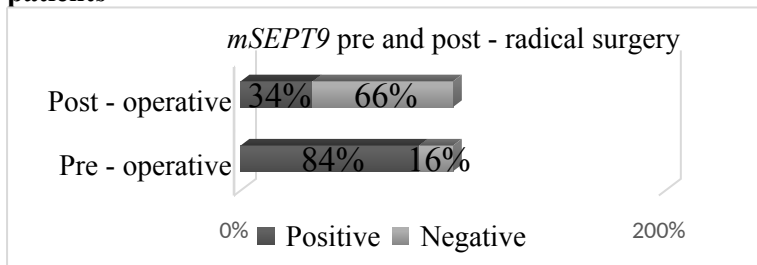


Figure 3.3. Changes in mSEPT9 before and after radical surgery

Testing mSEPT9 on 50 patients with stage III colorectal cancer before surgery and 7 days after radical surgery, the positive rate for plasma mSEPT9 after surgery was 34% (17/50) significantly

reduced compared to preoperative 84% (42/50), with a statistically significant change ($p = 0,027$).

Table 3.3. The relationship between mSEPT9 after surgery and progression after radical surgery in the group of stage III CRC

Object group	No progress n (%)	Progress n (%)	Total
mSEPT9 post - operative			
Negative	30 (90,9)	03 (9,1)	33 (100)
Positive	10 (58,8)	07 (41,2)	17 (100)
OR	7,000		
<i>p</i>	0,021 (95%CI: 1,515 - 32,333)		

Of the 33 mSEPT9-negative patients after surgery, 03 patients (9,1%) progressed, and of the 17 mSEPT9-positive patients, 07 patients (41,2%) progressed. Patients after radical surgery in the mSEPT9 positive group had a 7 times higher risk of recurrence and metastasis than in the mSEPT9 negative group after surgery (OR = 7, $p < 0,05$).

Table 3.4 Relationship of mSEPT9 before and after surgery with tumor stage, lymph node metastasis stage and pathology

Variable	n	Pre-operative			Post-operative		
		mSEPT9 (-) n (%)	mSEPT9 (+) n (%)	<i>p</i>	mSEPT9 (-) n (%)	mSEPT9 (+) n (%)	<i>p</i>
CRC	50	11 (22)	39 (78)		33 (66,0)	17 (34,0)	
Stage T				1,00			0,039
T1 + T2	12	2(16,7)	10(83,3)		11 (91,7)	1(8,3)	
T3 + T4	38	9(23,7)	29(76,3)		22 (57,9)	16 (42,1)	
Stage N				0,71			0,216
N1	35	7 (20)	28 (80)		25 (71,4)	10 (28,6)	
N2	15	4 (26,7)	11 (73,3)		8 (53,3)	7 (46,7)	
Pathology							
Adenocarcinom	41	5 (12,2)	36 (87,8)	0,14	24 (58,5)	17 (41,5)	0,02
Non adeno	9	3 (33,3)	6 (66,7)		9 (100)	0(0)	

From the table above, it can be seen that there was no statistically significant difference in mSEPT9 status before treatment in terms of tumor stage and lymph node stage, histological characteristics with $p > 0,05$, but after radical surgery, there was a statistically significant difference in mSEPT9 status

after surgery with T stage subgroup, histological characteristics with $p < 0,05$, and no difference was recorded in postoperative lymph node metastasis status with $p > 0,05$.

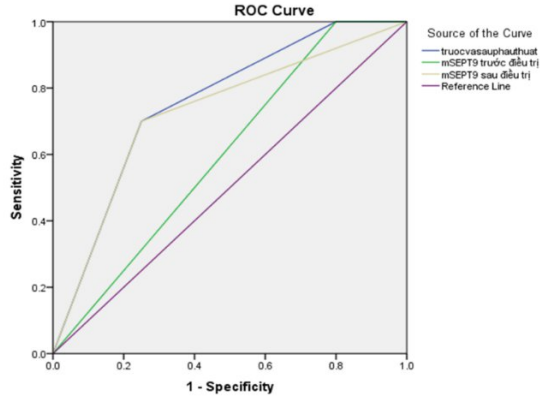
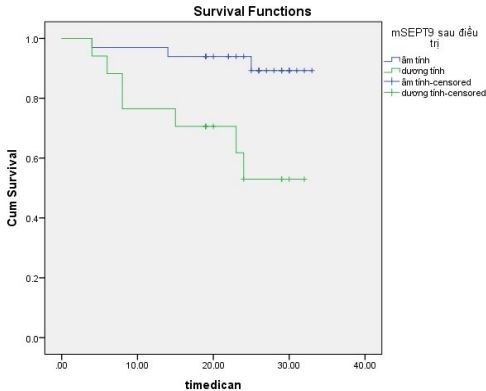
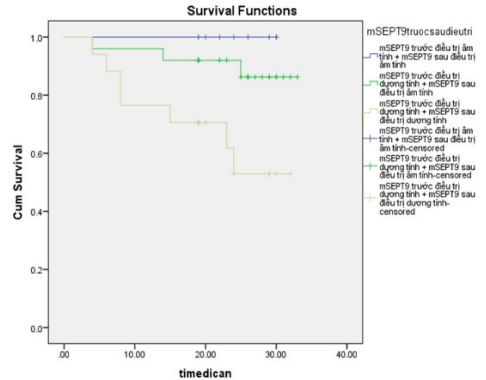


Figure 3.4. Analysis of the predictive value of postoperative progression when combining mSEPT9 before and after surgery in stage III CRC

ROC curve analysis showed that preoperative mSEPT9 was not meaningful in predicting the progression of stage III CRC after radical surgery with $AUC = 0,6$; $p > 0,05$. When combined with mSEPT9 after surgery, it has a fairly good value in predicting progression after radical surgery ($AUC = 0,755$, $p < 0,05$).



(a)



(b)

Figure 3.4 Estimate progression after radical surgery using the

Kaplan-Meier method. (a): mSEPT9 after surgery, (b): mSEPT9 before and after surgery

PFS in the mSEPT9 positive group after surgery was shorter than in the mSEPT9 negative group after surgery, with a statistically significant difference. Combining mSEPT9 before and after surgery showed the best prognosis for the negative mSEPT9 group before and after surgery; PFS was shortest in the mSEPT9 positive group both before and after surgery, the difference was significant with $p < 0,05$ (Log Rank test).

Table 3.5. Cox regression for PFS in stage III CRC

Variable	Progression-Free Survival			
	Univariate		Multivariate	
	PFS HR (95% CI)	<i>p</i>	PFS HR (95% CI)	<i>p</i>
Gender				
Female	1 (ref)	0,94	1 (ref)	0,85
Male	0,953 (0,26-3,37)		0,86 (0,19-3,82)	
Age				
≤ 60	1 (ref)	0,60	1 (ref)	0,96
> 60	0,72 (0,20-2,49)		0,96(0,24-3,73)	
Tumor location				
Right colon	1 (ref)		1 (ref)	
Left colon	2,21(0,26-18,43)	0,46	2,10(0,17-25,63)	0,56
Rectal	1,22(0,12– 11,75)	0,86	3,27(0,24-43,33)	0,36
Tumor length				
< 5 cm	1 (ref)	0,21	1 (ref)	0,23
≥ 5 cm	2,37 (0,61 – 9,19)		0,34(0,05-2,02)	
Tumor diameter				
< 4 cm	1 (ref)	0,09	1 (ref)	0,13
≥ 4 cm	3,20(0,82 – 12,44)		0,25(0,04-1,49)	
Tumor histology				
Non -Carcinoma	1 (ref)	0,35	1 (ref)	0,97

Carcinoma	0,03(0,00 – 41,09)		0,74(0,001-59,49)	
Degree of tumor differentiation				
Poor differentiation	1 (ref)		1 (ref)	0,84
High + moderate	0,57(0,07-4,55)	0,59	0,78(0,07-8,78)	
Stage T				
T1 + T2	1 (ref)	0,67	1 (ref)	0,10
T3 + T4	0,74(0,19-2,89)		7,63(0,66-86,99)	
mSEPT9 preoperative				
Negative	1 (ref)	0,36	1 (ref)	0,97
Positive	26,75(0,02-34300)		0,001(0,00-40,01)	
mSEPT9 postoperative				
Negative	1 (ref)	0,01	1 (ref)	0,035
Positive	5,67(1,45-22,11)		10,738(1,18– 97,69)	

Univariate analysis showed that mSEPT9 positivity after surgery (HR: 5,677, $p < 0,05$) was associated with shorter PFS compared with mSEPT9 negativity after surgery. Multivariate analysis noted that positive mSEPT9 after radical surgery (HR: 10,738, $p < 0,05$) was an independent factor predicting shorter PFS compared to the negative mSEPT9 group after surgery.

CHAPTER 4 - DISCUSSION

4.1 General characteristics of the research target group

4.2 Determining the diagnostic value and relationship of plasma mSEPT9 with clinical and paraclinical characteristics of CRC using PCR technique with Extendable blocking probe

4.2.1 *Diagnostic value of plasma mSEPT9 test in diagnosing colorectal cancer*

To determine the diagnostic value of mSEPT9 in diagnosing colorectal cancer, we designed a case-control study and obtained results of test sensitivity of 76,3%, specificity of 89,1%, positive predictive value of 92,1%, negative predictive value of 62,1%, and good diagnostic value in differentiating colorectal cancer from healthy people. AUC reached 0,827 with $p < 0,0001$; OR = 26,42, $p < 0,0001$. Our research results are consistent with some previous studies, specifically: According to Sun et al (2019), when the mSEPT9 test screened and diagnosed 600 cases over 40 years old at high risk of colorectal cancer, 50 colorectal cancer patients before treatment, recorded the sensitivity and specificity of mSEPT9 as 73,0% and 94,5%, AUC 0,8. Our study achieved higher sensitivity but lower specificity than this study. According to Lu et al (2022), when studying a group of 616 patients with colorectal cancer and 122 healthy people tested for mSEPT9, they recorded sensitivity of 72,94%, specificity of 81,97%, AUC of 0,826. This study's sensitivity and specificity are both lower than our study. In terms of AUC values, there are similarities.. According to Liang Min et al (2023) performed a meta-analysis of 79 studies on the value of mSEPT9 in diagnosing colorectal cancer using the commercial test kit Epi procolon 2.0, recording a sensitivity of 0,679 (95% CI: 0,622–0,732), specificity of 0,903 (95% CI: 0,878–0,923), AUC of 0,8.

In addition, the diagnostic sensitivity of mSEPT9 is also higher than the sensitivity of the CEA marker with a threshold value of 5

ng/ml at all stages. In our study, the sensitivity for detecting stage I (53,3%), stage 2 (71,8%), stage 3 (84%), stage 4 (90,9%). Our results are also consistent with other studies that have reported higher sensitivity for patients with stage II–IV disease, with improved detection sensitivity in stage I. According to Sabine Leerhoff et al (2023), a study on 120 patients with CRC also noted that the detection sensitivity of *mSEPT9* was higher than the detection sensitivity of CEA at all stages of the disease with the positive rate of *mSEPT9*, CEA in stage I was 33% compared to 12%, stage II was 68% compared to 35%, stage III was 48% compared to 27%, stage III was 48% compared to 27%. stage IV is 84% compared to 46%.

According to Quian Sun et al (2024), the detection value of *mSEPT9* according to stage of colorectal cancer was recorded: stage I is 53%, stage II is 76,5%, stage III is 72,3%, stage IV is 88,9%. At the same time, in our study, when dividing the early stage group (stage I, stage II) when the tumor is localized locally and the late stage group (stage III, stage IV) when the tumor progresses in areas with lymph node metastases and distant metastases, it was noted that the diagnostic value of *mSEPT9* combined with CEA reached AUC 0,686, $p < 0.05$, sensitivity 86,7%, specificity 36,2%, OR = 3,719, $p < 0,001$.

Therefore, *mSEPT9* detected in peripheral blood is a valuable diagnostic test for early detection of colorectal cancer consistent with the pathogenesis of DNA methylation leading to transcriptional inactivation and repression of oncogenes, which is an important process in the early stages of carcinogenesis. Among them, the *SEPT9* gene is located on chromosome 17q25.3 and is widely expressed in human cells. Results of research on methylation testing at tissue and plasma levels showed that the *SEPT9-v2* promoter region was almost completely methylated in patients with colorectal cancer and pre-cancer. With the Nested - PCR testing technique

using extendable blocking probe, high sensitivity is achieved. At the same time, economical testing costs create opportunities to expand testing to integrate with clinical practice in treating patients..

4.2.2 Relationship of mSEPT9 characteristics before treatment with clinical and paraclinical characteristics of the study patient group

Note the statistically significant difference in mSEPT9 status when grouped by tumor length with a threshold of 5cm; classify tumor diameter according to the threshold of 4cm; Tumor stage grouping, lymph node metastasis stage grouping, colorectal tumor distant metastasis stage grouping, TNM stage grouping, CEA level grouping before treatment with a threshold of 5 ng/ml with $p < 0,05$. Similar to studies Jie S. et al (2021) mSEPT9 peripheral blood positivity was associated with TNM stage, total tumor volume, and microsatellite instability. However, while there was a significant difference between positive mSEPT9 and tumor stage and distant metastasis stage, no difference was seen in the N1 and N2 lymph node stage groups.

4.3 Characteristics of mSEPT9 fluctuations before and after radical treatment in the group of stage III CRC

To study the fluctuations of mSEPT9 before and after radical treatment and initially evaluate the ability to predict progression after surgery, we took peripheral blood from colorectal cancer patients to test for mSEPT9 before treatment and 7 days after surgery, followed longitudinally to record the time of recurrence and metastasis, if any. The study found that the mSEPT9 positive group after surgery had a higher risk of progression than the mSEPT9 negative group after surgery (OR = 7, $p < 0,05$). Survival analysis using the Kaplan-Meier method showed that the mSEPT9 positive group after surgery had a significantly shorter PFS than the mSEPT9 negative group after surgery. At the same time, multivariate analysis found that mSEPT9 after radical surgery (HR: 10,738, $p < 0,05$) was an independent factor predicting PFS of patients with colorectal cancer. Our study results are consistent with some previous studies. According to Yuan et al.

(2022), post-operative colorectal cancer patients who were positive for *mSEPT9* were significantly associated with poorer PFS (HR = 6,21, $p < 0,001$) and *mSEPT9* was still the strongest independent predictor in the multivariate Cox regression analysis (HR = 5,83, $p < 0,001$), significantly outperforming CEA, CA19-9 and CA24-2. According to Hua Gao et al (2023), the rate of metastasis in patients with positive *mSEPT9* after surgery is 81,25% (13/16), the rate of metastasis in patients with negative *mSEPT9* after surgery is 5,19% (04/77). Thus, the rate of metastatic progression in the group of patients with positive *mSEPT9* after surgery is much higher than the group of patients with negative *mSEPT9*. In our study, 41,2% (07/17) of patients with progression in the group of *mSEPT9* positive after surgery were higher than 9,1% (03/33) of patients with progression in the group of negative *mSEPT9* after surgery. According to Rong Li et al (2024), monitoring 30 colorectal cancer patients before surgery and 7 days after surgery also noted that the positive rate for plasma *mSEPT9* in the week after surgery decreased significantly compared to the preoperative period (23,33% compared to 66,67%), with a statistically significant change.. Clinical research results on *mSEPT9* characteristics before and after radical surgery show compatibility with the pathogenesis of colorectal cancer caused by the increase in *mSEPT9* status leading to transcriptional inhibition, loss of tumor inactivation function, activation of important signaling pathways such as Wnt/ β -catenin or PI3K/AKT promoting colorectal cancer development and metastasis..

Therefore, *mSEPT9* characteristics after surgery can be used to evaluate the effectiveness of surgery as well as predict the risk of metastatic recurrence after surgery in colorectal cancer patients..

CONCLUDE

1. Determining the diagnostic value and relationship of plasma mSEPT9 with clinical and paraclinical characteristics of CRC using PCR technique with Extendable blocking probe

- mSEPT9 test results achieved sensitivity of 76,3% with 95% CI: 68,75-82,82, specificity of 89,1% with 95%CI: 80,91-94,66. Positive predictive value reached 92,06% with 95%CI: 86,52-95,44; Negative predictive value reached 69,49% with 95%CI: 62,92-75,35.

- The mSEPT9 test has a good value in diagnosing colorectal cancer from healthy people with an AUC of 0,827 with $p < 0,0001$, 95% CI (77,3% - 88,2%). Patients found to be mSEPT9 positive were 26,42 times more likely to have colorectal cancer than the negative mSEPT9 group (OR = 26,42, $p < 0,05$).

- In the relationship between mSEPT9 status in plasma and clinical and paraclinical characteristics, there was a statistically significant difference between positive mSEPT9 status when grouped by gross tumor length, tumor diameter, TNM disease stage, tumor stage, lymph node stage, distant metastasis stage, and CEA status before treatment. Patients with cervical cancer with positive mSEPT9 are 3,719 times more likely to have late-stage cancer with lymph node metastasis or distant metastases than the negative mSEPT9 group (OR = 3,719, $p < 0,001$).

2. Characteristics of mSEPT9 fluctuations before and after radical treatment in the group of stage III CRC

- The plasma mSEPT9 positivity rate in the week after surgery decreased significantly compared to before surgery (84% vs. 34%), a statistically significant change ($p = 0,027$).

- Analyzing the difference in mSEPT9 status in the group of stage III colorectal cancer patients according to tumor stage subgroups, the pathological type characteristics recorded no difference with the mSEPT9 characteristics before treatment, and there was a statistically significant difference with the mSEPT9 expression

status after surgery.

- Stage III colorectal cancer patients with *mSEPT9* positive after radical surgery have a 7 times higher risk of recurrence and metastasis than the *mSEPT9* negative group after surgery (OR = 7, $p < 0,05$).

- ROC curve analysis showed that *mSEPT9* after treatment had a fairly good value in predicting progression after radical surgery (AUC = 0,725, 95% CI 0,542 – 0,908; $p < 0,05$). When combined with a positive *mSEPT9* before surgery, it has a fairly good predictive value for progression after radical surgery (AUC = 0,755, 95% CI 0,597 – 0,913; $p < 0,05$);

- Kaplan - Meier curve analysis of PFS time in stage III CRC after radical surgery with *mSEPT9* after treatment showed that the PFS of the group of patients with positive *mSEPT9* after treatment was significantly shorter than the group with negative *mSEPT9* after treatment. The PFS time in the groups was statistically different with $p < 0,05$ (Log Rank test).

- Multivariate analysis showed that *mSEPT9* positivity after radical surgery (HR: 10,738; 95% CI: 1,18– 97,69, $p < 0,05$), was an independent predictive factor for significantly shorter PFS in the *mSEPT9*-positive colorectal cancer group after surgery. Analyzing the predictive value of *mSEPT9* after surgery with the risk of death, no statistical significance was found.

RECOMMENDATION

Our research results have demonstrated the value of the *mSEPT9* marker in peripheral blood using the new generation PCR method in diagnosing colorectal cancer differentiated from healthy people, especially confirming the role of *mSEPT9* as an effective biomarker to monitor recurrence and prognosis after surgery for colorectal cancer, and combined with the CEA test to significantly improve the diagnostic efficiency as well as monitor the progression of recurrence and metastasis in colorectal cancer. These findings encourage integration of *mSEPT9* testing into routine clinical practice and set the stage for broader research.

LIST OF PUBLISHED WORKS
RESEARCH RESULTS OF THE THESIS

1. **Linh Thuy Duong, Trang Thuy Dao, Hoai Thi Bui, Ung Dinh Nguyen, Ung Tien Hoang, Duc Viet Tran, Ba Van Nguyen, and Tho Huu Ho** (2024). “Innovative Semi-Nested Realtime PCR Assay with Extendable Blocking Probe for Enhanced Analysis of *SEPT9* Methylation in Colorectal Cancer”. *Biomedinines*, 12,1458. <https://doi.org/10.3390/biomedicines12071458>.
2. **Linh Thuy Duong, Ba Van Nguyen, Tho Huu Ho** (2024). “Research on detecting SEPT9 gene methylation in colorectal cancer patients using Semi - Nested PCR method of linear spectrum analysis”. *Viet Nam Medical Journal*, Number 545, December, 2024, 488 - 497.
3. **Linh Thuy Duong, Hai Anh Vu, Hung Van Nguyen, Tho Huu Ho, Ba Van Nguyen** (2025). “Research on the value of methylation of the *SEPT9* gene in plasma for the diagnosis of colorectal cancer”. *Journal of Military Medicine and Pharmacy*, number 5/2025, 97 – 106. ISSN 1859 – 0748. <https://doi.org/10.56535/jmpm.v50i5.1266>
4. **Linh Thuy Duong, Hoanh Viet Ho, Van Khanh Nguyen, Tho Huu Ho, Ba Van Nguyen** (2025). “Research on the characteristics of *SEPT9* gene methylation in plasma before and after surgery in patients with in stage III colorectal cancer”. *Journal of Military Medicine and Pharmacy*, number 6/2025, 100 – 108. ISSN 1859 – 0748. <https://doi.org/10.56535/jmpm.v50i6.1276>.