Diagnostic Value of Serum and Tissue Eosinophil in Diagnosis of Asthma Among Patients with Chronic Rhinosinusitis

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Dissertation Submitted in Partial Fulfilment of the Requirements of the Degree of Master of Medicine (Otorhinolaryngology – Head and Neck Surgery)

SCHOOL OF MEDICAL SCIENCES
UNIVERSITI SAINS MALAYSIA
2022

ACKNOWLEDGEMENT

Bismillahirrahmanirrahim

Alhamdulillah, praise to Allah, The Almighty, and The Most Beneficent.

First and foremost, I would like to show my sincere gratitude to my supervisor Dr. Norasnieda Md Shukri for her effort in guiding me to complete my study as scheduled. Special appreciation also goes to my co-supervisors Prof. Dr. Baharudin Abdullah, senior consultant in Department of Otorhinolaryngology-Head and Neck Surgery USM, Dr. Sakinah Mohamad, a specialist in Department of Otorhinolaryngology-Head and Neck Surgery USM, Assoc. Prof. Dr. Sharifah Emilia Tuan Sharif senior consultant in the Department of Pathology USM, Dr. Rosdi Ramli senior consultant in Department of Otorhinolaryngology -Head and Neck Surgery HRPZ II, Dr. Mat Zuki Mat Jaeb senior consultant in Department of Internal Medicine HRPZ II, Dr. Murni Hartini Jais specialist in Department of Pathology HRPZ II, Assoc. Prof. Dr. Najib Majdi Yaacob medical lecturer in Department of Biostatistics and Research Methodology USM and Dr. Mohd Yusran Yusoff medical officer in Department of Clinical Research Centre HRPZ II for their advice and countless support in assisting me while getting my research done.

My gratitude also goes to all lecturers, my colleagues well as the ORL-HNS clinic staff for their cooperation, assistance, and friendly support in numerous ways in the course of my study.

I would like to dedicate my work to my parents Che Mat Derasid and Masdiyah Jemain, my parent in law; Ahamad Shukri Daud and Nik Fauziah Tusa'adah who have devoted their lives to ensure that we get the best of everything.

Last but not least, I thank my beautiful wife Dr. Nik Aina Ilyani; a dentist who has continuously inspired and supported me throughout my career, and our precious boy Che Umar Harraz.

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ABSTRACT (ENGLISH)

Background: Chronic rhinosinusitis (CRS) is one of the most common chronic inflammatory diseases of sinonasal mucosa. Asthma among CRS patients is often underdiagnosed and causes management of CRS more challenging. Therefore, using serum and tissue eosinophil as an indicator and prediction of asthma in CRS patients are valuable for further preventing recurrent and increase the effectiveness of treatment for CRS.

Objective: To determine the association and diagnostic ability of serum and tissue eosinophils in the diagnosis of asthma among CRS patients.

Method: A cross-sectional study was conducted involving 24 CRS patients with and without asthma respectively from the Otorhinolaryngology clinic of two tertiary hospitals located in the East Coast of Peninsular Malaysia. Serum and tissue eosinophils (obtained from nasal polyp) between both groups were compared. Association between serum and tissue eosinophils with asthma evaluated using logistic regression analysis, adjusting for important sociodemographic characteristics. The diagnostic ability of serum and tissue eosinophil was then evaluated by assessing the receiver operating characteristic (ROC) curve.

Results: A total of 48 CRS patients with a mean [SD] age of 47.50 [14.99] years were included. Patients with asthma had significantly higher serum [0.48 vs 0.35 x109/L] and tissue eosinophil [100 vs 8.5 per HPF]. Tissue eosinophils were found to be an independent predictor of asthma with adjusted OR=1.05, $p<0.001$, after adjusting for age and serum eosinophils. The area under the ROC curve for serum eosinophil was 69.0%. At optimal cut-off value (0.375 x109/L), the sensitivity and specificity for serum eosinophil was 75.0% and 70.8%. The area under the ROC curve for tissue eosinophil was 93.4%. At optimal cut-off value (58.0 per HPF), the sensitivity and specificity for tissue eosinophil were 79.2% and 91.7% respectively.

Conclusion: A significantly higher level of serum and tissue eosinophil are seen in CRS with asthma. However, there was no correlation between serum and tissue eosinophil in both groups. The CRS patient needs to be screened for asthma if the level of serum eosinophil is more than 0.375 x 10^9/L and tissue eosinophil more than 58 per HPF.

Keywords: Asthma, Chronic, Rhinosinusitis, Serum Eosinophil, Tissue Eosinophil, Nasal Polyp, Endoscopy, Surgery, Paranasal Sinus, Inflammatory.

ABSTRAK (BAHASA MALAYSIA)

Latar Belakang: Rhinosinusitis kronik (CRS) adalah salah satu penyakit keradangan kronik yang paling utama pada mukosa rongga hidung and muka. Pesakit CRS sering kali tidak mendapat perhatian untuk asma dan menyebabkan pengurusan CRS lebih mencabar. Oleh itu, dengan menggunakan serum and tisu eosinofil sebagai tahap ukur dan meramalkan pesakit CRS mempunyai asma ataupun tidak untuk menjadikan perubatan menjadi lebih berkesan .

Objektif: Untuk menentukan hubungan dan kemampuan serum dan tisu eosinofil dalam mengenal pasti penyakit asma dalam kalangan pesakit CRS.

Kaedah: Satu kajian keratan rentas dilakukan yang melibatkan pesakit CRS dengan asma dan tanpa asma masing-masing dari klinik telinga, hidung dan tekak di dua hospital utama yang terletak di Pantai Timur Semenanjung Malaysia. Eosinofil serum dan tisu (diperoleh dari polip hidung) antara kedua-dua kumpulan dibandingkan. Hubungan antara eosinofil serum dan tisu dengan asma dinilai menggunakan analisis regresi logistic dan dipadankan dengan ciri sosiodemografi tertentu. Keupayaan diagnostik serum dan tisu eosinofil kemudian dinilai dengan menilai keluk ciri operasi penerima (ROC).

Keputusan: Sebanyak 48 pesakit CRS dengan usia purata [SD] 47.50 [14.99] tahun. Pesakit dengan asma mempunyai serum yang lebih tinggi [0.48 vs 0.35 x109 / L] dan eosinofil tisu [100 vs 8.5 per HPF]. Tisu eosinofil didapati sebagai peramal bebas asma dengan OR = 1.05, p <0.001 yang disesuaikan, setelah disesuaikan dengan usia dan eosinofil serum. Kawasan di bawah keluk ROC untuk eosinofil serum adalah 69.0%. Pada nilai pemotongan optimum (0.375 x109 / L), kepekaan dan kekhususan untuk eosinofil serum adalah 75.0% dan 70.8%. Kawasan di bawah keluk ROC untuk eosinofil tisu adalah 93.4%. Pada nilai pemotongan optimum (58.0 per HPF), kepekaan dan kekhususan untuk eosinofil tisu masing-masing adalah 79.2% dan 91.7%.

Kesimpulan: Kajian ini menunjukkan tahap eosinofil serum dan tisu yang lebih tinggi dalam CRS dengan asma. Walau bagaimanapun, tidak ada hubungan antara serum dan eosinofil tisu pada kedua-dua kumpulan. Berdasarkan kajian ini, pesakit CRS perlu menjalani pemeriksaan asma sekiranya tahap eosinofil serum melebihi 0.375 x 109 / L dan eosinofil tisu melebihi 58 per HPF.

Kata kunci: Asma, Kronik, Rhinosinusitis, Darah Eosinofil, Tisu Eosinofil, Hidung Polip, Endoscopy, Pembedahan, Sinus Paranasal, Jangkitan.

Chapter 1:

INTRODUCTION

1.1 INTRODUCTION

Chronic rhinosinusitis (CRS) is one of the inflammatory diseases of the paranasal sinuses [1]. CRS is the disease of the upper airways, not only in western countries but also growing in Asia. CRS has an essential outcome on health-related quality of life. In the United States, chronic rhinosinusitis affects 4 billion in health care costs every year [2]. The prevalence of CRS in Europe is 2-4% while in the United States about 4.2% [3,4]. However, in Asian countries, the prevalence was 2.6% in Korea and 1.1% in China [5,6].

According to the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2020, chronic rhinosinusitis can be diagnosed by symptoms present at twelve weeks or more [7]. The symptoms typically include nasal blockage, nasal discharge, reduction of smell sensation, and facial pain. By endoscopically, the sign of CRS included nasal polyps, edema of middle meatus mucosa, or mucopurulent discharge. Radiographic imaging is also included in EPOS guidelines by the presence of mucosal changes within the osteomeatal complex or sinuses. Chronic rhinosinusitis is divided into two types; polyp (CRSwNPs) or without polyp (CRSsNPs) based on endoscopically.

One of the contributing factors to CRS is asthma. Asthma is a common medical illness seen at entire stages of health care in Malaysia, and it is often related to chronic rhinosinusitis. It is related to increased morbidity and mortality because it will cause mucous secretion, bronchoconstriction, and airway narrowing [8]. Asthma has two vital defining features which are a history of respiratory symptoms such as chest tightness, shortness of breath, wheeze, and cough with expiratory airflow limitation [9]. In Asian countries, there is a variation of prevalence in asthma ranging from 0.7% to 11.9% [10]. Meanwhile in Malaysia, based on the latest National Health and Morbidity Survey in 2011 the prevalence of asthma was 6.4% [11].

Eosinophils are granulocyte lineage derived from bone marrow cells. They have an estimated 18 hours of half-life in the bloodstream. They mainly exist in tissues where they can persist for several weeks. They have several functions including degranulation, antigen presentation, cytokine mediators for chronic inflammation, responses to the parasite, and homeostatic immune responses [12]. Trung et al. reviewed most of the asthmatic patients will have an average of serum eosinophil more than greater than 400 cells/μL [12].

At present, there is no study on cut-off levels for eosinophil counts to predict CRS patients at high risk of asthma. Owing to this reason, bronchial asthma in CRS patients might be under diagnose and not correctly managed. CRS symptoms are almost similar to bronchial asthma, such as cough and difficulty in breathing. Having a mechanism to predict bronchial asthma in CRS patients will assist the clinician in having a better treatment strategy.

Early detection of asthma in CRS patient help strategies in the method of treatment and preventive measurement can be applied. After all, it will improve the outcome of the management of patients. CRS patients with untreated asthma have a poor quality of life and worse outcome than those without asthma. Early treatment of asthma will improve quality of life and contribute to better management of CRS symptoms. Therefore, our study aims to determine the association between serum and tissue eosinophils with asthma as well as to determine the diagnostic accuracy of both markers in differentiating CRS patients with asthma from those without asthma.

Chapter 2:

OBJECTIVES

2.1 General objective

To study the eosinophilic count (blood & tissue) in CRS patients with asthma

2.2 Specific objectives

I. To compare the different serum eosinophil and tissue eosinophil levels between CRS patients with asthma and without asthma
II. To correlate serum eosinophil level and tissue eosinophil level in CRS patients with asthma and without asthma

Chapter 3:

STUDY PROTOCOL

3.1 Study protocol submitted for ethical approval

JEPeM Code: USM /JEPeM/19120846

Study Title: Eosinophil Count In Chronic Rhinosinusitis With Asthma.

Investigators:

Principal Investigator: Dr. Che Mohd Hilmi Che Mat (MMC No: 65100)

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Introduction

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Early detection of asthma in CRS patient help strategies in the method of treatment and preventive measurement can be applied. After all, it will improve the outcome of the management of patients. CRS patients with untreated asthma have a poor quality of life and worse outcome than those without asthma. Early treatment of asthma will improve quality of life and contribute to better management of CRS symptoms. Therefore, our study aims to determine the association between serum and tissue eosinophils with asthma as well as to determine the diagnostic accuracy of both markers in differentiating CRS patients with asthma from those without asthma.

Study rationale & Benefit to Participants and Community

Managing CRS with asthma is more complicated than CRS without asthma. Many studies showed there was an increase in eosinophil count in CRS or asthma. Nevertheless, a survey regarding the correlation of the CRS with asthma using the eosinophil count is very limited and inadequate. Furthermore, this study is carried out to identify the relationship of eosinophil in blood and tissue of CRS with asthma.

Asthma is a clinical diagnosis. Furthermore, CRS patient with asthma has a poor outcome. These are because asthma makes the quality of life worse. However, at this moment, we do not know the cut-off value to predict asthma in CRS patients. Recently, no study shows the level of eosinophil count as a prediction for CRS patients to get asthma.

Sometimes, CRS patient is underdiagnosing as asthma because we do not know the patient had asthma or not because asthma and CRS share the same symptoms like coughing and sputum production.

The value of this study, we can have a cut-off point of eosinophil value as a predictor for asthma in CRS patients. Then we should be worried and concerned that the CRS patient might have asthma because the symptoms are not manifested yet at that time, or they are underdiagnosed. They also might have silent bronchial asthma. We will refer them to the chest specialty for further evaluation. They might need a lung function test.

All literature is well known that eosinophil count is associated with the severity of asthma, but we do not repeat the same study like that.

This study has a high value because we do not have data yet. We are doing a new study. There is no data to predict asthma in CRS patients. We probably need to diagnose asthma early because it will give a good outcome for CRS patients. Underdiagnosis of asthma makes the CRS worse.

When the data is achieved, this study will give benefit the community. The community will get proper treatment. Thus, the quality of life becomes better, and the community becomes productive. This will contribute to the general well-being and national building. It also can contribute to surveillance, monitoring, early diagnosis, and management of asthma leading to improve quality of life.

Therefore, this study will contribute to proper treatment and cost-effective management in patients CRS with asthma.

Hypothesis

There is a significant correlation of eosinophilic count as predictors of asthma in CRS patients.

Research Question(s)

What is the correlation between the serum eosinophil level and tissue eosinophil level in CRS patients with asthma or without asthma?

Objective

General: To study the eosinophilic count (blood & tissue) in CRS patients with asthma

Specific:

1. To compare the different serum eosinophil and tissue eosinophil levels between CRS patients with asthma and without asthma
2. To correlate eosinophil level and tissue eosinophil level in CRS patients with asthma and without asthma

Literature review

CRS and asthma are inflammatory diseases of the respiratory epithelium **(14)**. Many researchers have reported an association between CRS and asthma, but not explicitly using eosinophil as a biomarker **(15,16)**. Bresciani M et al. reviewed that most asthmatic patients also grieve for CRS **(17)**. Lamblin et al. stated that 30% of nasal polyps suffered from asthma **(18)**. CRS in Europe and the United States is classified into two types, which are CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) **(19)**. CRSwNP shows an increase in either serum neutrophils or eosinophils **(20)**. Therefore, CRSwNP is divided into two subtypes which are ECRS and non-ECRS **(21)**. Non-ECRS can be managed by macrolide therapy, while ECRS is unresponsive to it and respond well to steroid therapy **(22)**. Once the treatment of ECRS differs from non-ECRS, then diagnostic criteria are needed to differentiate both **(23)**.

The relationship between CRS and asthma is described via stimulating the bone marrow with cytokines to produce eosinophils, which finally travel to the airway mucosa and cause inflammatory response **(24)**.

In our study, we want to determine if there is a correlation between CRS and asthma by using eosinophil as a biomarker. The patient will divide into CRS with asthma and CRS without asthma. The level of eosinophil will determine whether there is a correlation between CRS and asthma. However, there was a limited study to correlate CRS and asthma by using eosinophil count.

The closest research was done by Han Kim et al. 2009 "Predictors of bronchial hyperresponsiveness in CRS with nasal polyp" **(25)**. Bronchial hyperresponsiveness (BHR) is always a risk factor for asthma. It is a state in which excessive narrowing of the airways occurs

in response to varying stimuli. Asthma without BHR is uncommon. Han Kim et al. 2009, stated that the prevalence of BHR was higher in CRS patients. Therefore, the evaluation of BHR was useful for the prediction of the surgical outcome in CRS patients. They attempt to identify clinical predictors as a guideline on whether CRS patients should investigate for BHR or not. Hence, in their study, the serum eosinophil was found to be a predictor of BHR with CRS.

The mean values and the ratio of serum/tissue eosinophils found raised in the BHR group than the non-BHR group. Besides, serum eosinophil and tissue eosinophil ratio showed a correlation due to eosinophil infiltration from the blood into the target tissue. In the conclusion of their study, raised eosinophil count could be used as a predictor for BHR in CRS patients. Thus, preoperative assessment of BHR is suggested in CRS patients with high serum eosinophil count.

From this study, once the clinical benefit of level eosinophil count in CRS with asthma is established, it can prevent unfortunate postoperative consequences that frequently occur after endoscopic sinus surgery. The importance of this is because treatment in CRS with asthma is different from CRS without asthma. Furthermore, CRS with asthma has a high recurrent rate of illness and a high chance for the formation of the polyp. In the future, all CRS patient filters by using eosinophil count to predict if there is asthma or not. If the level of eosinophil is high, the CRS patient will be referred early to the physician for the lung function test and given proper treatment before the disease becomes worst.

Risk, potential side effects, and benefits to the patient

All nasal polyps need to take a biopsy for HPE; it is a standard routine procedure in the clinic for CRS patient based on EPOS guideline 2016 regardless patient agree or not for operation. The biopsy is a normal standard routine procedure for a nasal polyp in CRS. This is because polyp can be inflammatory, inverted papilloma, mucocele, or malignant. Hence, every patient who had polyp needs to take a biopsy, and it is a standard routine procedure.

There is no serious adverse event (SAE) about the biopsy procedure; the patient may complain of minimal discomfort only. Bleeding is rare because polyp is not vascular. It is not a new intervention procedure. It is a normal standard procedure of treatment and management. The patient will be explained the risk and potential side effects before joining this study. The patient will be signing a consent as approval before starting the investigation.

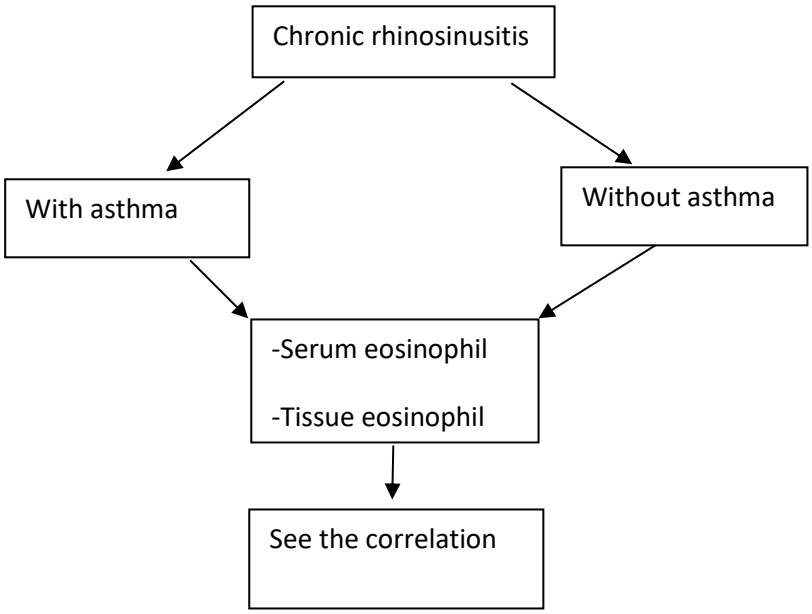
At this moment, we do not have any data yet that supports a certain level of eosinophil will predict asthma. Once a level of eosinophil is established to predict asthma, we will revise back the patient in the group of CRS without asthma. If some of them (group of CRS without asthma) have a level of eosinophil which is suggestive of asthma, we will refer them to a

lung function test/chest physician. This will give direct benefit to the patient and also generate new knowledge for humankind.

There is a direct benefit to the patient once this study establishes. Furthermore, this study generates new knowledge for the surgeon or physician to detect early asthma in CRS by using eosinophil as a marker. For this study, several eosinophils count in tissue and blood of CRS with asthma will be compared to CRS without asthma. The benefit to the patient and community is to detect early asthma in CRS with a polyp, thus helping strategies of treatment, and preventive measures can be applied before the asthmatic patient becomes worst and recurrent of the polyp.

Conceptual framework

Blood taking and biopsy are standard procedures in managing the CRS. The only patient who agrees to involve and consented to biopsy will be involved in this study.



Research design

Prospective cross-sectional study using clinical and laboratory data.

Study area

Group CRS with asthma (study group) and without asthma (control group) in the ORL clinic at HRPZ II and Hospital USM.

Study population

Patients who attended the ORL-HNS clinic will diagnose with CRS based on the European Position paper for Rhinosinusitis 2012, will be enrolled. Determination of asthma status depends on which patient already follows up with the physician or by pulmonary function status-FEV1/FVC test (<80%).

Subject criteria

Inclusion criteria

1. CRS patient based on EPOS criteria (nasal blockage, nasal discharge, reduced smell, and facial pain more than three months)

2. CRS patient who agrees to involve in this study (as FBC and biopsy is a standard procedure in CRS)

3. Age 18-70

Exclusion criteria

1. Pregnancy

2. Other types of nasal mass besides nasal polyps

3. Patient with bleeding tendency as suggestive by history

4. COAD

5. Inadequate sample biopsy for histopathology

6. Severe Asthma

7. Recent fungal/parasite skin infection

Note: If the patient is in doubt, unknown status of asthma, has a history of nebulizer, wheezing and family history of asthma, the lung function test will be tested to verify the asthma condition and later will be referred to the physician.

Sample size estimation

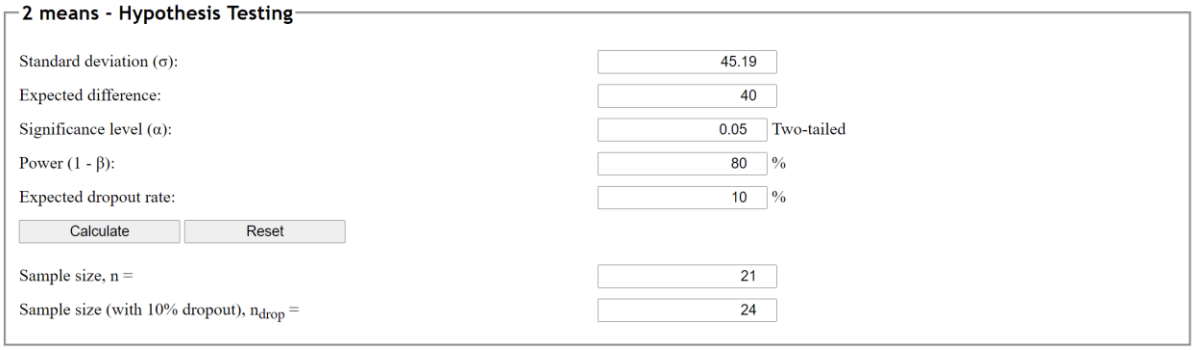
The sample size was calculated by using online https://wnarifin.github.io/ssc_web.html

Objective 1: To compare the different serum eosinophil and tissue eosinophil levels between CRS patients with asthma and without asthma

We figured by Two-mean comparison (independent).

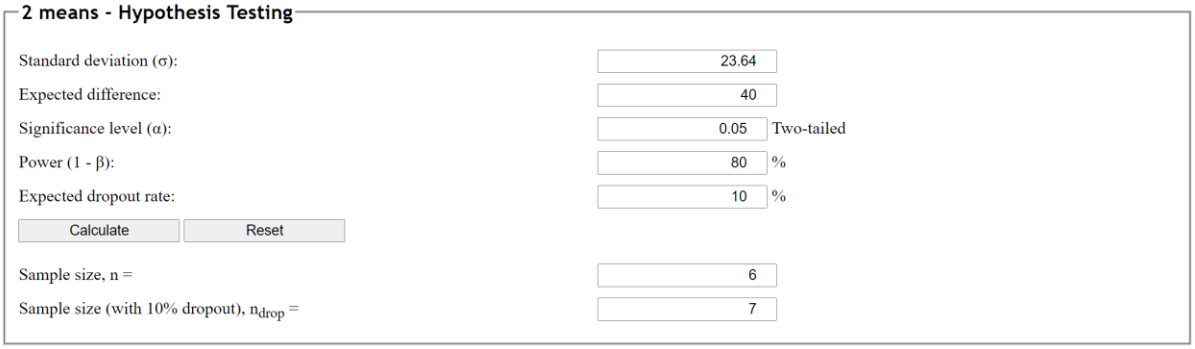
According to **Han Kim et al 2009**, by using serum eosinophil count for mean and standard deviation, there was 430.54 ± 45.19 (BHR/asthma) and 243.92 ± 23.64 (non BHR/asthma)

1. **CRS with asthma.**



Sample size = 24

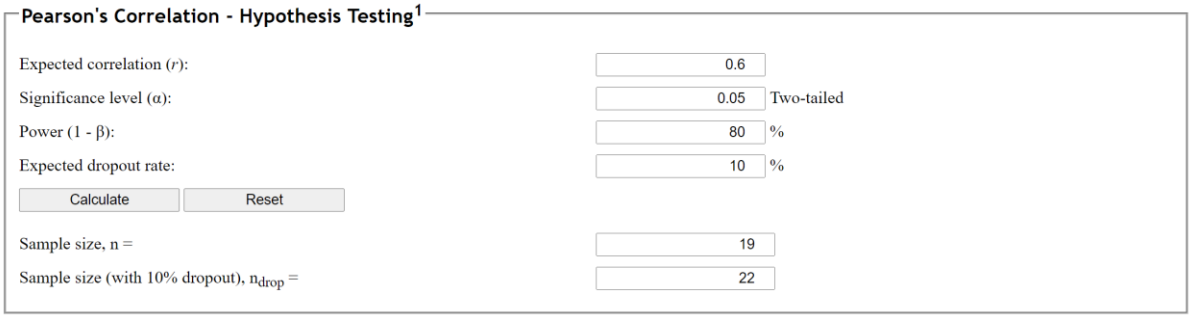
2. **CRS without asthma**



Sample size = 7

Objective 2: To correlate serum eosinophil level and tissue eosinophil level in CRS patients with asthma and without asthma

In this objective, we use Pearson's correlation.



Sample size = 22

Therefore, we choose the largest number = 24

So, the sample size estimated will be 24 for each group (CRS with and without asthma)

Sampling method and subject recruitment

Subjects recruit from ORL-HNS clinic in Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, and ORL-HNS clinic in Hospital Raja Perempuan Zainab 2, Kota Bharu Kelantan. The subject will be diagnosed to have CRS based on EPOS guidelines in ORL. Those who consented, the patient will be asked regarding the presentation of asthma, history using bronchodilator, or already follow up by a physician**.** If the patient who is a positive family history of asthma, has a chesty cough, wheezing, history of using an inhaler (silent bronchial asthma or under-diagnosis), but they are not under follow up chest physician, we will refer them to the lung function test.

A lung function test will be done to support the diagnosis of asthma (asthma is a clinical diagnosis; however, the gold standard to detect asthma is by lung function test). If the ratio of **FEV1/FVC** value is less than 80%, patients diagnosed with asthma. Then the patients will be separated into two groups: study group (CRS with asthma) and control group (CRS without asthma)

The patient also will proceed with nasoendoscopy. Nasoendoscopy is a clinical examination done routinely in all patients attending the rhinology clinic. The findings in CRS based on EPOS are nasal polyp and or mucopurulent discharge primarily from middle meatus and edema or mucosal obstruction mostly in the middle meatus.

Then, if the patient agrees to an operation, a tissue biopsy will be taken in the OT from a polyp or ethmoidal mucosa. The patient will be asked regarding the hematological disorder and anti-platelet medication. If the patient agreed to the operation, which is Functional endoscopic sinus surgery (FESS), the patient will be referred to the anesthesia team for a pre-operative assessment. In the operation theatre (OT), the biopsy will be taken as a part of the FESS procedure from a polyp or ethmoidal mucosa. Postoperatively, a merocele or nasopore will be inserted to control any nose bleed. Bolster charts will be applied to monitor the nose bleeding.

If the patient is not going to undertake surgery but agrees to participate in this study, a tissue biopsy for eosinophil will be taken under local anesthesia in the clinic. Patients sprayed with co-phenylcaine to anesthetize the nose. Then, using the biopsy forceps, the nasal polyp will be biopsied. If there are no polyps, the ethmoidal mucosa (in the region of ethmoidal bulla) will be biopsied. The sample was collected, put in formalin, and sent to a pathologist for analysis.

At the same time, blood (3 ml) for the eosinophilic count will be taken and sent to the pathology lab for analysis. The eosinophils count will be placed in a container with EDTA as the anticoagulant before it is sent for review in the laboratory immediately on the day of blood withdrawal.

In the case of an asthmatic patient with underlying CRS who is on steroid, antihistamine, and montelukast dependent, they need to go through a washout period for 2 weeks before blood eosinophil is taken **(26,27)**. If their asthma symptoms attack during this washout period, then they are excluded from this study.

For CRS patients who are on nasal sprays like Avamys (fluticasone furoate) and Nasonex (mometasone furoate), they need to withhold nasal spray at least 24hour before the biopsy is taken. As the intranasal spray is a local effect, the elimination half-life for Avamys is 15.1 hours and Nasonex is 5.8 hours (base on https://www.drugbank.ca)

Research tool

Clinical Definition of Acute and Chronic Rhinosinusitis with and without Nasal Polyps

Rhinosinusitis in adults
Rhinosinusitis in **adults** is defined as:

- inflammation of the nose and the paranasal sinuses characterised by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip):
  - ± facial pain/pressure
  - ± reduction or loss of smell

and either

- endoscopic signs of:
  - nasal polyps, and/or
  - mucopurulent discharge primarily from middle meatus and/or
  - oedema/mucosal obstruction primarily in middle meatus

and/or

- CT changes:
  - mucosal changes within the ostiomeatal complex and/or sinuses

Study Proforma

Study ID						
	Chronic rhinosinusitis				Age:	Sex:
	Block	Discharge	Facial Pain	Reduce Smell	Race:	Asthma Status:
	Endoscopic findings				Serum eosinophil count	Tissue eosinophil count
	Polyp	Discharge	Edema	OTHERS		

Data collection method

All polyp needs the biopsy, and it is a standard procedure in CRS patients. FBC is also taken as a standard routine procedure in the clinic because CRS is an inflammatory disease. During the clinic visit, patients will be informed regarding this study. If they are willing to participate, the consent forms will be signed and dated. If they need to, they can take the information sheet home to consult with their family members, and another day for getting consent arranged blood taking would be done and sent for serum eosinophilia. The blood sample takes by trained staff and will be sent and processed at the pathology laboratory. The results can be traced through an online system and documented.
If the patient is not going for an operation, a tissue biopsy from polyp will be taken, and the sample will be sent to the pathology clinic together with a blood sample on the same day. If the patient is going for an operation, a tissue biopsy will take in the operating theatre. The operation is Functional Endoscopic Sinus Surgery (FESS) will be done if the CRS symptoms persist after failed medical treatment. The polyp will take intraoperatively.

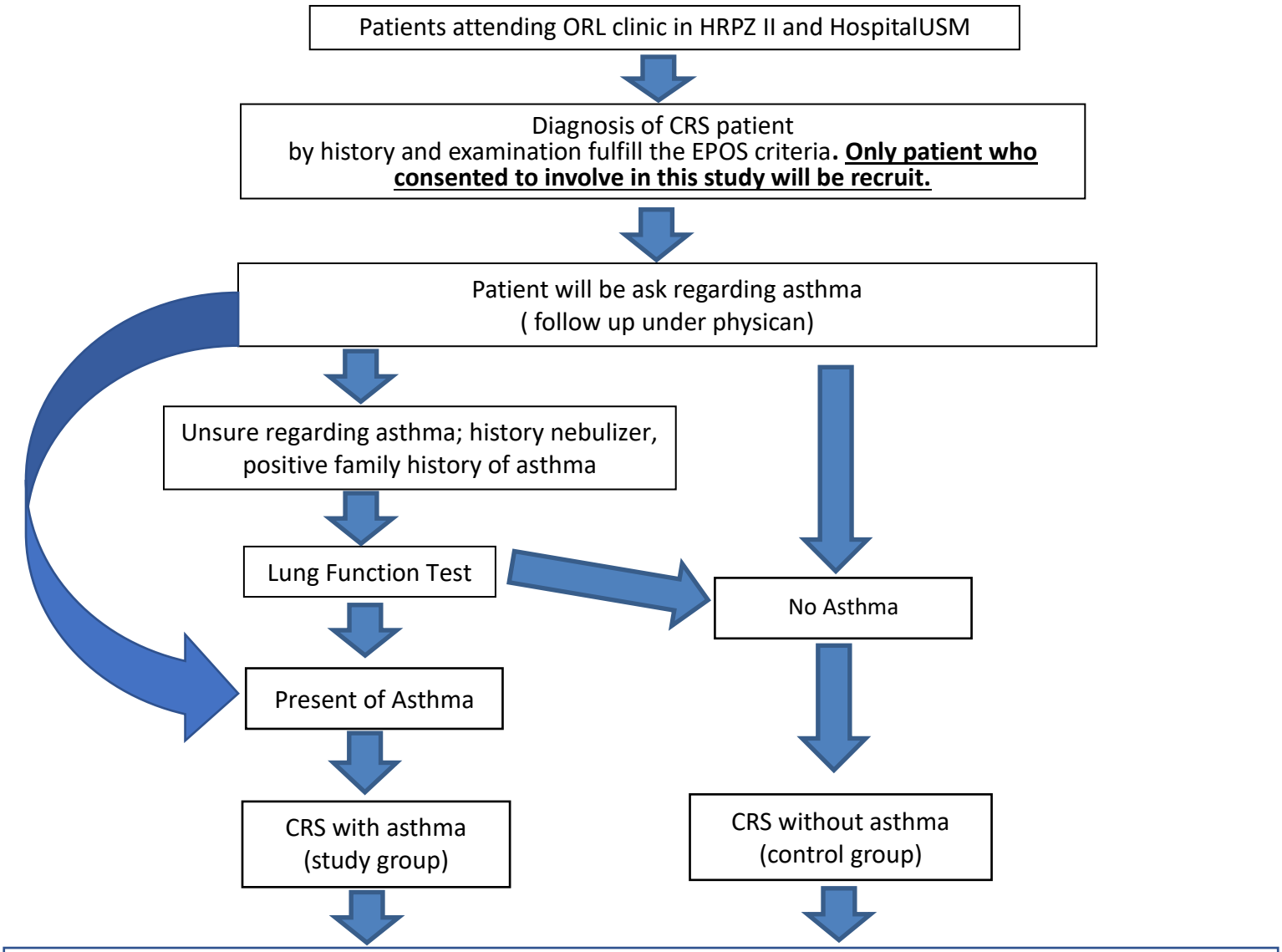
In the case of CRS patients who is underlying asthma and are on treatment, the need to withhold their systemic medication like steroids, antihistamine, and montelukast for 2 weeks before the biopsy is taken. If the symptoms of an asthma attack in this period, they can

continue the systemic medication, and they will be excluded from this study. Those who use an inhaler are not affected because it takes via the mouth.

Based on the website https://www.drugbank.ca, the elimination half-life for Avamys is 15.1 hours and Nasonex is 5.8 hours. Thus, CRS patients who are on nasal sprays like Avamys (fluticasone furoate) and Nasonex (mometasone furoate), need to withhold nasal spray at least 24hour before a biopsy is taken.

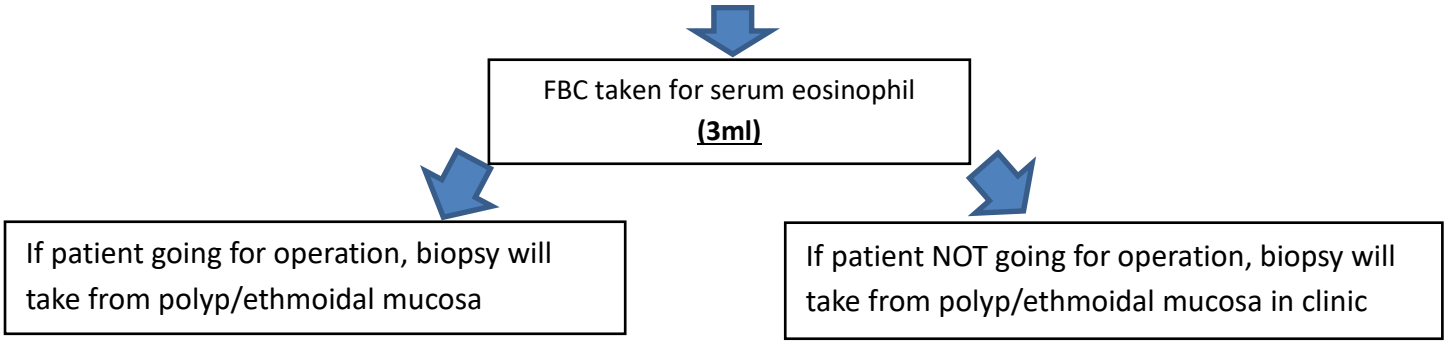
Only consented CRS patients will be involved in the study; those who have not consented will exclude. Those who have not consented still get the same treatment as FBC and biopsy is standard procedure in CRS.

Study flowchart



WASHOUT PERIOD

1. CRS with asthma on systemic medication like steroid, anti-histamine and montelukast need to withhold medication for 2 week. If the symptoms asthma attack in this period, they can continue the systemic medication, and they will be excluded from this study. Those who use an inhaler is not affected because it takes via the mouth.
2. CRS patient who on nasal spray like Avamys® (fluticasone furoate) and Nasonex (momethasone furoate), they need to withhold nasal spray at least 24hour before biopsy is taken.



Eosinophil Count in Histopathology:

According to Sydney ENT Clinic from St Vincent's Hospital (based at Darlinghurst Sydney, Australia), they used a high-power field (HPF) as a unit for eosinophil count in histopathology.

They divide into four categories which are absent, mild, moderate, and severe.

Absent: no eosinophil is seen

Mild: less than 10 HPF

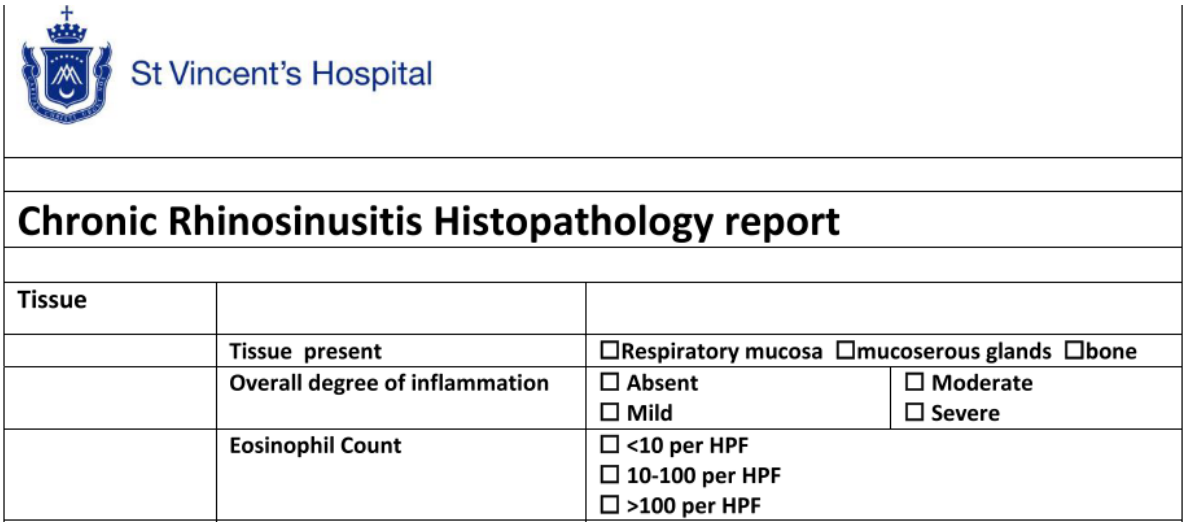
Moderate: 10-100 HPF

Severe: more than 100 HPF

However, in our study, we do not count eosinophil for the classification, but we want to get a cut point in which CRS patients may predict to get asthma.

The High-power field (HPF) is a reference unit for us to count the eosinophil in our study.

Website:https://sydneyentclinic.com/richard-harvey/wp-content/uploads/2017/06/Chronic-Rhinosinusitis-Histopathology-report-2012-version.pdf



St Vincent's Hospital

Chronic Rhinosinusitis Histopathology report

Tissue			
	Tissue present	☐Respiratory mucosa ☐mucoserous glands ☐bone	
	Overall degree of inflammation	☐ Absent ☐ Mild	☐ Moderate ☐ Severe
	Eosinophil Count	☐ <10 per HPF ☐ 10-100 per HPF ☐ >100 per HPF	

Notes on grading			
Absent: virtually no inflammatory cells in subepithelial stroma	**Mild:** single and "small" groups of inflammatory cells identified focally, typically in locations such as perivascular, amongst mucoserous glandular tissue or in the superficial subepithelial stroma. The inflammatory infiltrate does not distort mucosal structures.	**Moderate:** Inflammatory cells form larger, more confluent aggregates, yet the distribution is still patchy. There may be some distortion of mucosal structures, such as separation of mucoserous glandular acini. There may be stromal oedema of any degree from	**Severe:** Confluent, often dense aggregates and sheets of inflammatory cells which distort, expand or obscure normal mucosal structures. Oedema is usually mild to severe. Some areas of relatively absent inflammation may still be observed, and are not incompatible with a designation of severe.

Eosinophil count	The original intention was to make counts at three random areas of the mucosa. In practice due to the often patchy nature of the infiltrates, which may also be separated by severe stromal oedema, we have chosen the approximately 3 most dense collections of eosinophils in the stroma.	**<10 per HPF:** may have 1 field only >10	**10-100 per HPF:** 10-100 eosinophils per HPF, in 2 or more areas	**>100 per HPF:** >100 eosinophils per HPF, in 2 or more areas

Data analysis

Data will be entered and analyzed via SPSS version *22*. Descriptive statistics will be used to summarise the socio-demographic features of subjects. Numerical data presented as mean (SD) based on their normality distribution. An independent t-test and correlation test also will be used.

Expected result(s)

Table 1: Sociodemographic profiles of the patient (N= 48)

	N (%)	Mean ± SD
Age		
- CRS with asthma		
- CRS without asthma		
Sex		
-Male		
-Female		
Asthma status		
-Yes	24 (50%)	
-No	24 (505)	

Table 2: Comparison of the different serum eosinophil and tissue eosinophil levels between CRS patients with asthma and without asthma

Variables	CRS with asthma Median (IQR)	CRS without asthma Median (IQR)	P-value
Blood eosinophil			
Tissue eosinophil			

Table 3: Correlation serum eosinophil level and tissue eosinophil level in CRS patient with asthma using Spearman correlation test.

Variable	**Tissue eosinophil with asthma**	***p*-value**
Blood eosinophil with asthma		

Table 4: Correlation serum eosinophil level and tissue eosinophil level in CRS patient without asthma using Spearman correlation test.

Variable	**Tissue eosinophil without asthma**	***p*-value**
Blood eosinophil without asthma		

Gantt chart & milestone

	Oct 2019 - Nov 2019	Nov 2019 - Jan 2020	Jan 2020 - May 2020	June 2020 - July 2020	Aug 2020 - Sept 2020	Sept 2020 -Dec 2020
Planning & Proposal presentation						
Ethical approval						
Data collection						
Data analysis						
Thesis writing						
Submission						

Budget proposal: Proposed budget from the grant. As the biopsy of polyps and FBC procedure is a standard of care for all patients who come with nasal polyps, no charge will be undertaken. However, based on the grant; each patient who is included in this study will get a monetary honorarium of RM30 each for their commitment to the study.

Vote	**Budget Details & Justification**	**Amount requested by the researcher (RM)***)*
14	Elaun lebih masa untuk analisa serum eosinophil count dan tissue eosinophil count	RM 646.00
21	Perjalanan dan Sara Hidup	RM 3940.00
23	Utilities	RM 300.00
27	Bekalan dan bahan lain	RM 5375
29	Perkhidmatan ikhtisas dan lain	RM 10 620.00
TOTAL AMOUNT		RM20,881.00

Ethical considerations:

1. **Subject vulnerability**

The subject is a patient in which under doctor observation. However, the patient has full freedom to participate or not without affecting his/her medical condition management and care. The data will be independent; it will not be used for any achievement assessment and decision-related to work.

2. **Declaration of an absence of conflict of interest**

The investigators declare they have no conflict of interest. Even if a patient refuses to join the study or wishes to drop out of the study, optimal treatment will give to the patient. It will clearly explain to the patient before enrolling in the study.

3. **Privacy and confidentiality**

The subjects' names will be kept on a password-protected database and will be linked only with a study identification number for this research. The identification number instead of patient identifiers will be used on subject datasheets. All data will be entered into a computer that is password protected. On completion of the study, data on the computer will be copied to CDs and the data in the computer erased. CDs and any hardcopy data will be stored in a locked office of the investigators and maintained for a minimum of three years after the completion of the study. The CDs and data will be destroyed after that period of storage. Subjects will not be allowed to view their study data, as the data will be consolidated into a database. Subjects can write to the investigators to request access to study findings

4. **Publication Policy**

No personal information will be disclosed, and the subjects will not be identified when the findings of the survey are published

5. **Honorarium and incentives**

Token of appreciation given to all responders.