# RESEARCH PROPOSAL


## THE ROLE OF GUT MICROBIOME AND CHRONIC INFLAMMATION IN YOUNG-ONSET COLORECTAL CANCER

**Next-Generation Sequencing (NGS) as screening method**

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# INTRODUCTION

## Background

In 2025 the World Health Organization (WHO) estimates that cancer will have a higher mortality rate than coronary heart disease and stroke. It is estimated there are more than 20 million new cases of cancer each year in low- and middle-income countries due to global epidemiological changes. Colorectal cancer is the third most prevalent cancer worldwide and is in the fourth place in cancer mortality rate. The incidence of colorectal cancer is estimated to be 1.2 million each year with more than 630.000 number of deaths, accounting for 8% of deaths caused by cancer. In 2030 it is estimated there will be 2.2 million number of new cases and 1.1 million number of deaths caused by colorectal cancer. Based on Jakarta Cancer Registry in 2012 in Indonesia, colorectal cancer is the second most common cancer in men and the fourth most common in women. The incidence of colorectal cancer was 3.15 per 100.000 of populations in women and 4.13 per 100.000 of populations in men.

Previous studies indicated that colorectal cancer patients in Indonesia were younger compared to developed countries and were sporadic. Sporadic colorectal cancer generally resulted from somatic mutation without any correlation to family history. In comparison to patients in developed countries, colorectal cancer patients in Indonesia also showed differences in several characteristics including distal localization (rectum), higher clinical staging, and poor prognosis.

Increasing number of colorectal cancer cases make a profound impact on national health expenditures. In the era of *Jaminan Kesehatan Nasional /* JKN (the National Health Insurance in Indonesia), there are limitations on budgets for colorectal cancer management. Thus, numerous efforts are needed on the early screening and prevention of colorectal cancer.

Gastrointestinal system comprises of more than 200 $m^2$ of mucosal surface. The immune system in gastrointestinal mucosa has been studied extensively until this day. The microflora or the microorganism in gastrointestinal system, also commonly known as the gut microbiome, is a part of the immunity system in gastrointestinal mucosa. Around 20% of malignancy are linked to microorganism. The gut microbiome is suspected to have a role in genetic and epigenetic changes that cause dysplasia, clonal expansion, tumor growth, and cancer. In addition, chronic

inflammation is also suspected to have a role in colorectal carcinogenesis. One of the chronic inflammation markers linked with carcinogenesis is NF-κB.

Several studies showed that dysbiosis or imbalance of gut microbiome is linked with mucosal barrier damage, chronic inflammation and production of carcinogenic metabolite causing neoplasm. Thus far, the association between gut microbiome and colorectal cancer has provided an opportunity to develop new strategies in the screening method of colorectal cancer.

Non-invasive methods for colorectal cancer screening are currently being developed to replace colonoscopy. One of the screening methods is *fecal immunochemical test* (FIT) using fecal samples. This method has a sensitivity of 87.5% in detecting colorectal cancer cases. Another screening method uses blood sample to evaluate *carcinoembryonic antigen* (CEA) level in blood serum. An increase in CEA serum level of more than 5 ng/mL is found in 17-47% of colorectal patients and is associated with poor prognosis.

## Research Objectives

### General Objectives

To investigate the role of gut microbiome pattern and inflammation marker NF-κB in colorectal cancer patients

### Specific Objectives

1. To obtain data on gut microbiome pattern on young-onset colorectal cancer patients and colorectal cancer patients aged >50 years old.
2. To explore the role of inflammation marker NF-κB on young-onset colorectal cancer patients and colorectal cancer patients aged >50 years old.
3. To obtain CEA values of young-onset colorectal cancer patients and colorectal cancer patients aged >50 years old.
4. To obtain data on gut microbiome which could be used as screening methods in patients suspected with colorectal cancer

## Hypothesis

1. There are differences in gut microbiome pattern on young-onset colorectal cancer patients and colorectal cancer patients aged >50 years old compared to healthy individuals
2. There are differences in NF-κB on young-onset colorectal cancer patients and colorectal cancer patients aged >50 years old compared to healthy individuals
3. There are differences in CEA values on young-onset colorectal cancer patients and colorectal cancer patients aged >50 years old compared to healthy individuals

## Research Benefits

### For Researchers

Researchers will expand their knowledge on gut microbiome pattern in colorectal cancer patients.

### For Institutions

This study will provide a contribution of science in national and international scale. The result of this study could be used as a guideline on utilizing gut microbiome data on colorectal cancer management.

### For Communities

The result of this study is expected to improve the management of colorectal cancer as a screening method that would be applicable in daily clinical practice.

# METHODOLOGY

## Study Design

This is a cross-sectional study.

## Time and Place

This study is conducted in Dr. Cipto Mangunkusumo Hospital: Digestive Endoscopy Center, Department of Internal Medicine, Department of Clinical Pathology, Department of Anatomical Pathology, and Eijkman Institute of Molecular Biology from May 2019 to May 2022.

## Population

### Target Population

Patient suspected with colorectal cancer in Indonesia.

### Accessible Population

Patients suspected with colorectal cancer who come to Digestive Endoscopy Center in Dr. Cipto Mangunkusumo Hospital to conduct colonoscopy procedure and histopathological examination.

## Subject Criteria

### Inclusion Criteria

1. Age $\geqslant$ 35 years old
2. Suspected with colorectal cancer and undergoing a colonoscopy procedure
3. No history of colorectal cancer treatment

### Exclusion Criteria

1. Unwilling to provide fecal and blood sample
2. Incomplete colonoscopy procedure due to any reasons

## Estimated Sample Size

Since this is a pilot study to obtain gut microbiome pattern on colorectal cancer patients, the number of samples is determined by the researchers: 100 subjects for neoplasm and 50 subjects for non-neoplasm (according to histopathology report).

**Randomisation**

The sampling will not be randomized. All patients suspected with colorectal cancer who come to Digestive Endoscopy Center in Dr. Cipto Mangunkusumo Hospital to conduct colonoscopy procedure and histopathological examination who fulfilled the criteria will be recruited for this study and will undergo a series of examinations

## Operational Definition

| No. | Variable | Definition | Source | Scale |
|---|---|---|---|---|
| 1. | Patient suspected with colorectal cancer | Patient suspected with colorectal cancer by clinician based on clinical findings such as gastrointestinal bleeding, chronic constipation, chronic diarrhea, nonspecific abdominal pain, weight loss, palpable abdominal mass | Clinical judgement | Nominal |
| 2. | Age | Patient's age (in years) | Medical record | |
| 3. | Sex | Patient's sex | Medical record | Nominal |
| 4. | Family history of colorectal cancer in a first-degree relative | First-degree relative: parents, sibling, children | Medical record and history taking | Nominal<br>1= No<br>2= Yes |
| 5. | History of smoking | Past or current history of smoking | Medical record and history taking | Nominal<br>1= No<br>2= Yes |
| 4. | FIT results | The result of Fecal immunochemical test (FIT) | Fecal immuno-chemical test (FIT) | Nominal<br>1= Negative<br>2= Positive |
| 5. | Gut microbiome results | The result of gut microbiome examination | Next Generation Sequencing (NGS) | Nominal<br>1: Fusobacterium<br>2: Bacteroides<br>3. Lachnospiraceae |

| | | | | |
|---|---|---|---|---|
| 6. | NF-κB results | The results of NF-κB examination: positive if accumulated score ≥3 | Examination with immune-histochemical method | Nominal<br>1= Negative<br>2= Positive |
| 7. | CEA results | The results of Carcinoembryonic antigen (CEA) examination | Examination with ELISA method | Nominal<br>1= Negative<br>2= Positive |
| 7 | Histo-pathological results | The results of histopathological examination from biopsy samples are used as gold standard of colorectal cancer diagnosis | Medical record | Nominal<br>1 = Colorectal cancer<br>2 = Non-colorectal cancer |

**Ethical Approval**

This study will be obtained ethical approval from the Ethics Committee of Faculty of Medicine, University of Indonesia.

**Research Flow**

Patients suspected with colorectal cancer who come to Digestive Endoscopy Center in Dr. Cipto Mangunkusumo Hospital to conduct colonoscopy procedure and histopathological examination who fulfilled the criteria will be recruited for this study and will undergo a series of examinations Each patient will be given an explanation of this study and will be asked his/her willingness to participate on this study by signing the informed consent form. Baseline characteristics data will be obtained from medical record. Before the colonoscopy procedure is conducted, each patient will undergo a score assessment, blood sampling, and fecal sampling.

1. Score Assessment

   Asia Pacific Colorectal Screening (APCS) is a validated tool to predict the risk of colorectal cancer in asymptomatic Asian population. The scoring system comprises of three categories: low risk (score 0-1), moderate risk (score 2-3) and high risk (score 4-7). Patients with moderate and high risk will undergo further examinations

**Table.** Asia Pacific Colorectal Screening (APCS).

| Risk Factor | Criteria | Score |
|---|---|---|
| Age (in years) | < 50 | 0 |
| | 50 – 69 | 2 |
| | ≥ 70 | 3 |
| Sex | Female | 0 |
| | Male | 1 |
| Family history of colorectal cancer in a first-degree relative | Absent | 0 |
| | Present | 2 |
| History of smoking | Never | 0 |
| | Current or past | 1 |

2. Blood Sampling

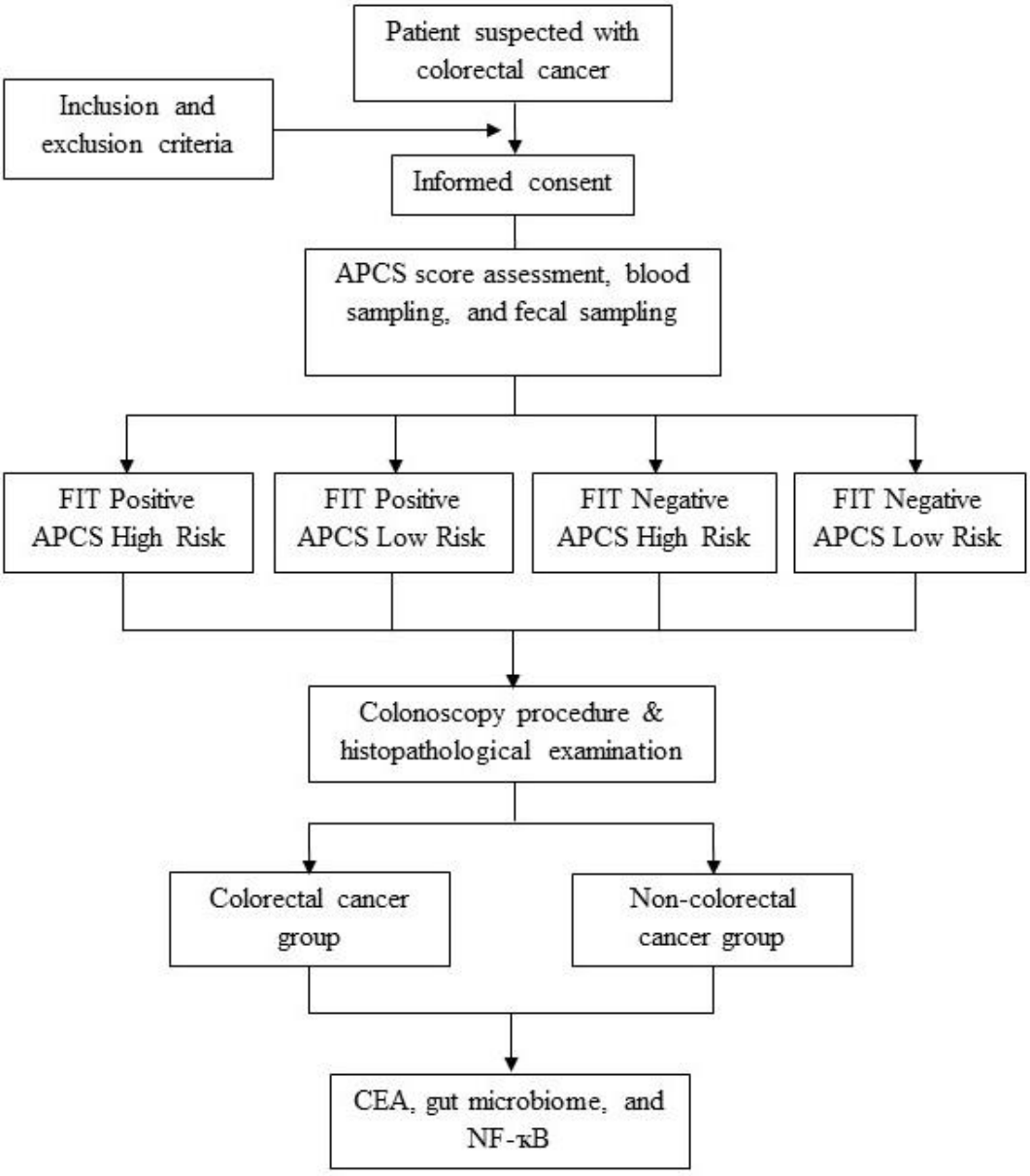
   Blood samples will be taken before colonoscopy procedure to evaluate the level of serum CEA by ELISA method and to evaluate the presence of NF-κB by immunohistochemical method.

   a. Carcinoembryonic antigen (CEA) is a well-known marker for colorectal cancer. A pre-treatment serum CEA level of ⩾ 5 ng/mL is associated with poor prognosis in colorectal cancer patients.
   b. NF-κB is a chronic inflammation marker found in colorectal cancer patients

3. Fecal Sampling

   Fecal samples will be taken before colonoscopy procedure to be tested for FIT and to evaluate the gut microbiome.

   a. Fecal immunochemical test (FIT) is a recommended screening method for colorectal cancer. Detection of hemoglobin over a certain level in fecal samples indicated a positive FIT. Patients with positive FIT will undergo further examinations.
   b. Gut microbiome examination will be conducted with next generation sequencing (NGS) method.




Blood samples and fecal samples will be tested in Department of Clinical Pathology Dr. Cipto Mangunkusumo Hospital, while gut microbiome will be evaluated in Eijkman Institute of Molecular Biology. Colonoscopy procedure will be conducted in Digestive Endoscopy Center Dr. Cipto Mangunkusumo Hospital. Histopathological examination be conducted in Department of Anatomical Pathology Dr. Cipto Mangunkusumo Hospital. The results of colonoscopy procedures and histopathology report will be obtained from medical record.

Gut microbiome examination from fecal samples will be initiated by DNA isolation. After extraction material is obtained, PCR will be performed with 16S RNA Sequencing. Metagenomic study will be carried out by analysing prokaryotic 16S ribosomal RNA gene (16S rRNA) which has a length of 1500 base pairs and consists of 9 variable regions that are between conserved regions. Variable regions that are generally used for phylogenetic analysis are V3 and V4 for classification to the level of genus or species. To obtain gut microbiome pattern, Next-Generation Sequencing (NGS) examination will be conducted.

# TIMELINE

First Year

| No | Activities | Month | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| 1 | Obtaining ethical approval | X | X | | | | | | | | | | |
| 2 | Obtaining location permit | | X | X | | | | | | | | | |
| 3 | Sampling | | | | X | X | X | X | X | X | X | X | X |
| 4 | FIT, CEA, NF-kB, gut microbiome examinations | | | | X | X | X | X | X | X | X | X | X |

Second Year

| No | Activities | Month | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| 1 | Sampling | X | X | X | | | | | | | | | |
| 2 | FIT, CEA, NF-kB, gut microbiome examinations | X | X | X | | | | | | | | | |
| 3 | Data analysis and report writing | | | | X | X | X | X | X | X | X | X | X |

Third Year

| No | Activities | Month | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| 1 | Data analysis and report writing | X | X | X | | | | | | | | | |
| 2 | Scientific publication | | X | X | X | X | X | X | X | X | X | X | X |

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