THE USE OF TUMOUR M2-PYRUVATE KINASE AS A STOOL BIOMARKER FOR DETECTION OF COLORECTAL CANCER IN HOSPITAL UNIVERSITI SAINS MALAYSIA

BY

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(Surgery)

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II. ABSTRAK

Pengenalan

Ujian tinja (najis) telah digunakan sebagai ujian saringan untuk mengenalpasti kanser kolorektal. Ujian ‘guaiac FOBT’ adalah terbatas disebabkan oleh kadar sensitiviti dan spesifisiti yang rendah. Kehadiran ‘tumour M2-PK’ di dalam tinja pesakit-pesakit yang menghidap kanser kolorektal dan ‘polyp’ kolorektal sedang diselidik sebagai potensi menjadikannya ujian saringan kanser kolorektal.

Objektif

Untuk mengkaji penggunaan ujian tinja ‘tumour M2-pyruvate kinase’ dalam mengenalpasti kanser kolorektal dan membandingkannya dengan ujian saringan tinja yang digunakan pada masa kini iaitu ujian ‘guaiac FOBT’.

Kaedah

Pesakit-pesakit simptomatik yang dirujuk untuk pemeriksaan kolonoskopi elektif di HUSM memberikan sampel tinja untuk dianalisa berkenaan kehadiran ‘tumour M2-PK’ dan kehadiran darah di dalam tinja tersebut. Untuk ujian M2-PK, sampel tinja tersebut diperiksa menggunakan ujian komersial yang tersedia, ‘Schebo M2-PK Quick’. Untuk ujian ‘gFOBT’, sampel tinja yang sama dihantar ke makmal HUSM untuk dianalisa berkenaan kehadiran darah di dalamnya. Tiada perbatasan makanan dilakukan bagi kedua-dua ujian. Keputusan kedua-dua ujian dibandingkan dengan keputusan kolonoskopi (atau/dan dengan keputusan histopatologi).

Keputusan

Seramai 85 subjek (purata umur ± sisihan piawai, 56.8 ± 15.3 tahun) direkrut. 17 pesakit kanser kolorektal dan 10 pesakit ‘adenoma’ kolon dikesan. Sensitiviti ujian M2-PK terhadap pengesanan kanser kolorektal adalah lebih tinggi berbanding ujian gFOBT (100.0% vs 64.7%). Walau bagaimanapun, ujian M2-PK mempunyai spesifisiti yang lebih rendah apabila dibandingkan dengan ujian gFOBT (72.5% vs 88.2%). ‘Positive predictive value’ dan ‘negative predictive value’ bagi ujian M2-PK masing-masing adalah 47.2% dan 100.0%, manakala bagi ujian gFOBT masing-masing adalah 57.9% and 90.9%. Kedua-dua ujian M2-PK dan ujian gFOBT mempunyai sensitiviti yang rendah dalam mengenalpasti kehadiran ‘adenoma’ kolon (20.0% vs 30.0%). Spesifisiti ujian M2-PK adalah 54.7%, iaitu lebih rendah berbanding ujian gFOBT yang mempunyai spesifisiti 78.7%.

Kesimpulan

Ujian M2-PK Quick mempunyai sensitiviti yang sangat tinggi sebagai penanda tinja bagi pengesanan kanser kolorektal apabila dibandingkan dengan ujian gFOBT. Ianya cepat dan mudah dilakukan di peringkat klinikal, maka ianya sesuai dijadikan sebagai ujian awal saringan untuk kanser kolorektal. Bagi mereka yang mempunyai ujian positif, mereka perlu menjalani ujian penilaian yang seterusnya. Walau bagaimanapun, peranannya dalam pengesanan ‘adenoma’ kolorektal adalah sangat terbatas.

III. ABSTRACT

Introduction

Stool tests have been used in colorectal cancer screening. Guaiac FOBT is limited by its low sensitivity and specificity. The determination of tumour M2-PK in stool of patients with colorectal cancer and colorectal polyp has been investigated as a potential stool test for colorectal cancer screening.

Objectives

To evaluate the use of faecal tumour M2-pyruvate kinase test in detection of colorectal cancer and to compare it with the current surveillance tool, guaiac faecal occult blood test.

Methodology

Symptomatic subjects who were referred for elective colonoscopy in HUSM provided stool samples for analysis of tumour M2-PK and occult blood. For M2-PK test, the stool sample was tested using a commercially available rapid test, ScheBo M2-PK Quick. For gFOBT, the stool sample was sent to HUSM's laboratory following usual practice to detect faecal hemoglobin. No dietary restrictions were applied for both tests. The results of both tests were compared with colonoscopic result (and/ or with histopathology report).

Results

85 subjects (mean age ± SD, 56.8 ± 15.3 years) were enrolled. A total of 17 (20.0%) colorectal cancer and 10 (11.8%) colorectal adenoma were detected. The sensitivity of M2-PK test towards detection of colorectal cancer was higher than gFOBT (100.0% vs 64.7%). However, M2-PK test had a lower specificity when compared with gFOBT (72.5% vs 88.2%). The positive predictive value and negative predictive value of faecal M2-PK test were 47.2% and 100.0% respectively, whereas for guaiac FOBT were 57.9% and 90.9% respectively. Both M2-PK test and gFOBT had low sensitivity towards detection of colorectal adenoma (20.0% vs 30.0%). The specificity of M2-PK test was 54.7%, which was lower than gFOBT which had higher specificity of 78.7%.

Conclusion

Faecal M2-PK Quick test has a high sensitivity for detection of colorectal cancer as a stool biomarker when compared with gFOBT. It is fast and easy to be performed in clinical settings, thus suitable for initial screening tool for colorectal cancer. Those with positive result should undergo further complete diagnostic evaluation. However, its role for detection of colorectal adenoma is very limited.

1 – INTRODUCTION

1.1 LITERATURE REVIEW

M2 Pyruvate Kinase and Cancer Metabolism

In contrast to normal proliferating cells, tumour cells consume more glucose and generate high amounts of lactate to produce energy. Tumour cells exhibit a high rate of glycolysis in generating adenosine triphosphate (ATP), rather than using oxidative phosphorylation, even when abundant of oxygen is presence. This phenomenon is known as aerobic glycolysis or Warburg effect, which provides energy and metabolic intermediates to the tumour cells for the synthesis of amino acids, nucleic acids, lipids and other macromolecules to facilitate growth and survival of tumour cell. Cancer cells are shown to express pyruvate kinase type M2, and play a crucial role in the Warburg effect (Yang *et al.*, 2014).

Pyruvate kinase is an enzyme that catalyzes the final step of glycolysis by conversion of phosphoenolpyruvate and adenosine diphosphate (ADP) to pyruvate and ATP, and production of lactate. Unlike mitochondrial respiration, production of energy by pyruvate kinase is independent of oxygen and allows survival of cells under condition of insufficient oxygen, which is an important metabolic ability in tumour cells (Mazurek, 2008).

Tumour cells have to survive in unfavorable atmospheres with lack of oxygen and nutrient. The expression of M2- pyruvate kinase (M2-PK) allow anaerobic metabolism to maintain metabolic activities and ATP production in the deficient-oxygen environment, allowing the tumour cells to proliferate, grow and survive. High lactate production by aerobic glycolysis has been found to increase metastasis of tumour cells and angiogenesis by stimulating cytokines, such as vascular endothelial growth factor (VEGF) and interleukin-8

(IL-8). Tumour cells also produce bicarbonic and lactic acids, which favor acidic environment that enhance tumour metastasis (Zhou *et al.*, 2012).

The Isoforms of Pyruvate Kinase

There are 4 types of pyruvate kinase isoforms (L, R, M1 and M2) that are expressed in different cells and tissues depend on its metabolic activities. Pyruvate kinase type L is responsible in gluconeogenesis and thus, found in liver and kidney. Pyruvate kinase type R is dominant in erythrocytes. M1 isoenzyme is present in tissues that require large amounts of ATP such as in skeletal muscle and brain, whereas M2 is mainly expressed in lung tissues and cells with high rates of nucleogenesis such as adult stem cells, embryonic cells and especially tumour cells (Mazurek *et al.*, 2005).

M2-PK exists in 2 oligomeric forms. The tetrameric form of M2-PK is present in lung and normal proliferating cells, while dimeric form is predominate in tumour cells, and have been termed tumour M2-PK (Hardt and Ewald, 2008; Mazurek *et al.*, 2005).

Proliferating cells express pyruvate kinase type M2 and is progressively replaced by other isoforms during tissue differentiation. However, during tumourigenesis the tissue specific isoenzymes, such as L-PK in liver, disappear and M2-PK is expressed (Iqbal *et al.*, 2014).

Tumour M2-PK: a Tool for Early Detection of Tumour

Tumour M2-PK is released by tumour cells into the blood and also into the stool of patients with gastrointestinal tumour, most probably due to tumour necrosis and cell turnover, providing the opportunity of diagnostic approaches (Mazurek, 2008). Higher amount of tumour M2-PK is detected in the EDTA plasma of patients with renal cell carcinoma, melanoma, esophageal, lung, pancreatic, cervical, ovarian, breast, gastric, and colorectal cancer. The presence of tumour M2-PK also has been detected in stool of patients with

colorectal and gastric cancer, and correlate with colorectal cancer stages (Mazurek, 2008). The presence of M2-PK in plasma and stool has been used to monitor failure, success or recurrence of disease during therapy (Mazurek, 2012).

Tumour M2-PK can be measured and quantified in blood by using enzyme-linked immunosorbent assay (ELISA) technique. The test is based on 2 monoclonal antibodies that react specifically with tumour M2-PK and do not cross-react with other pyruvate kinase isoenzymes (type R, L and M1). A reference value of 15U/ml in EDTA plasma correspond to a sensitivity of 57.3%, specificity of 89%, positive predictive value of 85.7% and negative predictive value of 64.8% for colorectal cancers. For gastric/ esophageal cancers, the sensitivity was 62.1%, specificity was 89%, positive predictive value was 88% and negative predictive value was 64%, whereas for pancreatic cancers, , the sensitivity was 72.5%, specificity was 89%, positive predictive value was 58% and negative predictive value was 94% (Kumar *et al.*, 2007).

Tumour M2-PK can be detected in faeces by a similar ELISA technique used in plasma assay. A reference value of 4 U/ml corresponds to a sensitivity of 73-92% and specificity of 83% (Kumar *et al.*, 2007).

M2-PK in Stool: a Marker for Colorectal Cancer Screening

M2-PK is overexpressed in colon cancer cells compared to normal cells. Overexpression of M2-PK is associated with later tumour stage and more lymph node metastasis in colorectal cancer patients, indicating that M2-PK is involved in tumour growth and metastasis. M2-PK is also found in healthy colon but at a lower level, suggesting that M2-PK is unspecific to cancer cells (Zhou *et al.*, 2012).

Tumour M2-PK can be measured in faeces of GI malignancy patients, mainly in colorectal cancer by using enzyme-linked immunosorbent assay (ELISA) technique,

favouring a possible colorectal cancer tumour marker. Furthermore, the M2-PK test can detect bleeding or non-bleeding GI cancer and polyps (Tonus *et al.*, 2012).

There are several studies concerning the use of tumour M2-PK in colorectal cancer screening.

Hardt et al conducted a study on 206 patients to determine the use of faecal tumour M2-PK as a screening tool for colorectal tumours. They revealed a significantly higher level of faecal tumour M2-PK in patients with colorectal cancer than in the control group without colorectal cancer. Overall sensitivity is 73% and specificity is 78%. There was a strong correlation between the levels of faecal tumour M2-PK and staging. The sensitivities increased from 57% and 59% in the case of T1 and Dukes' A, respectively, to 78% in the case of T4 and 90% in the case of Dukes' D (Hardt *et al.*, 2004).

Abdullah et al recruited 328 patients with high-risk and symptomatic group to evaluate the performance of faecal tumour M2-PK as a diagnostic biomarker for colorectal cancer screening. The sensitivity and specificity of the faecal tumour M2-PK test towards colorectal cancer were 71.4% and 71.0% respectively. However, it has a low sensitivity for detecting adenomas (Abdullah *et al.*, 2012).

In a different study, Haug et al performed a comparative analysis to estimate the potential of faecal tumour M2-PK in discriminating patients with colorectal cancer from unselected older adults aged 50-75 years. The study recruited 65 colorectal cancer patient and 917 unselected older adults. The results showed sensitivity of 85% for colon cancer and 56% for rectal cancer. The specificity for detecting colorectal cancer was estimated to be 79% (Haug *et al.*, 2007).

Koss et al recruited 55 patients to assess faecal tumour M2-PK levels along the adenoma-carcinoma sequence and to evaluate its levels in different colorectal cancer stages. The study reported sensitivity of 20% for polyps smaller than 10mm and 60% for polyps

greater than 10mm with specificity of 92%. The sensitivity for colorectal cancer was 91% and specificity was 92%. There was no association found between tumour size and faecal tumour M2-PK level. The faecal tumour M2-PK level was noted to be higher in patients with colorectal cancer stages Duke's B and C than Duke's A. The study also observed significant reduction in faecal tumour M2-PK levels at 6 months following successful surgical intervention (Koss *et al.*, 2007).

Haug et al recruited 1082 average-risk subjects aged 55 years or older for screening colonoscopy to investigate the potential of the faecal tumour M2-PK to detect colorectal adenoma. The study reported a low sensitivity of 22% for advanced adenoma and 23% for other adenoma, whereas specificity was 82%. They concluded that the tumour M2-PK has a very limited role in detection of colorectal adenoma (Haug *et al.*, 2008).

A similar result reported by Ewald et al, they found that the overall sensitivity of adenoma was 45.9%, increasing to 61.1% for adenomas larger than 1 cm (Ewald *et al.*, 2007).

A meta-analysis performed by Tonus et al reported a relatively good performance of faecal tumour M2-PK in colorectal cancer screening with a mean sensitivity and specificity of 80.3% and 95.2% respectively. For adenoma, the sensitivity were 25% for adenoma <1cm in diameter, 44% for adenoma >1cm and 51% for adenoma of unspecified diameter. The author recommended the faecal tumour M2-PK as a routine test for colorectal cancer screening as faecal M2-PK is able to detect bleeding and non-bleeding tumours and adenoma with high sensitivity and specificity (Tonus *et al.*, 2012).

Recently, Sithambaram et al performed a validation study on the use of a rapid, point of care stool test M2-PK for the detection of colorectal cancer. The faecal M2-PK was tested in 100 colorectal cancer patients and 200 subjects with normal colonoscopy. The performance of M2-PK Quick revealed sensitivity of 93%, specificity of 97.5% and

diagnostic accuracy of 96%. The test was found to be highly accurate regardless of the tumour stages and location (Sithambaram *et al.*, 2015).

1.2 RATIONALE FOR THE STUDY

A highly sensitive and specific yet non-invasive and acceptable screening tool for colorectal cancer is of major importance for lowering the incidence of colorectal cancer. The aim of this study is to evaluate the potential use of faecal tumour M2-PK to detect colorectal cancer and compare it with the current surveillance tool, guaiac FOBT.

2 – STUDY PROTOCOL

2.1 DOCUMENT SUBMITTED FOR ETHICAL APPROVAL

PROPOSED PROJECT OF STUDY

FOR

MASTER OF MEDICINE (GENERAL SURGERY)

THE USE OF TUMOUR M2 PYRUVATE KINASE AS A STOOL BIOMARKER FOR DETECTION OF COLORECTAL CANCER IN HOSPITAL UNIVERSITI SAINS MALAYSIA

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A. Project Identification

Title of Study:

The Use of Tumour M2 Pyruvate Kinase as a Stool Biomarker for Detection of Colorectal Cancer in Hospital Universiti Sains Malaysia.

B. Introduction

According to World Health Organisation's GLOBOCAN 2012, estimated about 37,400 persons were diagnosed with new cancer in Malaysia and colorectal cancer (CRC) is the second most common cancer in men (14.1%) and the third in women (10.2%)[1]. Approximately, 10.6% patients died due to CRC that year, which makes it the third most common death from cancer (after lung cancer with approximately 19.1% death and breast cancer 11.9% in 2012)[1]. The cumulative lifetime risk of developing colon cancer in males was 1:38 and 1:50 for females[2]. The incidence and mortality of CRC is rising rapidly in Asia[3], with majority of patients are above 50 years old[4]. Many Asian countries, including China, Japan, South Korea, and Singapore, have an increase of two to four times in the incidence of CRC during the past few decades[5], despite availability of screening methods.

Most CRC can be prevented by detection of early-stage adenocarcinoma, and detection and removal of adenomatous polyps, and survival is significantly better when CRC is diagnosed while still localized[6]. Incidence and mortality due to CRC can be effectively reduced with early diagnosis, if regular screening is done[6]. However, a study by Harmy et al, 2012 showed that participation in CRC screening among average risk in Malaysia is extremely low mainly due to embarrassment, uncomfortable and some patients mentioned never being advised to do screening[7].

Current gold standard screening tool for early detection of CRC is colonoscopy, which has the most sensitivity and specificity. It allows direct mucosal inspection of the

entire colon and provides the opportunity to perform an excision for an adenoma and to take a histological sample from a suspected neoplasia[6]. However, most patients refuse to participate because of its invasiveness. Non-invasive faecal test are much more acceptable as they are less invasive and less uncomfortable[7,8]. Currently available stool tests for CRC screening are faecal occult blood test (FOBT), genetic stool test and M2-pyruvate kinase (M2-PK) stool test. The most widely used is FOBT[9]. According to Malaysian clinical practice guidelines on CRC screening 2001, the best approach to screen for CRC is by using FOBT as an annual screening to those at average risk. However, FOBT is limited by its low sensitivity towards colorectal polyps and frequent false-positive results[10]. Stool DNA test is not recommended by the Asia Pacific Consensus on CRC as this test is still in experimental phase and is not ready to be used as a routine screening test[11].

Pyruvate kinase is an enzyme that involved in glycolysis by catalysing the ATP regenerating dephosphorylation of phosphoenolpyruvate to pyruvate[12]. Type M2-PK is a special isoenzyme of pyruvate kinase which is expressed by proliferating cells[12,13]. The dimeric form, termed tumour M2-PK is predominantly overexpressed in tumour cells[12,14,15]. M2-PK is essential in tumour growth of CRC by regulating cell proliferation and migration of colon cancer cells[13]. Greater amount of this isomer is found at later tumour stage[13,16] and associated with lymph metastasis in CRC patients[13]. Tumour M2-PK has also been found in the stool of patients with CRC[9] and colorectal polyps[12]. The amount of M2-PK in stool can be quantified by ELISA[12] and thus faecal M2PK has been proposed as a tool for detecting CRC[17,18,19].

C. Justification

A highly sensitive and specific yet non-invasive and acceptable screening tool for CRC is of major importance for lowering the incidence of CRC. This study is aimed to evaluate the potential of the faecal tumour M2-PK test to detect CRC and compare it with guaiac-based FOBT.

D. Objectives of Study

General objective

- To evaluate the use of faecal M2-pyruvate kinase test in detection of colorectal cancer.

Specific objectives

- To determine the sensitivity specificity, positive predictive value and negative predictive value of faecal M2-pyruvate kinase test towards detection of colorectal cancer.
- To compare the diagnostic accuracy of faecal M2-pyruvate kinase with current surveillance tool, faecal occult blood test (FOBT) in detecting colorectal cancer.

E. Research Question

Is faecal M2-PK test is a more accurate test compare to guaiac-based FOBT for the detection of colorectal cancer?

F. Hypothesis

Faecal M2-PK test has high sensitivity and specificity in detecting colorectal cancer.

Faecal occult blood test has low sensitivity and specificity in detecting colorectal cancer.

G. Null Hypothesis

Faecal M2-PK test has lower sensitivity and specificity compare to guaiac-based FOBT in detecting colorectal cancer.

H. Research Methodology

Study design

This is a cross-sectional study which would be carried out in Hospital Universiti Sains Malaysia after the approval by the university Ethics Committee.

Study population

All adult patients who referred for elective colonoscopy for various indication at Hospital Universiti Sains Malaysia.

Patient selection

- The subjects of the study will be consented, explained the aim and the risks of the study.
- Adult patients undergoing colonoscopy for various indications like CRC screening, investigation of colonic symptoms, high risk for CRC, family history of CR neoplasia, and clinical suspicion of CRC will be considered for enrolment into the study.
- Exclusion criteria consist of the following: patient who presents as emergency cases (eg: obstruction), past history of colectomy, patient known to have CRC and came for surveillance colonoscopy, underwent chemotherapy for CRC.

Patients' recruitment

Patients attending and/or referred to surgical out-patient clinics at Hospital Universiti Sains Malaysia and planned for elective colonoscopy.

Sample size calculations

From the literature review, a study by Hajhamad et al reported the sensitivity of a positive faecal tumour M2-PK test for colorectal cancer are 100%[30]. Sithambaram et al, reported a specificity of 97.5%[29]. Based on this, sample size calculated:

Sample size (n) based on sensitivity $n = \frac{Z_{\alpha/2}^2 \, S_N \, (1-S_N)}{L^2 \, P}$

S_N = Sensitivity

L = Absolute precision

P = Prevalence

S_P = Specificity

Sample size (n) based on specificity $n = \frac{Z_{\alpha/2}^2 \, S_P \, (1-S_P)}{L^2 \, (1-P)}$

Alpha	=	5%	n for sensitivity	=	84.7
$Z_{\alpha/2}$	=	1.95	n for specificity	=	8.7
Precision	=	5%			
Prevalence	=	13%	Final sample size	=	84.7
Sensitivity	=	100%	Anticipate dropout	=	10%
Specificity	=	98%	Final sample size	=	95

Measurement Tool

- Patients' demographic will be collected using standard data form.
- The subjects who have consented to participate in the study will be given a clean plastic container and a plastic spoon for stool sample collection.
- They have to collect 2 stool samples before bowel preparation for colonoscopy as they present for scope day.
- The stool received will be analysed within 2 days after sampling, as recommended by the manufacturer.
- For FOBT, the stool sample will be sent to laboratory following usual practice to detect haemoglobin in the faeces.
- For M2-PK test, random stool sample will be collected according to the manufacturer's instructions and the faecal tumour M2-PK will be detected using a commercially available rapid test, ScheBo M2-PK Quick (ScheBo® Biotech AG, Giessen, Germany) based on monoclonal antibodies against dimeric M2-PK.
- Another stool sample will be send to HUSM's laboratory for faecal occult blood testing.
- No dietary restrictions are required prior to stool collections.
- Colonoscopy will be performed by experienced endoscopists according to the current practice at Endoscopy Unit, HUSM, with visualization of the entire colon up to caecum using a standard colonoscope after routine bowel preparation.
- Patients with incomplete colonoscopies will be proceed with CT colonography or barium enema or repeat colonoscopy to complete colonic examination.
- After colonoscopy is performed, reports on colonoscopy will be collected and information will be extracted in a standardised manner.

- Any found lesions during colonoscopy (or repeat colonoscopy) will be biopsied and the tissue biopsy will be studied by Pathology Department at HUSM according to current practice and histological reports will be collected and information will be extracted in a standardised manner.
- The results of M2-PK test and FOBT will be labelled as positive or negative and both will be analysed and compared to the colonoscopic findings with or without histopathology examination.

Statistical analysis

Data will be analysed using Statistical Package for the Social Sciences (SPSS) version 22. Descriptive analysis will be done for patients' demographic on age, gender and ethnicity to give an overall picture of epidemiology trend of this study. Cross tabulation will be done to determine the true positive, true negative, false positive and false negative results to calculate the sensitivity and specificity values of faecal M2-PK test. Calculation of sensitivity and specificity values are as below:

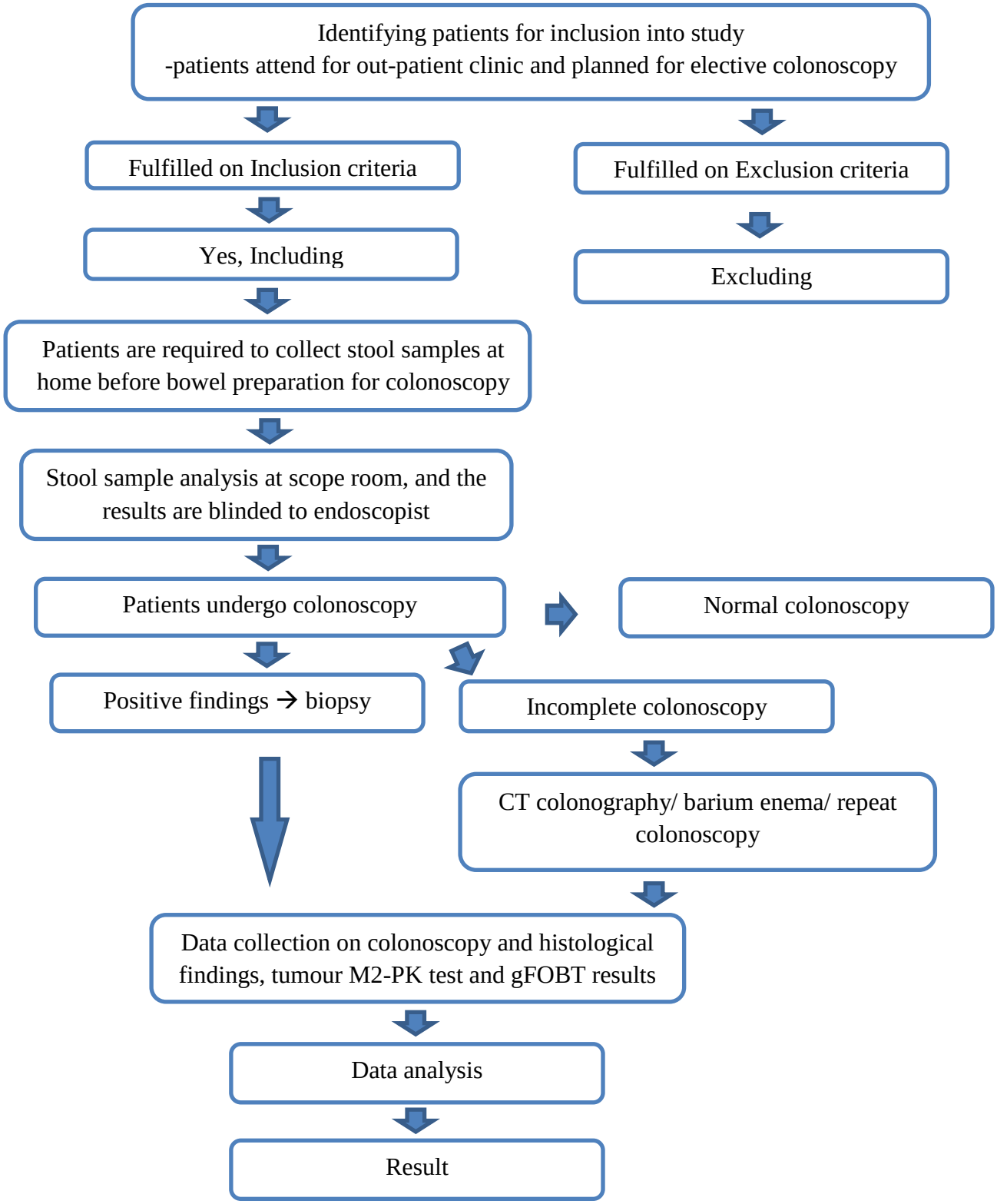
Sensitivity = TP / (TP + FN)

TP = true positive data, FN = false negative data

Specificity = TN / (TN + FP)

TN = true negative data, FP = false positive data

Figure 1: Methodology Flowchart



I. Expected Results

Table 1 – Patients' Demographics

Patients' demographics	**No.**	**(%)**
Frequency		
Age (years)		
Gender Male Female		
Ethnicity Malay Chinese Indian Others		

Table 2 – Result of faecal M2PK test in relation to colonoscopy findings

Colonoscopy findings	**Total**	**M2PK test positive**		**M2PK test negative**	
	No.	**No.**	**(%)**	**No.**	**(%)**
Colorectal cancer					
Colorectal adenoma					
Normal or non-neoplastic lesion					

Table 3 – Result of FOBT test in relation to colonoscopy findings

Colonoscopy findings	**Total**	**FOBT positive**		**FOBT negative**	
	No.	**No.**	**(%)**	**No.**	**(%)**
Colorectal cancer					
Colorectal adenoma					
Normal or non-neoplastic lesion					

Table 4 – Performance characteristics of faecal tumour M2PK and FOBT in different diagnostic groups

Parameter	Tumour M2PK	gFOBT
Sensitivity (%) Colorectal cancer Colorectal adenoma		
Specificity (%) Colorectal cancer Colorectal adenoma		
Positive predictive value (%) Colorectal cancer Colorectal adenoma		
Negative predictive value (%) Colorectal cancer Colorectal adenoma		
Positive likelihood ratio (%) Colorectal cancer Colorectal adenoma		
Negative likelihood ratio (%) Colorectal cancer Colorectal adenoma		

J. Sample Data Collection Form

PROFORMA

A prospective study of faecal M2-PK in detecting CRC in HUSM

Study ID: Age:

Gender: male ( ), female ( ) Race: Malay ( ), Chinese ( ), India ( ), Others ( )

Indications for colonoscopy:

( ) gastrointestinal bleeding

( ) abdominal pain

( ) change in bowel habits

( ) CRC screening

( ) abdominal mass

Faecal M2-PK test: positive ( ), negative ( ) gFOBT: positive ( ), negative ( )

Colonoscopy results: (complete/ incomplete)

carcinoma ( ), adenoma ( ), colitis ( ), others pathology ( ), normal ( )

CT colonography/ Barium enema/ Repeat colonoscopy result:

K. Gantt Chart

Month	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**
Year	**2014**							**2015**												**2016**				
Data collection	X	X	X	X	X	X	X	X	X	X	X	X												
Data analysis								X	X	X	X	X	X	X										
Report writing													X	X	X	X	X	X						
Submission of paper																			X	X	X			
Publication of research																						X	X	X

Milestones of Research Activities:

1. Completion of data collection : May 2015
2. Completion of data analysis : July 2015
3. Completion of report writing : November 2015
4. Completion of paper submission : February 2016
5. Completion of research publication : May 2016