UNIVERSITI PUTRA MALAYSIA

EVALUATION OF AFB/FUNGAL BODIES IN CHRONIC GRANULOMATOUS INFLAMMATION CASES IN HOSPITAL SERDANG FROM 2009 UNTIL 2012

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By

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EVALUATION OF AFB/FUNGAL BODIES IN CHRONIC GRANULOMATOUS INFLAMMATION CASES IN HOSPITAL SERDANG FROM 2009 UNTIL 2012

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ABSTRACT

Introduction: Granulomatous inflammation is a common histopathology diagnosis that can be due to multifactorial causes. Two most common causes of granulomatous inflammation are tuberculosis and fungal infection. However, the histopathologic features of granulomatous inflammation usually differ depending on its underlying causes.

Objective: To evaluate granulomatous inflammation on histologic specimen. The distribution of sociodemographic (age, gender, race) and clinicopathologic (type of granuloma, site of tissue sample) parameters of the patients was determined. Besides, the frequency of positive acid fast bacilli (AFB) and fungal infection was also determined. Relationship between histopathologic features with acid fast bacilli (AFB)/ fungal bodies was investigated. The sensitivity and specificity of Ziehl-Neelsen (ZN) Stain and fungal stain (GMS/PAS) were also determined in this study.

Method: Subjects comprised of cases that have been diagnosed with chronic granulomatous inflammation in Hospital Serdang in 2009 to 2012 that were obtained from the list of medical records using a standardized pro forma. Data was analyzed by SPSS version 20. Age of patient was presented as mean and standard deviation. Gender, positive cases of AFB, and positive cases of fungus was presented as frequency and Chi square test was used to determine the association between the parameters.

Result: Most of the respondents are females (51%) and majority of the respondents were Malays (63%). Most of the cases presented with necrotizing granulomatous inflammation (81%). Besides that, majority of tissue sample are obtained from lymph node (37%). 44 of the cases was positive Acid Fast Bacilli while 14 cases was positive fungus. There was

significant relationship between Langhan's giant cells with tuberculosis and fungal infection (p=0.001). While, there was no significant relationship between caseous necrosis with tuberculosis and fungal infection (p=0.687). The sensitivity of Ziehl-Neelsen's stain is 51.16% and the specificity is 100%. The sensitivity of fungal stain calculated in the data is 92.6% while the specificity is 97.7%.

Conclusion: There was significant relationship between Langhan's giant cell with tuberculosis and fungal infection in term of histopathologic features. Ziehl-Neelsen's stain was still lack of sensitivity in detecting the presence of acid fast bacilli (AFB). On the other hand, fungal stain has a higher sensitivity as compared to Ziehl-Neelson's stain.

Keywords: granulomatous inflammation, Acid Fast Bacilli, fungal infection, histopathologic features, Langhan's giant cell, caseous necrosis, sensitivity, specificity, Ziehl-Neelsen's stain, fungal stain

PENILAIAN AFB/KULAT DI DALAM KES KERADANGAN GRANULOMATUS YANG KRONIK DI HOSPITAL SERDANG DARI TAHUN 2009 HINGGA 2012

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ABSTRAK

Pendahuluan: Keradangan granulomatus adalah diagnosis histopatologi yang biasa dan disebabkan oleh pelbagai punca. Dua penyebab utama keradangan granulomatus adalah batuk kering dan kulat. Walaubagaimanapun, ciri-ciri histopatologi pada keradangan granulomatus ini kebiasaannya berbeza bergantung kepada jenis penyakit.

Objektif: Mengkaji keradangan granulomatus pada specimen histology. Mengenalpasti taburan sociodemografi (umur, jantina, kaum) dan parameter klinikopatologi (jenis granuloma, tapak sampel tisu). Selain itu, kekerapan positif 'acid fast bacilli' (AFB) dan jangkitan kulat turut sama dikenalpasti. Hubungkait di antara ciri-ciri histopatologi dengan 'acid fast bacilli' (AFB)/ kulat juga dikaji. Sensitiviti dan spesifisiti pewarnaan Zielh-Neelsen dan pewarnaan kulat (GMS/PAS) dikenalpasti di dalam kajian ini.

Langkah kajian: Subjek kajian ini terdiri daripada kes-kes yang disahkan dengan keradangan granulomatus yang kronik di Hospital Serdang dari tahun 2009 hingga 2012. Subjek diperoleh daripada senarai rekod perubatan dengan menggunakan pro forma yang diseragamkan. Data dianalisis menggunakan SPSS versi 20. Umur pesakit telah dibentangkan sebagai min dan sisihan piawai. Jantina, kes positif AFB, dan kes positif kulat telah dibentangkan sebagai kekerapan dan 'Chi square test' telah digunakan untuk menentukan hubungkait antara parameter.

Keputusan: Majoriti responden adalah berjantina perempuan (51%) dan berketurunan Melayu (63%). Kebanyakan kes yang diperolehi merupakan kes keradangan granulomatus yang bernekrosis (81%). Selain itu, kebanyakan sampel tisu diperoleh daripada nodal limfa (37%). Sejumlah 44 daripada sampel merupakan positif 'acid fast bacilli' manakala

hanya 14 daripada sampel adalah positif kulat. Terdapat hubungan yang signifikan di antara sel gergasi Langhan dengan batuk kering dan jangkitan kulat (p=0.001). Manakala, tiada hubungan yang signifikan yang diperoleh di antara nekrosis kaseous dengan batuk kering dan jangkitan kulat (p=0.687). Sensitiviti pewarnaan Ziehl-Neelsen adalah sebanyak 51.16% dan spesifitinya pula sebanyak 100%. Sensitiviti pewarnaan kulat adalah sebanyak 92.6% manakala spesifisitinya pula adalah 97.7%.

Kesimpulan: Terdapat hubungan yang signifikan di antara sel gergasi Langhan dengan batuk kering dan jangkitan kulat. Pewarnaan Ziehl-Neelsen masih kurang dari segi sensitiviti bagi mengesan kehadiran 'acid fast bacilli' di dalam sampel tisu. Sensitiviti bagi pewarnaan kulat pula adalah lebih tinggi jika dibandingkan dengan pewarnaan Ziehl-Neelsen di dalam kajian ini.

Kata kunci: keradangan granulomatus, Acid Fast Bacilli, jangkitan kulat, ciri-ciri histopatologi, sel gergasi Langhan, nekrosis kaseous, sensitiviti, spesifisiti, pewarnaan Ziehl-Neelsen, pewarnaan GMS/PAS

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LIST OF ABBREVIATION

AFB: Acid fast bacilli

BCG: bacillus Calmette–Guérin

GMS: Grocott Methenamin Silver

HIV: Human immunodeficiency virus

H&E stain: Hematoxylin and eosin stain

PAS: Periodic acid-Schiff

PCR: Polymerase chain reaction

TB: Tuberculosis

ZN: Ziehl-Neelson

Chapter 1: Introduction

Granulomatous inflammation is a distinctive pattern of chronic inflammation characterized by aggregates of activated macrophages that assume an epithelioid appearance. (Kumar V. et al., 2007) It is formed as the result of an immune response against antigens that cannot be eliminated or that are only slowly degradable. (Cardoso J. C., Calonje E., 2013) The formation of granuloma involves transformation of histiocytes into epitheliod cells. The transformation will result in decreased phagocytic activity of macrophages as well as increased the capacity of secreting cytokines. The cytokines is responsible for activation of T-cells and induce recruitment and futher transformation of macrophages lineage. T-cells play an important role in all phases of granuloma formation. (Cardoso J. C., Calonje E., 2013) Granulomas can be divided into sarcoidal, tuberculoid, foreign body-type, necrobiotic, suppurative, xanthogranulomas and miscellaneous. (Cardoso J. C., Calonje E., 2013) However in this study, we only focus of granuloma that is due to tuberculosis and fungal cause. This is because the most common infectous causes that present with granulomatous inflammation are *Mycobacterium tuberculosis* followed by fungal causes and can be easily found in cases in hospital settings. This is basically what we want to study and the histologic specimen of these two causes will be evaluated. Other common causes include Sarcoidosis, Wegener's granulomatosis, Actinomycosis, Crohn's disease, Histoplasmosis, foreign body and Langerhans cell histiocytosis. (M.MudassarMajeed, M. Bukhari, 2011) (Guler M. et al., 2011) These causes will not be studied as the cases are rare compared to tuberculosis and fungal.

Histologic evaluation of granulomatous inflammation and granulomas must include special stain in order to exclude or include the presence of fungi and acid fast

bacilli (AFB). (Haque A., 2010) The special stain used for acid fast bacilli is Ziehl-Neelson (ZN) stain. On the other hand, Grocott Methenamin Silver (GMS) and Periodic acid-Schiff (PAS) stain are two most common stains used to detect the present of fungi in tissue. ZN and GMS stains are very specific for their respective diagnosis. (Haque A., 2010) However, the sensitivity in ZN stain is low and the exact sensitivity is not frequently studied and determined. Therefore this will leads to inconclusive diagnosis using ZN stain if the stain is of negative results as we cannot exclude the tuberculosis cause due to limitation of sensitivity.

Other than histologic evaluation, the distribution of sociodemographic of granulomatous inflammation which include age, sex and race in Hospital Serdang is not determined. In this study, we hope to find out the distribution pattern of granulomatous inflammation so that to help us find out which demographic is of common one. The distribution of clinicopathologic parameters will also be determined. In clinicopathology, we will include site of tissue sample and also type of granuloma that will only be divided into granuloma with caseous necrosis and granuloma without caseous necrosis.

1.1 Problem statements

The distribution of sociodemographic and clinicopathologic parameters of patients in Hospital Serdang was not determined. Granulomatous inflammation is a common histopathology diagnosis. It is of multifactorial causes. The use of special stains is important to delineate the possible cause. However in some cases the stains are inconclusive due to the limitation of sensitivity.

1.2 Objectives

1.2.1 General objective

To evaluate granulomatous inflammation on histologic specimen in Hospital Serdang from 2009 until 2012

1.2.2 Specific objective

1. To determine the distribution of sociodemographic (age, sex, race) and clinicopathologic (type of granuloma, site of tissue sample) parameters of the patients.

2. To determine the frequency of positive acid fast bacilli (AFB) and fungal infection.

3. To determine the relationship between histopathologic features with acid fast bacilli (AFB)/ fungal bodies.

4. To determine the sensitivity and specificity of Ziehl-Neelson (ZN) Stain and fungal stain (GMS/PAS).

1.3 Research hypothesis

1.3.1 Null hypothesis

There is no relationship between histopathologic features and acid fast bacilli (AFB) or fungal infection.

1.3.2 Alternative hypothesis

There is a relationship between histopathologic features and acid fast bacilli (AFB) or fungal infection.

Chapter 2: Literature review

2.1 Granulomatous inflammation

Granulomatous inflammation is a distinctive pattern of chronic inflammation characterized by aggregates of activated macrophages that assume an epithelioid appearance. (Kumar V. et al., 2007) Granulomas are often present in certain specific pathologic states. Recognition of granulomatous pattern is important because of limited number of condition that cause it and some of the condition can be life-threatening. (Kumar V. et al., 2007)

Morphologically, the epitheliod cells in granulomas have pink, granular cytoplasm with indistinct cell boundaries. Lymphocytes that surrounded the aggregates of epitheliod macrophages secrete the cytokines. These cytokines are responsible for continuing the macrophages activation. Fibroblasts and connective tissue may also found in older granulomas. Some diseases are present with multinucleated giant cells which are found in the granulomas with a diameter of 40 to 50 μm. Central zone of necrosis which is a combination of hypoxia and free-radical injury is present in granulomas associated with certain infectious organism particularly the tubercle bacillus. Caseous necrosis that has a granular, cheesy appearance can be seen grossly in some of the disease. The necrosis appears as amorphous, structureless, granular debris with complete loss of cellular details under the microscope. A quite extensive fibrosis can occur during healing process of the granulomas. (Kumar V. et al., 2007) Below is some example of disease with granulomatous inflammation. (Kumar V. et al., 2007)

Table 1: Example of diseases with granulomatous inflammation

Disease	Cause
Bacterial	
Tuberculosis	*Mycobacterium tuberculosis*
Leprosy	*Mycobacterium leprae*
Syphilitic gumma	*Treponema pallidum*
Cat-scratch disease	*Bartonella henselae*
Fungal	
Histoplasmosis	*Histoplasma capsulatum*
Blastomycosis	*Blastomyces dermatidis*
Unknown	
Sarcoidosis	Unknown etiology
Inorganic metal or dust	
Silicosis	Inhalation of silica
Berylliosis	
Foreign body	Sutures, breast prosthesis, vascular graft

2.2 Tuberculosis

Tuberculosis is an infectious chronic disease caused by *Mycobacterium tuberculosis.* This disease usually affects lungs (pulmonary tuberculosis) but can also affect other parts of human body (extrapulmonary tuberculosis). The transmission usually through inhalation of respiratory droplet of actively or latently infected individuals. (Miranda M. S., Breiman A., 2012) Generally, only small amount of people infected with *Mycobacterium tuberculosis* will develop tuberculosis. However, the probability of developing the disease becoming much higher among people infected with the human immunodeficiency virus (HIV). (Global Tuberculosis Report, 2012)

The most common method for diagnosing tuberculosis worldwide is through sputum smear microscopy. In this method, the bacteria in sputum samples are observed under a microscope. The treatment for new cases of drug-susceptible tuberculosis consist of a 6 months regimen with four first line drugs (isoniazid, rifampicin, ethambuthol and pyrazinamide). While patient with multidrug resistant tuberculosis requires more expensive and toxic drugs. (Global Tuberculosis Report, 2012)

Epidemiology of tuberculosis

The incidence of tuberculosis was estimated about 8.7 million of cases globally in 2011 with equivalent to 125 cases per 100000 population. In Asia, the number of cases occurred about 59% followed by Africa with 26% while the rest occurred in Eastern Mediterranean Region (7.7%), the European Region (4.3%) and the least in the Region of America with only 3%. Among the 8.7 million incident cases, 0.5 million of cases were children and about 2.9 million cases reported were among women. The highest cases reported according to country were India (2.0 million-2.5 million) followed by China (0.9 million-1.1 million). The third place were South Africa (0.4 million-0.5), Indonesia (0.4 million-0.5 million) and lastly Pakistan (0.3 million-0.5 million). The African Region also reported with the highest number of individual having tuberculosis coinfected with HIV which is about 39%. (Global Tuberculosis Report, 2012)

In Malaysia, the incidence reported was about 81.4 per 100000 populations in year 2010. The number of new cases also increases from 15000 reported in 2005 to 19251 cases reported in 2011. Besides that, the commonest form of tuberculosis in Malaysia is pulmonary tuberculosis. According to age group, majority of patient infected with tuberculosis are in the 21-60 years age group with 69.5%. This is followed by age group more than 65 years old with 13.3% of cases and the least is among age group below than 14 years old with 2.7%. Other than that, male cases recorded are more than female cases with 65%. Among all the cases recorded, 13.9% of cases are contributed by the foreigners. The highest prevalence of tuberculosis cases was noted in Sabah, followed by Selangor and Sarawak.

Causative organism

Tuberculosis is an infection cause by aerobic acid-fast rod bacteria, *Mycobacterium tuberculosis*. It is typically measure 0.5 μm by 3μm. (Knechel, 2009) The bacteria contain high lipid content of cell wall, which prevent dyes used in Gram stain from staining the organism. (Levinson, 2010) The well develop cell wall contain fatty acid, mycolic acid which attach covalently to the underlying peptidoglycan-bound polysaccharide arabinogalactan that provide an extraordinary lipid barrier. The compositions of the cell wall component affect the bacteria's virulence and growth rate. The peptidoglycan polymers provide cell wall rigidity while lipoarabinomannan, a carbohydrate structural antigen on the outside of the organism is immunogenic and contribute towards the survival of the bacteria in the macrophages. (Knechel, 2009)

Pathogenesis

Once the infectious droplet being inhaled, *Mycobacterium tuberculosis* move to the lower part of respiratory tract. (Miranda M. S., Breiman A., 2012) The bacteria that reach the alveoli then are quickly surrounded and engulfed by alveolar macrophages. (Knechel, 2009) The uptake of the bacteria later will trigger the innate immune signaling pathways. This will result in production of various chemokines and cytokines which then promotes production of other immune cells such as more macrophages, dendritic cells, and lymphocytes to the infection site. (Miranda M. S., Breiman A., 2012) The adaptive immune response to the bacteria results in the formation of granuloma. (Jose L., Ganguli S., Kirschner D, 2004) A granuloma comprises of infected macrophages at the center surrounded by foamy epitheliod macrophages, monocytes and multinucleated giant cells are form at the infection site. (Mi-Jeong Kim et al, 2010) The granuloma represents a stable structure that contains a fairly constant bacterial load but does not cause any sign of disease towards the infected individuals. (Mi-Jeong Kim et al, 2010)

The bacteria can survive for a long period of time inside the granuloma in a latent state. The reactivation of the bacteria occurs due to some environmental factors such as malnutrition and HIV or genetic factor which is thought to result in the death of infected macrophages. The death of infected macrophages induced the formation of necrotic zone called caseum in the centre of granuloma. (Miranda M. S., Breiman A., 2012) The necrosis of granuloma increases until the center liquefies and ruptures allowing the bacteria to spread into the other parts of lungs and more lesion will be formed. (Mi-Jeong Kim et al, 2010) Then, infection will also be transmitted to other people through free bacilli exhaled into the atmosphere. (Miranda M. S., Breiman A., 2012)

Signs and Symptoms

Early signs and symptoms are very important so that to recognize the disease earlier and give immediate treatment. Tuberculosis usually affects the lungs, but can also affects any parts of the body depends on the severity of the disease. Tuberculosis can be divided into two different stages, the latent stage and active stage. Not all who infected with tuberculosis may develop signs and symptoms. People with no previous exposure but infected with tuberculosis is considered to have primary tuberculosis. They usually develop latent tuberculosis but can also develop progressive lung disease and military tuberculosis. (Gates, 2003)

People who have latent tuberculosis infection do not feel sick and remains asymptomatic. Although asymptomatic, they may still develop fever and nonproductive cough due to the immune reaction. Abnormalities can be seen on chest X-ray. Ghon focus is usually seen in chest X-ray which is the infection of lung. A Ghon complex, usually with lymph nodes involvement is calcification seen in pulmonary parenchyma (usually midlung) resulting from earlier, usually childhood, infection with tuberculosis. (Gates, 2003) (B. Jana, 2003)Tuberculosis can remains in latent stage for years. It must be aware that a healthy looking person may still be infected with tuberculosis. People who had pervious infection are considered to have secondary tuberculosis. Latent tuberculosis can progress to active tuberculosis. This is mainly due to the immune system of the infected person is weakened and signs and symptoms start to develop. Tuberculosis will have many different consequences. The symptoms for active tuberculosis depend on which part of the body the bacteria are growing. Symptoms of tuberculosis include persistent cough for at least 3 weeks with green, yellow or bloody sputum, chest pain, hemoptysis, lethargy,

loss of appetite, weight loss, chills, fever, shortness of breath and night sweat. (David L. Longworth, James K) (Gates, 2003) (Ilese J, Chatman, 2008) Tuberculous pleuritis may occur in 10% of people who infected with tuberculosis. This condition is due to rupture of infected area into the pleural space. These people will have nonproductive cough and chest pain in this condition. In people who have weakened immune systems, the disease may progress to military tuberculosis and spread through blood to various part of the body. They may produces fever, weakness, loss of appetite and weight loss. (David L. Longworth, James K) Coughing and difficulty in breathing are less common. About 15% of people may develop tuberculosis in an organ other than lungs. The common sites for infection other than lungs include lymph nodes, genitourinary tract, bone and joint sites, meninges and the gastrointestinal tract. (David L. Longworth, James K) The occurrence of additional symptoms depends on the extent the disease has spread. Spreads through lymph nodes cause lymphadenopathy and enlargement of glands at the sides of the neck or axilla. If tuberculosis is spread to bones and joints, it can cause back pain and swelling of hip or knee joint. Spreading to genitourinary tract causes pain in the flank with urinary discomfort, urinary frequency and hematuria. (David L. Longworth, James K)

Risk Factor

Tuberculosis test is not tested for all individual in normal situation unless they are at increased risk for getting the disease. Anyone can get the disease, but certain factors may higher the risk of getting the disease. Generally, the people who are at higher risk can be categorized into two categories, which is people who have been to endemic area, people with medical conditions especially those who are immunocompromised.

Only people who have active tuberculosis can spread the disease. The bacteria are spread when we cough, sneeze or even talking. A healthy immune system is required to fight the infection. A weakened immune system lower down the body resistance to infection and our body cannot mount an effective defense against the infection. Medication or other conditions can also weaken the immune system. Conditions that cause weakened immune system and predisposed to tuberculosis include HIV, diabetes mellitus, cancers, corticosteroid therapy, malnutrition, pregnancy, radiotherapy, and extreme ages. (Coker R, McKee M et al., 2006) (P.D.O. Davies., 2005)Tuberculosis risk is also higher to those who always travels, especially people who travel to country that is epidemic of tuberculosis. Country that is of high rates of tuberculosis include Sub-Saharan Africa, India, Mexico, parts of Southeast Asia and parts former Soviet Union. Poverty or substance abuse may also increase the risk of getting the disease. For example, country that is not economically stable, the medical service provided can be limited. People who live in remote area of low fixed income, the access to the medical care are poor. (Coker R, McKee M et al., 2006) Therefore, they may not aware of the important of the disease. Even if they are infected with the disease, it is hard for them to do preventive measures. For substance abuse, long term drugs or alcohol can weaken our immune

system. (P.D.O. Davies., 2005) Other than that, tobacco use is also proven to have greatly contributed to the risk of getting the disease. (P.D.O. Davies., 2005) The environment that we are in also contributed to the risk. For example, those health care providers who work in a hospital setting will have an increase risk as they will contact with patient who is infected with tuberculosis regularly. (P.D.O. Davies., 2005) This increases the chance of exposure to the bacteria. Therefore, many safety precautions in hospital setting like wearing mask and frequent washing should be followed to reduce the risk of getting tuberculosis. People who work or live in a residential care facility are also at increased risk of getting tuberculosis. For example, people who work or live in prison may contact with people of backgrounds and origin. Immigration centers where regular contact with immigrants from country of high rate of tuberculosis. (P.D.O. Davies., 2005) (Coker R, McKee M et al., 2006) Even nursing home can be a risk for tuberculosis. This is also partially contributed by overcrowding of the environment and poor ventilation of the surroundings. (P.D.O. Davies., 2005) Refugees who live in a refugee camp or shelter may also have higher risk of getting tuberculosis as they are living in a crowded, unsanitary and malnutrition conditions. (Coker R, McKee M et al., 2006) Their immune system is also weaken and at especially high risk of tuberculosis infection. However, those who injected with BCG vaccine show about 75% protection for 15 years and therefore a protective risk for tuberculosis. (P.D.O. Davies., 2005) (Soysal A, Millington KA. et al, 2005)

2.3 Fungi

Fungi are eukaryotic organisms that consist of two type; yeast and mold. Yeast is single cells fungi that reproduce by buding process. While, mold consist of long filaments of cells called hyphae and reproduce by cell division process. Some of fungi are dimorphic which mean they can exist as yeast or mold depending on the temperature. Fungi have two cell structures that are medically important; the cell wall and cell membrane. The cell wall of fungi is made up of chitin that is a polysaccharide composed of long chains of N-acetylglucosamine. It also consists of other polysaccharide, β-glucan that is a long polymer of D-glucose. B-glucan is important as it serve as site of action for antifungal drug caspofungin. Besides that, fungal cell membrane contains ergosterol which is differing from cholesterol that is present in human. This is important as the selective action of antifungal drug such as amphotericin B and azole drug towards fungi is based on this difference in membrane sterols. (Levinson, 2010)

Fungal infection can be classified into superficial, subcutaneous, systemic and opportunistic depending on the degree of invasion of the host. (Nicki R. Colledge, Brian R. Walker, Stuart H. Ralston, 2010) Below is some example of features of important fungal disease. (Levinson, 2010)

Table 2: Features of important fungal diseases

Type	Anatomic Location	Representative Disease	Genus of causative organism(s)
Cutaneous	Dead layer of skin	Tinea versicolor	*Malassezi*
	Epidermis, hair, nails	Dermatophytosis (ring worm)	*Microsporum, Trichopyhton, Epidermophyton*
Subcutaneous	Subcutis	Sporotrichosis	Sporothrix
		Mycetoma	
Systemic	Internal organs	Coccididiomycosis	*Coccididiodes*
		Histoplasmosis	*Histoplasma*
		Blastomycosis	*Blastomyces*
		Paracoccididiomycosis	*Paracoccididiodes*
Opportunistic	Internal organs	Crytococcis	*Cryptococcus*
		Candidiasis	*Candida*
		Aspergillosis	*Aspergillus*
		Mucormycosis	*Rhizopus*

Some of fungi species invade the subcutaneous tissue causing formation of abscess or granulomas. While, deep fungal infections usually heal or remain latent in normal host. However, the deep fungal infection can spread systemically, invade tissues and destroying vital organ in immunocompromised host. (Kumar V. et al., 2007)

2.4 Diagnosis

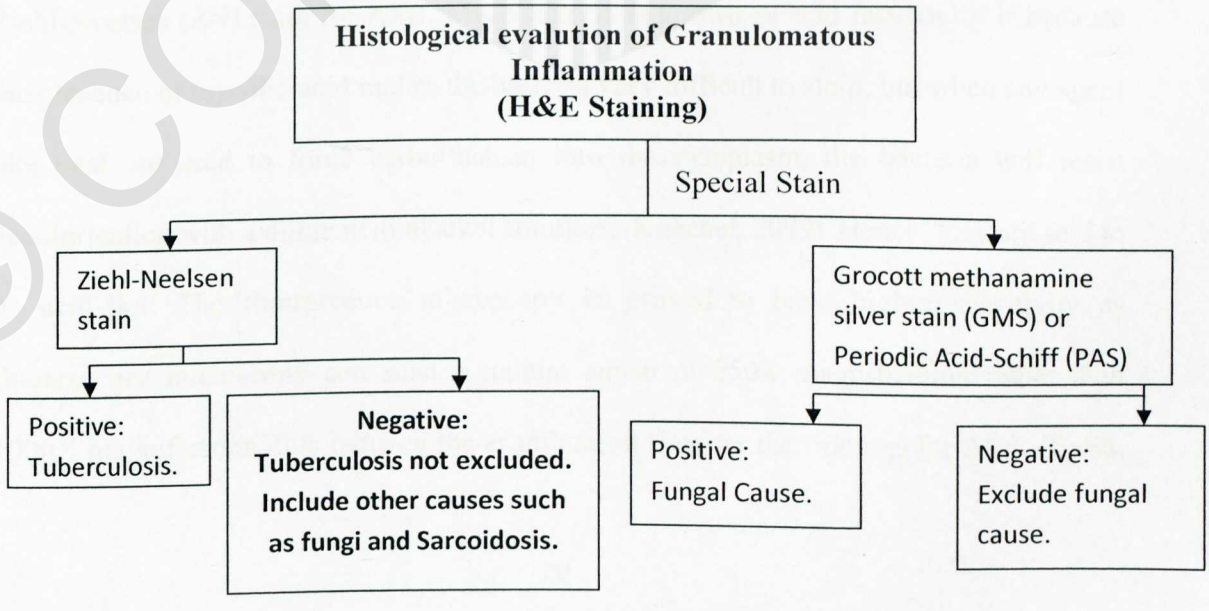
Granulomatous lesion is very commonly encountered abnormalities found in clinical settings especially in pulmonary pathology. It is a distinctive pattern of chronic inflammation featured by aggregation of macrophages. (Kumar V. et al., 2007) Skin, Subcutaneous tissues, lymph nodes and lungs are the most common sites for surgical pathologist to encounter granulomatous lesion. (S Mukhopadhyay, AA Gal.) There are many causes of granulomatous lesion. Granulomas can form in the setting of persistent T-cell reaction towards certain microbes such as mycobacterium tuberculosis, *T. pallidum*, or fungi. (Kumar V. et al., 2007) In fact, there are many types of distribution of granuloma, which include lymphatic routes (eg : sarcoidosis), randomly (miliary infection : mycobaterial and fungal infections) or along the airways (hypersensitivity pneumonitis). (Cancellieri, A., Dalpiaz, G., 2010) Some example of diseases with granulomatous inflammation is listed in Table 3. (Kumar V. et al., 2007) (S Mukhopadhyay, AA Gal.)

Table 3. Example of diseases with granulomatous inflammation

Disease	**Cause**	**Tissue Reaction**
Tuberculosis	*Mycobacterium tuberculosis*	Noncaseating tubercle (granuloma prototype) : a focus of epitheloid cells, rimmed by fibroblasts, lymphocytes, histiocytes, occasional giant cells Caseating tubercle: central amorphous granular debris, loss of all cellular detail; acid-fast bacilli
Leprosy	*Mycobacterium leprae*	Acid-fast bacilli in macrophages; noncaseating granulomas

Syphilis	*Treponema pallidum*	Gumma: microscopic to grossly visible lesion, enclosing wall of histiocytes; plasma cell infiltrate; central cells are necrotic without loss of cellular outline
Cat-scratch disease	Gram-negative bacillus	Rounded or stellate granuloma containing central granular debris and recognizable neutrophils; giant cells uncommon
Sarcoidosis	Unknown etiology	Noncaseating granulomas with abundant activated macrophages
Crohn disease (inflammatory bowel disease)	Immune reaction against intestinal bacterial, self-antigens	Occasional noncaseating granulomas in wall of intestinal, with dense chronic inflammatory infiltrate
Cryptococcosis	*Cryptococcus neoformans*	These ganulomas in the lung can be necrotizing or non-necrotizing.
Histoplasmosis	*Histoplasma capsulatum*	Granuloma in form of histoplasmosis, usually typical necrotizing granulomatous inflammation. Disseminated histoplasmosis shows ingestion of organism by macrophages without granuloma formation.
Aspergillosis	*Aspergillus* spp.	A rim of epithelioid cells surrounding a purulent center of PMNs
Pneumocystosis	*Pneumocystis carinii*	Varied from ill-defined granulomatous pneumonia to well-formed necrotizing granulomas. The typical intraalveolar eosinophilic frothy exudate is absent.
Coccidioidomycosis	*Coccidioides* immitis	Single or multiple areas of airspace consolidation to the formation of nodules or cavities, which may further progress to diffuse reticulonodular lung disease and upper-lobe scarring
Blastomycosis	*Blastomyces dermatitidis*	Similar to coccidioidomycosis

The step by step approach to granulomas is to identify the organism, look for histological features (H&E stain) of noninfectious granulomatous diseases, and if does not yield a specific diagnosis, proceed with special stains. (S Mukhopadhyay, AA Gal.) Hematoxylin & Eosin is a versatile stain that enables the pathologist to evaluate the host response, to detect other micro-organisms, to identify their natural pigments and to evaluate the cells morphology. (Haque A., 2010) Microphages are the cells that define a granuloma. They often fused to form langhans giant cell (multinucleated giant cell) and being referred as epitheloid cells. Langhans giant cell are not same as ordinary microphages as they often resembles slipper and shoes. (Janeway's Immunobiology, 2008) The process of interpreting and identifying presence of an organism is quite challenging. Many pathologist find it hard to interpret with special stains namely Ziehl-Neelsen (ZN) stain for Mycobateria and Grocott methenamine silver (GMS) stain for fungi, the task if even more complicated when the organism is not detected by staining techniques. The lack of identifiable organisms in tissues raises questions about the next appropriate steps for pathologist to pursue, whether to further investigate with polymerase chain reaction (PCR) or continue with differentiate diagnosis.



The flow chart above shows the special stains and the results. The final decision taken for ZN stain and GMS stain is not the same. This is because the sensitivity in ZN stain is low and in cases of negative results we still may not exclude tuberculosis as a cause of granulomatous inflammation.

Mycobacterium tuberculosis remains a significant organism in granulomatous lesion because of the importance of Tuberculosis (TB). TB remains a significant health problem as 8 million of new cases and 3 million deaths due to the disease every year around the world. (Ulukanligil M., Aslan G., Tasci S) (Kumar V. et al., 2007) It is recommended to have at least three specimens, preferably in three consecutive days to be collected for the laboratory diagnosis of acid fast bacilli. (Ulukanligil M., Aslan G., Tasci S) The detection of acid fast bacilli (AFB) can be in direct smears prepared with concentrated sputa, urine, tissues, and any other specimens that are considered to have clinical and epidemiological values. Detection of AFB remains the most widely used rapid test for TB in most developing countries. (Ulukanligil M., Aslan G., Tasci S) In the present, there are two AFB staining methods, one is fluorescence microscopy which uses auramine O and rhodamine stain, and another one is bright-field microscopy, which uses Ziehl-Neelsen (ZN) stain for AFB. Mycobacteria is known as acid fast bacilli is because the presence of mycolic acid makes the bacteria very difficult to stain, but when any agent like heat are used to force carbolfuchsin into the cytoplasm, the bacteria will resist decolorization with a dilute acid-alcohol solution. (Knechel, 2009) Hence, they are said to be acid-fast. The fluorescence microscopy is proved to have higher sensitivity as fluorescence microscopy can scan a sputum smear at 250X magnification rather than 1000X magnification, this reduced the examination time for the findings for AFB. (F. Ba,

H. L. Rieder, 1999) Fluorescence microscopy is also proven through numerous studies to be at least 10% more sensitive than traditional light microscopy. Thus, test characteristic is very important especially in staining time is very important in overall laboratory efficiency in reporting results. (Cindy H., Kim D., 2009) Although fluorescence microscopy is more sensitive in testing AFB, it is recommended that any positive result is reexamined with Ziehl-Neelsen (ZN) stain when fluorescence method is introduced. (P. Kubica G, 1980) The sensitivity of AFB staining methods however are primarily depends on individual qualities and attentiveness of workers evaluating the slides. (P. Kubica G, 1980)

Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitis which are dimorphic fungi are commonly seen in infected immunocompetent individuals, or disseminated condition in immunocompromised persons and causes granulomatous lesion. Therefore, people who with HIV are more prone to systemic disease. [9] Each dimorphic fungus has a typical geographic distribution. Fungal stains are used to stain degenerated fungi that their morphology cannot be demonstrated by H&E staining. Furthermore, H&E staining is difficult to identify poorly stained fungi. Grocott methenamine silver (GMS) and Periodic Acid-Schiff (PAS) are two most commonly used stain in cytology specimens to differentiate fungal infection. (Haque A., 2010) GMS is more preferred for screening purpose, because the contrast it gives is more prominent. GMS can stain algae, cyst wall of *Pneumocystis carinii* or even pathological free amoebas. (Haque A., 2010) Prolonged staining with silver nitrate may be required to demonstrate presence of yeast-like cells such as *Histoplama capsulatum* in granuloma. The disadvantage of GMS is that they can mask the lesion and therefore cannot determine whether the agent is dermaticeous

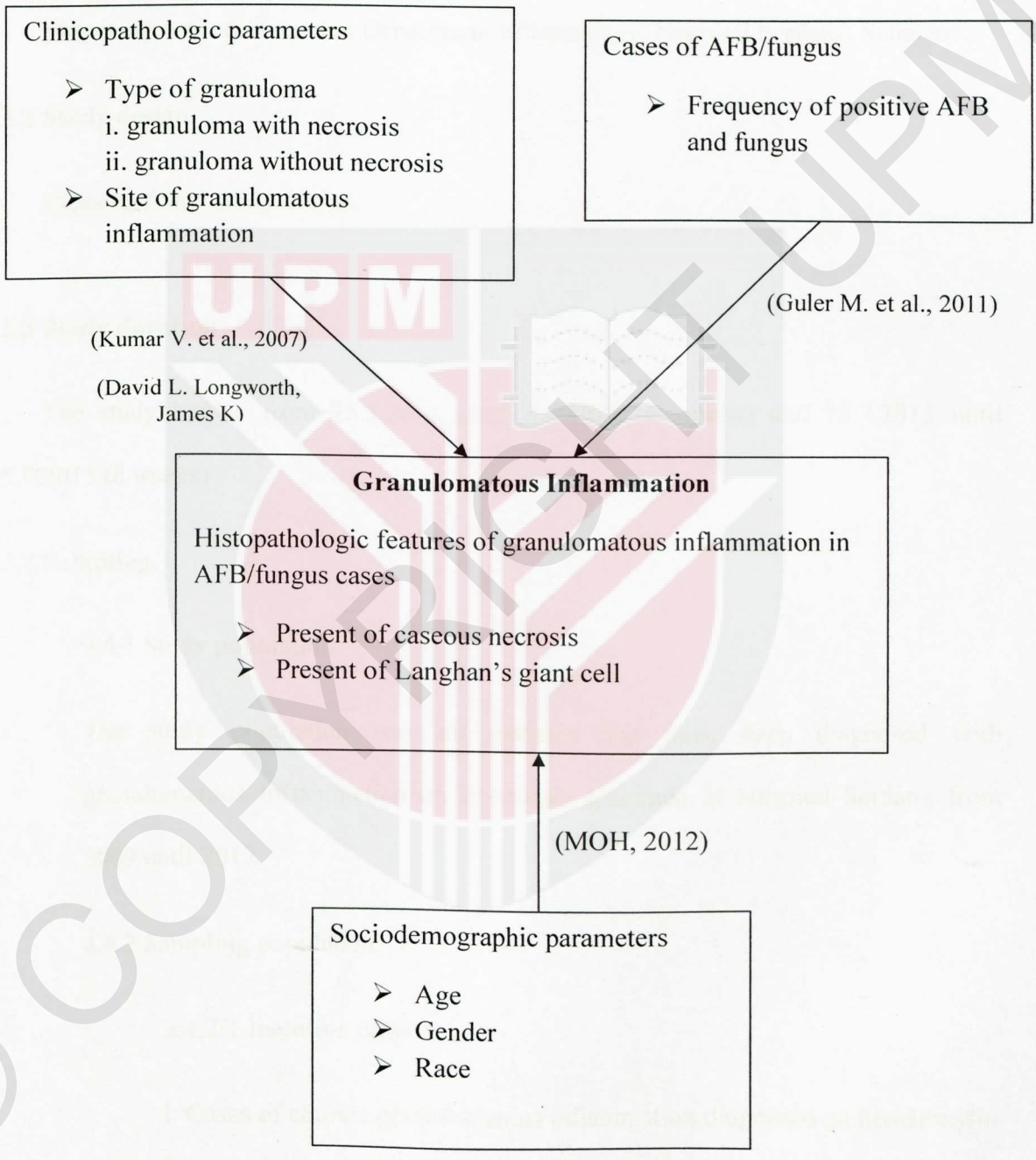
(pigmented). (Haque A., 2010) PAS perform as well as GMS, which also stains degenerated fungi that may not be visible on H&E stain. Calcific bodies which are found during caseating granulomas are also stained by PAS and can be mistaken for yeast-like fungi and therefore leads to misinterpretation. This is why GMS is more preferred over PAS. (Haque A., 2010) Another drawback for using PAS for fungal staining is that it will stain polysaccharides wherever they appear in tissues. Therefore, intracellular components (eg : glycogen, neutral mucosubstances, some epithethial sulfomucins and sialomucins, colloid of the thyroid) of normal cells are also stained. (Coulson R., 2005) The differential diagnosis of fungi may require narrow-spectrum stains. For example, mucin stains such as alcian blue, and Mayer's, or Southgate's mucicarmine, are used to demonstrate the mucoid capsule of Cryptococcus neoformans. These stains differentiate Cryptococcus from other fungal agents of similar morphology. In some cases, poorly encapsulated crytococcus may not stain positive with mucicarmine stain. (Haque A., 2010) The diagnosis of fungal granulomatous lesion is more complicated compared to mycobacterial granulomatous lesion. Knowledge is required to give specific diagnosis as there are more fungal agents compared to bacteria that causes granulomatous lesion.

2.5 Treatment

The drugs treatment of tuberculosis can be divided into first line, second line and third line. The first line drug for tuberculosis is ethambutol, isoniazid, pyrazinamide, and rifampin. Streptomycin which originally considered as first line drug is withdrawn out due to its high rates of resistance. The second lines of drugs are considered as the reserved therapy for tuberculosis treatment. The second line drugs are only used when in special conditionssuch as when the patient is resistant to first line drugs. There are six classes of drugs in second line drugs, namely terizidone, cycloserine, thioamides, fluoroquinolones, polypeptides and aminoglycosides. (Geneva, 2010) The third line drugs are not as effective, they are rifabutin, macrolides, linezolid, thioacetazone, thioridazine, arginize and vitamin D. (Rahman A)

The most effective antifungal drug are amphotericin B and various type of azole drug exploit the present of ergosterol in the cell membrane of fungi for its action as the ergosterol cannot be found in bacteria or human cell membrane. The role of amphotericin B is to distrupt the fungal cell membranes at the site of ergosterol while azole drugs inhibit the synthesis of ergosterol. The example of azole drugs are itraconazole, fluconazole and ketoconazole. Another antifungal drug is caspofungin. It work by inhibiting the synthesis of β-glucan which is found in the fungal cell wall. (Levinson, 2010)

2.6 Conceptual framework



Chapter 3: Methodology

3.1 Study location

This study was conducted in Department of Pathology, Hospital Serdang, Selangor.

3.2 Study design

Cross sectional study design.

3.3 Study duration

The study begins from 25.3.2013 until 3.5.2013 (6 weeks) and 15.7.2013 until 5.9.2013 (8 weeks).

3.4 Sampling

3.4.1 Study population

The study population was all patients that have been diagnosed with granulomatous inflammation on histologic specimen in Hospital Serdang from 2009 until 2012.

3.4.2 Sampling population

3.4.2.1 Inclusion criteria

i. Cases of chronic granulomatous inflammation diagnosed on hematoxylin & eosin stain in Hospital Serdang from 2009 until 2012.

ii. Cases that have been stained with Ziehl Neelson and Grocott/PAS stain.

3.4.2.2 Exclusion criteria

Other causes of granulomatous inflammation.

3.4.3 Sampling frame

All patients with granulomatous inflammation reported in Hospital Serdang from year 2009 until 2012.

3.4.4 Sampling unit

A patient with granulomatous inflammation reported in Hospital Serdang from year 2009 until 2012.

3.4.5 Sampling method

Universal sampling

3.5 Instrument and data collection

3.5.1. Instrument

Histopathological examination record (secondary data from data base access).

3.5.2. Data collection technique

The data were collected from the Patients and Laboratory Information System.

3.5.3 Quality control

Data collection and analysis were monitored by supervisor.

3.6 Data analysis

Data were analyzed by SPSS version 20. Age of patient was presented as mean and standard deviation. Gender, positive cases of AFB, and positive cases of fungus was presented as frequency and Chi square test was used to determine the association between the parameters.

Below is the calculation formula for sensitivity:

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100\%$$

$$\text{Specificity} = \frac{TN}{TN+FP} \times 100\%$$

$$\text{Positive Predictive Value} = \frac{TP}{TP+FP} \times 100\%$$

$$\text{Negative Predictive Value} = \frac{TN}{TN+FN} \times 100\%$$

3.7 Study ethics

Approval for ethical review was submitted to the Medical Research and Ethics Committee (MREC) and NMRR, Ministry of Health Malaysia.

3.8 Variables

Dependent variable: Cases diagnosed of AFB/fungus on special stain finding.

Independent variable: Granulomatous inflammation cases.

3.9 Definition of term

3.9.1 Chronic Granulomatous Lesion – Chronic lesion caused by a diverse group of hereditary diseases in which cells of immune system have difficulty forming the reactive oxygen compounds used to kill certain ingested pathogens. This will lead to the formation of granuloma in many organs. Granuloma is a tiny collection of immune cells as macrophages. Granulomas form when immune system attempts to wall off substances that it perceives as foreign but is unable to eliminate it. Such substances include infectious organisms such as bacteria and fungi as well as other materials such as keratin and suture fragments. A granulomatous lesion is therefore a special type of inflammation that occurs in wide variety of diseases.

3.9.2 Acid Fast Bacilli Stain - The acid-fast stain is a laboratory test that determines if a sample of tissue, blood, or other body substance is infected with the bacteria that causes tuberculosis and other illnesses. There are two type of staining, which is Ziehl-Neelsen stain and Fluorescence microscopy stain.

3.9.3 Positive for AFB - On ZN staining the acid fast bacilli will be labeled when pink, beaded, and rod-shaped organisms are found after comparing with control samples.

3.9.4 Positive for fungus - On GMS staining, presence of black colored septated or nonseptated hyphae (depending upon the species of Fungus) or spores against a greenish background would be labeled as positive for fungus. On PAS stain, presence of red- or purple-colored septated or nonseptated hyphae or spores would be labeled as positive for fungus.

Chapter 4: Result

4.1 Sociodemographic and clinicopathologic parameters

Table 1 shows the distribution of 100 respondents by age, gender and ethnicity. The overall mean age of 100 respondents was 35.25 years with a median of 31.50 years. The age of respondents ranged from 1 to 75 years. The respondents were divided into eight age groups, which were 1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, and 71-80 years old. Most of the respondents are females (51%). While, majority of the respondents were Malays (63%) followed by others (16%), Chinese (14%) and least with Indian (7%).

Most of the cases presented with necrotizing granulomatous inflammation (81%). Besides that, majority of tissue sample are obtained from lymph node (37%).

Table 4 : Sociodemographic and clinicopathologic parameters of respondent

Variables	Frequency(s)	Percentage(%)
Age group		
1-10	5	5.0
11-20	12	12.0
21-30	28	28.0
31-40	18	18.0
41-50	13	13.0
51-60	14	14.0
61-70	6	6.0
71-80	4	4.0
Total	**100**	**100.0**
Gender		
Male	49	49.0
Female	51	51.0
Total	**100**	**100.0**
Ethnicity		
Malay	63	63.0
Chinese	7	7.0

Indian	14	14.0
Others	16	16.0
Total	**100**	**100.0**
Type of granulomatous inflammation		
Necrotizing granulomatous inflammation	81	81.0
Non-necrotizing granulomatous inflammation	19	19.0
Total	**100**	**100.0**
Site of tissue sample		
Lymph node	37	37.0
Soft tissues	13	13.0
Skeletal muscle	1	1.0
Neck	8	8.0
Colon	2	2.0
Lungs	9	9.0
Bronchus	2	2.0
Skin	5	5.0
Small bowel	5	5.0
Pleura	8	8.0
Nasopharynx	2	2.0
Bone	3	3.0
Omentum	1	1.0
Eye appendages	1	1.0
Stomach	1	1.0
Chest wall	1	1.0
Spleen	1	1.0
Total	**100**	**100.0**

4.2 Frequency of AFB/Fungal on stain

Out of 100, 44 patients of granulomatous inflammation are positive AFB.

Table 5: Frequency of positive Acid Fast Bacilli

Acid Fast Bacilli	Frequency(s)	Percentage (%)
Positive AFB	44	44.0
Negative AFB	56	56.0
Total	100	100.0

From 100 cases of granulomatous inflammation, about 14 patients are positive fungus.

Table 6: Frequency of positive fungus

Fungus	Frequency(s)	Percentage (%)
Positive fungus	14	14.0
Negative fungus	86	86.0
Total	100	100.0

4.3 Association of histopathology and diagnosis

Table 7: Association between histopathology features and diagnosis using chi square test.

	Tuberculosis within the histopathology features (%)	Fungal Infection within the histopathology features (%)	x^2	p - value	Df	Fisher`s Exact Test
Histopathology Features						
Langhan`s Giant Cell	69 (95.8%)	3 (4.2%)	20.651	0.001	1	0.001[a]
Caseous Necrosis	14 (93.3%)	1(6.7%)	0.788	0.375	1	0.687[a]

[a] Fisher exact test is used.

There are total 72 cases of present with Langhan`s giant cell, 69 cases with Langhan`s Giant cell diagnosed with tuberculosis which account for 95.8%, while another 3 cases diagnosed with fungal infection which account for 4.2% of cases with Langhan`s giant cell. There are total 15 cases present with caseous Necrosis, 14 are diagnosed with tuberculosis which is 93.3% and only 1 (6.7%) is diagnosed with fungal infection. By using Chi square test in the case of Langhan`s giant cell, the chi square value is large which is 20.651, therefore the difference between the expected frequency and the observed frequency is large. P-value is 0.001 and degree of freedom is 1. In this case, as more than 20% of the cases with expected frequency less than 5, we have to use fisher`s exact test and the value is 0.001. As the significant value is less than 0.05, the alternative hypothesis is accepted.Chi square test is used in the case of caseous necrosis. The p-value is 0.375 and the degree of freedom is 1. As more than 20% of the cases with expected frequency less than 5, we have to use fisher`s exact test. The significant value is 0.687, as it is more than 0.05. The alternative hypothesis is rejected.

4.4 Sensitivity and specificity of Ziehl-Neelsen (ZN) stain.

Table 8: Crosstabulation of Ziehl-Neelsen stain with tuberculosis

		Tuberculosis		Total
		Positive	Negative	
Ziehl-Neelsen Stain	Positive	44	0	44
	Negative	42	14	56
Total		86	14	100

Specificity of Ziehl-Neelsen stain $= \frac{14}{14} \times 100\%$

$= 100\%$

Sensitivity of Ziehl-Neelsen stain $= \frac{44}{86} \times 100\%$

$= 51.16\%$

Positive Predictive Value $= \frac{44}{44} \times 100\%$

$= 100\%$

Negative Predictive Value $= \frac{14}{56} \times 100\%$

$= 25\%$

The specificity is 100%. The sensitivity of Ziehl-Neelsen stain calculated in the data is 51.16%, while the positive predictive value is 100%. This means that 100% of positively stained cases in our sample is true positive and diagnosed with tuberculosis. While the negative predictive value is only 25% which means only 25% of the cases are safe to consider as true negative.

4.5 Sensitivity and specificity of Fungal stain.

Table 9: Crosstabulation of Fungal stain with fungal infection

		Fungal		Total
		Positive	Negative	
Fungal stain	Positive	13	2	15
	Negative	1	84	85
Total		14	86	100

Specificity of Fungal stain $= \frac{84}{86} \times 100\%$

$= 97.7\%$

Sensitivity of Fungal stain $= \frac{13}{14} \times 100\%$

$= 92.6\%$

Positive Predictive Value $= \frac{13}{15} \times 100\%$

$= 86.7\%$

Negative Predictive Value $= \frac{84}{85} \times 100\%$

$= 98.8\%$

The specificity is 97.7%. The sensitivity of fungal stain calculated in the data is 92.6%, while the positive predictive value is 86.7%. This means that 86.7% of positively stained cases in our sample are true positive and diagnosed with fungal infection. While the negative predictive value is 98.8% which means 98.8% of the cases are safe to consider as true negative.

Chapter 5: Discussion

5.1 Sociodemographic and clinicopathologic parameters distribution

The three sociodemographic parameters that were being determined in this study are age, gender and ethnicity. Firstly, for the distribution of age of the 100 respondent, the overall mean age was 35.25 years with a median of 31.50 years. The age of respondents ranged from 1 to 75 years. The respondents were divided into eight age groups, which were 1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, and 71-80 years old. Most of respondent came from age group 21-30 (28%), followed by age group 31-40 (18%), 51-60 (14%), 41-50 (13%), 11-20 (12%), 61-70 (6%), 1-10 (5%) and the least age group was 71-80 years old (4%). From the research have been done by (Ismail, 2004), most of the respondent came from 61-70 age groups which can be categorized as elderly. As compared to our research, most of respondent was from young adult group age from 21 to 30 years old.

In terms of gender, most of the respondents were females (51%) while males respondents with (49%). From study by (Ismail, 2004), males are more predominance compared to females with a ratio of 60:40. However, research done by (M.MudassarMajeed, M. Bukhari, 2011) showed that female respondents (62%) were more that male respondent (38%) which is consistent with our research finding.

While, majority of the respondents were Malays (63%) followed by others (16%), Chinese (14%) and least with Indian (7%).

The clinicopathologic parameters that were being investigated in this study are type of granulomatous inflammation and site of tissue sample obtained. We divided the type of granulomatous inflammation into two categories that are necrotizing granulomatous

inflammation and non-necrotizing granulomatous inflammation. Most of the cases obtained were presented with necrotizing granulomatous inflammation (81%). Only a small percentage of the cases presented with non-necrotizing granulomatous inflammation (19%).

There were various site of tissue sample obtained which is about 17 different sites. However, majority of the tissue sample for the granulomatous inflammation cases was obtained from lymph node (37%) followed with soft tissues (13%), lungs (9%), neck and pleura each (8%), skin and small bowel each (5%), bone (3%), colon, bronchus and nasopharynx each (2%), and the least with (1%) are skeletal muscle, omentum, eye appendages, stomach, chest wall and spleen.

5.2 Frequency of AFB/Fungal on stain

Out of 100, 44 cases of granulomatous inflammation was positive AFB on Ziehl-Neelson's stain. While, the remaining 56 cases was negative AFB when stained with Ziehl-Neelson's stain. However, from the diagnosis of the patient, 42 out of 56 cases of negative AFB was false negative. Only 14 out of 56 cases are true negative AFB. So, the total of patients diagnosed with tuberculosis was 86. Research done by (M.MudassarMajeed, M. Bukhari, 2011) also showed similar result where the percentage of cases with positive AFB was less compared to cases with negative AFB on Ziehl-Neelson's stain.

On the other hand, 14 of the cases was positive fungus on GMS/PAS stain. The remaining 86 cases were negative for fungus. The total patients diagnosed with fungal infection were 14. So, there is no false negative result for fungal stain. This result showed that only a small percentage of cases was positive fungus on fungal stain. This finding is consistent with the result from research done by (M.MudassarMajeed, M. Bukhari, 2011) where only small percentage (5%) of respondent was positive fungus on fungal stain.

5.3 Association of histopathology and diagnosis

Based on our study, there was significant association between Langhan`s Giant Cell and cases of acid fast bacilli (AFB)/ fungal bodies (X^2=20.651, p=0.001). However, there was no significant relationship between Caseous Necrosis and cases of acid fast bacilli (AFB)/ fungal bodies (X^2=20.651, p=0.001). However, there was no previous study to support our finding.

According to (Phillip K. Peterson, Genya Gekker et al., 1996), the description of multinucleated giant cell in tuberculosis is attributed to Theodor Langhans in 1868, Tuberculous granulomas are associated with Langhans giant cells and the condition should not be confused with Langerhans' cell histiocytosis.

The sample we got for tuberculosis with Langhan`s Giant cell accounted 69% of the total sample which was large enough to get a significant results and clearly showed a significant association. However, when compared to sample of caseous necrosis, we only got 15 % of the sample that may not be large enough to show the association between caseous necrosis and acid fast bacilli (AFB)/ fungal bodies. This was due to caseous necrosis was very rare to identify in hospital settings, or maybe the clinicians failed to identify or was not reporting it in the patient report sheets although it was there. As there was no previous study on caseous necrosis, we still cannot concluded that there is no association between them due to the limitation of sample size unless we got a larger sample size to make the results more significant and representative of the population, but in this study, caseous necrosis showed no significant association with acid fast bacilli (AFB)/ fungal bodies.

5.4 Sensitivity and specificity of Ziehl-Neelsen (ZN) stain

Based on our study, the specificity of Ziehl-Neelsen stain towards TB was 100% while the sensitivity of Ziehl-Neelsen stain towards TB was 51.16%. In this study, we assume that all Acid fast bacilli (AFB) were tuberculosis. We also assume that the true negative cases for TB is of fungal causes as in our inclusion criteria we only include specimens that are of TB and fungal causes.

Based on study done by (Ulukanligil M., Aslan G., Tasci S), a total of 465 specimens obtained from 295 patients were analyzed and the Sixty-eight patients (23.1%) were diagnosed as having TB by culture. The Ziehl-Neelsen staining sensitivities towards TB were 67.6% (46/68). When three specimens or more were collected from the patients (76 patients, 25.8%), TB positivity was determined in 20 of them (26.3%) and the sensitivities were 80% for Ziehl-Neelsen stain. In the study done by (Ulukanligil M., Aslan G., Tasci S), it was clearly shown that the sensitivity of Ziehl-Neelsen is higher when more specimens were taken from the same patient. In this study, it was to compare how different staining methods may affect the results of sensitivity.

However in our study, the sensitivity of Ziehl-Neelsen stain was based on 100 specimen results that was diagnosed with TB and fungal and was collected from Hospital Serdang database and the sensitivity of Ziehl-Neelsen stain towards TB was 51.16%. All the specimens were collected from different patient. Moreover the sample we collected was small although the data was collected from range of four years. When we compared the sensitivity of Ziehl-Neelsen stain to the study done by (Ulukanligil M., Aslan G., Tasci S), it was clearly shown that there was slightly higher of sensitivity of Ziehl-Neelsen stain.

The differences might be due to different sampling method as suggested by (Ulukanligil M., Aslan G., Tasci S). The study done by (Ulukanligil M., Aslan G., Tasci S) in different country which was Turkey, difference in population may affected the sampling size we collected was different. Based on our study, which was done by hospital setting, it can only represent the distribution of cases from the hospital and the sensitivity calculated may varies from hospital to hospital as different area will have different prevalence of cases.

It was known that the specificity of Ziehl-Neelsen was always high while the sensitivity was always varies and therefore it was hard for clinician to exclude causes of tuberculosis in certain cases. Based on our study, only about half of the specimens in Hospital Serdang was correctly excluded. Therefore, clinician experience and skill were important in the consideration of diagnosis as Ziehl-Neelsen stain in our study had a moderate sensitivity.

5.5 Sensitivity and specificity of fungal stain

The specificity and sensitivity of fungal stain was 97% and 92.6% respectively. This was expected from our study as we knew that fungal stain was highly specific and sensitive. (Haque A., 2010) We must exclude fungal causes of granulomatous inflammation if it was negatively stained.

The remaining cases that were not fungal stain sensitive might be due to technical errors while staining. In our study, we took GMS and PAS stains as the fungal stain that we studied. . GMS and PAS were two most commonly used stain in cytology specimens to differentiate fungal infection. However, GMS was more preferred over PAS. (Haque A., 2010) But in the clinical settings, both of the stains were usually ordered together, while sometimes only GMS was used instead. GMS and PAS staining on fungi was based on different principles, and hence depends on different characteristic that was present from the fungi. There might be slight variation between them and hence contributed to uncertainties in staining. Overall, their specificity and sensitivity were relatively high.

5.5 Limitation

The result of this research does not represent the status of disease in this country population since the research is conduct in hospital based setting. Besides that, eventhough we manage to get the data for 4 years duration, the number of samples collected was still not big enough for us to draw an appropriate conclusion for this study.

5.6 Conclusion

Based on our present study, the distribution we got that most of respondent with granulomatous inflamation cases came from age group 21-30 and the least age group was 71-80 years old. Majority of the respondents were Malays (63%) followed by others (16%), Chinese (14%) and least with Indian (7%). In term of gender, male and female are of almost equal distribution. In term of clinicopathology features, most of the cases we obtained were of necrotizing granulomatous inflammation. There were many sites of tissues which granulomatous inflammation was arising but the most common site was lymph node. In term of frequency, tuberculosis had a frequency of 86 cases while fungal only had 14 cases.

Based on our study, we found that there was significant association between Langhan`s Giant cell and cases of acid fast bacilli (AFB)/ fungal bodies. So, research hypothesis which state that there is a relationship between histopathologic features (Langhan`s Giant cell) and acid fast bacilli (AFB) or fungal infection was accepted. There was no significant association between caseous necrosis and cases of acid fast bacilli (AFB)/ fungal bodies. So, research hypothesis which state that there is a relationship between

histopathologic features (caseous necrosis) and acid fast bacilli (AFB) or fungal infection was rejected.

The specificity of Ziehl-Neelsen stain to acid fast bacilli was expected high from previous study and same as calculated in our study. However, the sensitivity calculated in our study was 51.6%. The sensitivity may different from studies to studies due to different study settings. However, the results of sensitivity studies of Ziehl-Neelsen always showed that it was lack of sensitivity in detecting acid fast bacilli (AFB).

The specificity and sensitivy of fungal stain to fungal was expected high based on the finding from previous study. The specificity and sensitivity that we have calculated was 97% and 92.6% respectively and hence we can conclude that the statement that fungal stain were highly specific were true.

In summary, the pathologist might need to repeat the Ziehl-Neelsen's stain twice if it is negatively stain before on the cases of granulomatous inflammation with the present of Langhan's Giant cell. This is in order for us to rule out the possible causes and come out with an accurate diagnosis as there was significant relationship between Langhan's Giant cell with acid fast bacilli and fungal bodies.

5.7 Recommendation

We recommend our study to be done in future as this kind study can help the clinician in hospital to know the distribution of granulomatous inflammation cases in term of sociodemographic factors and frequency of granulomatous inflammation cases throughout the years. This information can be very helpful in term of logistic preparation of the hospital settings. We can also know whether there is a rise or fall in cases of granulomatous inflammation from years to years. Also, knowing the significant association between the histopathology and cases of TB/Fungal may further help clinician in diagnosis and put more attention in certain histopathology of suspected cases.

We would like to recommend that a larger sample size to be collected to get a more accurate and significant sensitivity. In term of methodology, we would like to recommend that to choose a hospital with more cases if known, if possible the study setting should be widen to an area instead of hospital setting so that to get more sample size. To get a better sensitivity that can represent for a population, the inclusion criteria should include granulomatous inflammation cases other than tuberculosis and fungal causes so that we can know the exact true negative and false negative other than assuming them. Also, different type of stains that stain for the same histopathology should be separately analyzed so that to know the sensitivity and specificity of each and which stain was more preferred according to the results.

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APPENDIX

Dummy Table

Table 10: Distribution of age of patients

Age of patients (years)	Granulomatous inflammation	Percentage of granulomatous inflammation (%)

Table 11: Distribution of gender of patients

Gender	Frequency	Percentage (%)
Male		
Female		

Table 12: Frequency of acid fast bacilli on granulomatous inflammation cases

Acid fast bacilli	Frequency	Percentage (%)
Positive for AFB		
Negative for AFB		
Total		

Table 13: Frequency of fungus on granulomatous inflammation cases

Fungus	Frequency	Percentage (%)
Positive for fungus		
Negative for fungus		
Total		

Table 14: Relationship of acid fast bacilli with caseation necrosis

Caseous necrosis	Acid fast bacilli		Total
	Positive for AFB	Negative for AFB	
Present			
Not present			
Total			

Table 15: Relationship of fungus with caseation necrosis

Caseous necrosis	Fungus		Total
	Positive for fungus	Negative for fungus	
Present			
Not present			
Total			

Table 16: Relationship of acid fast bacilli with Langhan's giant cells

Langhan's giant cell	Acid fast bacilli		Total
	Positive for AFB	Negative for AFB	
Present			
Not present			
Total			

Table 17: Relationship of fungus with Langhan's giant cells

Langhan's giant cell	Fungus		Total
	Positive for fungus	Negative for fungus	
Present			
Not present			
Total			

Gantt chart

Months / Events	MARCH	APRIL	MAY	JUNE	JULLY	AUGUST
Writing proposal	X					
Presentation		X				
Preparing ethical approval to organisation		X				
Data collection			X	X	X	
Data analysis					X	X
Presentation					X	
Report writing					X	X
Presentation						X

Proforma

Tick (√) the appropriate.

Patient ID: _____ **Age**: _____

Sex:

☐ Male

☐ Female

Race:

☐ Malay

☐ Indian

☐ Chinese

☐ Others: _____

Type of granuloma:

☐ Granuloma with necrosis

☐ Granuloma without necrosis

Site of tissue sample: _____

Special stain test:

i. Ziehl-Neelson stain:

☐ Positive

☐ Negative

ii. GMS/PAS stain:

☐ Positive

☐ Negative

Histopathologic features:

i. Caseous necrosis: ☐ Present ☐ Absent

ii. Langhan's giant cell: ☐ Present ☐ Absent

JKEUPM Ref No. : FPSK_Mei(13)13 (undergraduate)

Members of the JKEUPM who reviewed the documents:

Dr.Rojanah Kahar

Date of approval: 4/6/2013

Endorsed at JKEUPM Meeting on **7/6/2013**, attended by:

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Prof. DrZamberiSekawi	Medical Microbiologist & Deputy Dean of Research and Internationalization, Faculty of Medicine and Health Sciences	Male	
Prof. Dato' Dr. Lye Munn Sann	Medical Statistician, Dept of Community Health, Faculty of Medicine and Health Sciences	Male	√
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