UNIVERSITI PUTRA MALAYSIA

Immunophenotyping Analysis in Patient Suspected with Multiple Myeloma at Hospital Kuala Lumpur from year 2011 to 2012. (GROUP 31)

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IMMUNOPHENOTYPING ANALYSIS IN PATIENT PRESUMED WITH MULTIPLE MYELOMA AT HOSPITAL KUALA LUMPUR FROM YEAR 2011 TO 2012

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ABSTRACTS

INTRODUCTION: Multiple myeloma is a cancer of plasma cells. Current diagnosis criteria required morphological assessment of plasma cell percentage. Flowcytometryimmunophenotyping is a newer and additional method for diagnosis of multiple myeloma and other plasma cell disorder.

OBJECTIVE: This study aim to determine the type and frequency of antigen expression by flow cytometry in patients presumed with multiple myeloma and correlates its with patient sociodemographic and clinical presentations at diagnosis.

METHODOLOGY: This was a retrospective,cross-sectional study conducted in Kuala Lumpur Hospital from 25th March 2013 till 5thSeptember 2013. Standardized proforma were used to extract secondary data from immunophenotyping reports from laboratory haematology of Kuala Lumpur Hospital.

RESULTS:A total of 141 patients were included in this study. The age was range 19-55 years old. There were 82 male patients as compare to 59 female.Majority of them were Malays(55.3%) followed by Chinese (29.8%) and Indian(9.9%). Anaemia was the common presentations (57.4%). Analysis showed more than half of patients had aberrant antigen expression of CD33, CD56 and CD117 comprise of 14.2%, 59.6% and 37.8%, and respectively. There was a significant association between gender and CD33 and CD56 antigen expression of p=0.027,p= 0.020 respectively.

CONCLUSION: This study highlights the role of flowcytometry in providing a rapid and accurate result in addition to classical methods used in determining diagnosis and prognosis of patients with presumed multiple myeloma. However, further studies are needed to collaborate the relationship between FCM immunophenotype with the underlying genetic alterations.

KEYWORDS:immunophenotyping, multiple myeloma, antigen expression.

ANALISA *IMMUNOPHENOTYPING* DALAM KALANGAN PESAKIT YANG DI SUSPEK MENGHIDAPI *MULTIPLE MYELOMA* DI HOSPITAL KUALA LUMPUR DARI TAHUN 2011 KE 2012.

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Abstrak

PENGENALAN: *Multiple myeloma* adalah kanser plasma .Kriteria diagnosis semasa memerlukan penilaian morfologi peratusan sel plasma. *Flowcytometry Immunophenotyping* adalah satu kaedah baru dan tambahan untuk diagnosis *multiple myeloma* dan gangguan sel plasma yang lain.

OBJEKTIF: Matlamat kajian ini adalah untuk menentukan jenis dan kekerapan ekspresi antigen menggunakan *flowcytometry* di kalangan pesakit yang disuspek dengan multiple myeloma dan hubung kait dengan cirri-ciri klinikal dan faktor sosiodemografi semasa diagnosis.

METODOLOGI: Ini adalah kajian *Cross Sectional* yang dijalankan di Hospital Kuala Lumpur pada 25 Mac 2013 hingga 5 Sep 2013. Proforma yang diselaraskan telah digunakan untuk mendapatkan data sekunder daripada laporan *Immunophenotyping* dari makmal hematologi Hospital Kuala Lumpur.

KEPUTUSAN: Sebanyak 141 pesakit telah terlibat di dalam kajian ini. Antara jumlah itu, seramai 59 orang perempuan dan 82 orang lelaki, berumur dari 19 hingga 55 tahun telah terlibat dalam kajian ini. Kebanyakan mereka adalah orang Melayu (55.3%) diikuti oleh Cina (29.8%) dan India (9.9%). Anemia menunjukkan pada kadar umum (57.4%). Analisia menunjukkan lebih separuh daripada pesakit mempunyai antigen CD33, CD56 dan CD117 sebanyak 14.2%, 59.6% dan 37.8% masing-masing. Terdapat hubungan yang signifikan antara jantina dan CD33 dan CD56 dengan catatan $p = 0.027$, $p = 0.020$ masing-masing.

KESIMPULAN: Kajian ini menekankan peranan *flowcytometry* dalam menyediakan keputusan yang cepat dan tepat di samping kaedah klasik yang digunakan dalam menentukan diagnosis dan prognosis pesakit disuspek *multiple myeloma*. Walau bagaimanapun, kajian lanjut diperlukan untuk mengaitkan hubungan antara *immunophenotype flowcytometry* dengan perubahan genetik asas.

KATA KUNCI: *Immunophenotyping*, *multiple myeloma*, ekspresi antigen.

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Chapter 1: Introduction

1.1Title

Immunophenotyping Analysis in Patient Presumed with Multiple Myeloma at Hospital Kuala Lumpur from year 2011 to 2012.

1.2 Background

Multiple myeloma is a cancer of plasma cells. In Malaysia incidence rate of multiple myeloma in 2007 was about 0.7% in males and 0.5% in females(Malaysia National Cancer Registry Report 2007). Patients usually presented with recurrent infection, pathological fracture, and anaemia multiple myeloma is characterized by suppressed production of other blood cells and produced large quantities of abnormal immunoglobulins. These abnormal immunoglobulin is useless for immune reaction. On top of that, the abnormal immunoglobulin lead to hyperviscosity which may cause blockage of the blood vessels and can cause damage to the organs for example kidney damage(C.P. Dancaster et al,1959).

The traditional methods to diagnose multiple myeloma is based on 3 criteria,which are bone marrow aspirate,biochemical and radiology assessment. Bone marrow aspirate shows increased plasma cell up to more than 30% with presence of form of abnormal plasma cells. Biochemical analysis,showed raised serum paraprotein, and presence of free light chain protein in BJP. Radiological finding showed lytic lesion which may lead to pathological fractures . In advanced cases, the patient will also have decreased serum albumin and increased beta2 microglobulin. These laboratory findings are used for diagnosis and prognostic assessment in multiple myeloma(C.P. Dancaster et al,1959).

Flow cytometry is defined as the simultaneous measurement of multiple physical characteristics of a single cell as the cell flows in suspension through a measuring device.

Flow cytometry can identify different types of cell based on the antigen presented on the surface of the cells as different cells have different types of antigen presentation. However , as the cells undergoes a maturation process some of the antigen will change. Abnormal cells in bone marrow as found in lymphoma ,myeloma ,cancerous and non-cancerous cells can be identified via flow cytometry(B.Paiva et al,2010).

In flow cytometry, the cell is treated with specific antibodies. These antibodies tag with flurochromes will only bind to specific antigen of the cells. The treated cell will pass through a laser beam. The cells bond with the flurochromes antibodies will give off light and it will be counted by the computer(B.Paiva et al,2010).

Flow cytometry is a newer method being used for diagnosis and monitoring in patients with multiple myeloma. Normally the markers used to identify the myeloma cells are CD79a,CD138,CD38 and CD19. They helps to differentiate among normal,malignant and reactive plasma cells as well as evaluating the risk of progression from monoclonal gammopathy of unknown significance (MGUS) to multiple myeloma. Information gathered from flow cytometry is also used in identifying new targets for multiple myeloma therapy.Antigen expression in multiple myeloma is divided into aberrant and classical. The classical antigen are CD38 +, CD138+, CD19+/-, CD 20 +/- and CD45 +/-. While for the aberrant antigens are CD56, CD117 and CD33 (B.Paiva et al,2010).

Mutiple myeloma is treated with combination of several chemotherapy such as melphalan and thalidomide. Stem cell tansplantation is suitable for young patient. Supportive treatment such as rehydrate,dialysis,biphosphonate and a transfusion may also be given to prevent the complication from multiple myeloma(S A W Fadilah et al,2010).

1.3 Problem statement

Flow cytometry was the newer method being used to diagnose and monitoring patients with multiple myeloma (http://ajcp.ascpjournals.org/content/137/3/377.full(28 march 2013)).Majority of studies on phenotype of multiple myeloma and frequency of antigen expressions by FCM(flow cytometry) are from western countries. A study about

immunophenotyping analysis in patient presumed with multiple myeloma at Hospital Kuala Lumpur from year 2011 to 2012 has not been reported . Thus, reliability of identifying all multiple myeloma cases by FCM screening panel remains a challenge. This study can provide a baseline data on the antigen expression among patients with multiple myeloma in Malaysia. In this study, we assessed the type and frequency of antigen expression in multiple myeloma in Malaysia. The incidence of aberrant phenotypes is still controversial and divergent results have been found by different groups probably due to diversity of monoclonal antibody (MoAbs) panel and sampling size. We explored the occurrence of aberrant phenotypes and correlated these findings with patient's clinical features (demographic data and clinical presentations) (NWCI van de Donk 1et al,2012).

1.4. Objective

1.4.1General objective

– To determine the type and frequency of antigen expression by flow cytometry immunophenotyping in patients presumed with multiple myeloma

1.4.2 Specific objective:

-To determine the distribution of the respondent by:

a)socio demographic factor(gender,race and age)

b) Clinical presentation (pathological fracture ,anemia and recurrent infection)

c) Frequency of antigen expression by flow cytometry in patients presumed withmultiple myeloma

-To determine the association between socio demographic factors (gender,raceand age) and aberrant antigen expression.

- To determine the association between clinical presentation (pathologicalfracture,anemia and recurrent infection) and aberrant antigen expression.

1.5 Research Hypothesis

Null Hypothesis:

a) There was no association between socio demographic factors (gender,race and age) with aberrant antigen expression in multiple myeloma presumed patient.

b) There was no association between clinical presentations(pathological fracture,anemia and recurrent infection) with aberrant antigen expression in multiple myeloma presumed patient.

Alternative Hypothesis

a) H1: There was an association between socio demographic factors (gender,race and age) with aberrant antigen expression in multiple myelomapresumed patients.

b) H2: There was an association between clinical presentations (pathological fracture,anemia and recurrent infection) with aberrant antigen expression in multiple myelomapresumed patients.

Chapter 2 : Literature Review

2.1 Definition

Historically, multiple myeloma was described in the 1840s by physicians who observed softening of the bones and infiltrated bone marrow in postmortem specimens(Kyle RA et al,2003)The Bence Jones protein in urine was first described by Physician of British in 1847(Bence Jones H.chemical pathology.Lancet 1847).While the discovery of serum monoclonal protein in patients with plasma cell dyscrasias was made by Waldenstrom in 1960s(Waldenstrom J,1961) . Multiple Myeloma (myelomatosis) is defined as a malignant disease of the bone marrow (Oxford Conise medical dictionary Elizabeth A and Martin MA,sixth edition,2002).Myeloma is a clonal B cell malignancy characterized by aberrant expansion of plasma cells within the bone marrow, as well as cortical bone and other extramedullary sites(S A W Fadilah et al,2010).This neoplastic proliferation leads to excessive accumulation of abnormal plasma cell in the bone marrow, the presence of abnormal monoclonal protein in the serum or free light chain (Bence-Jones protein) in urine. It also causing tissue damage and typical lytic deposits in the bone that causes hole appearance on X-ray(Hoffbrand AV et al,2006).

2.2 Epidemiology

Multiple myeloma accounted for 0.8% of all cancer diagnoses and 0.9% death in cancer patients worldwide in 2002(Parkin DM et al,2002).Myeloma accounted for 20% of all deaths from haematological malignancies. (Kyle RA et al,2003)In addition, multiple myeloma was at 8th and 9thof cancer deathin males and females respectively worldwide. (World Health Organization : Burden of death in 2004).In South East Asia(SEA),multiple myeloma was ranked 4th and 19thfor males and females respectively. In Malaysia, 16.8 % of patientswerediagnosed with multiple myeloma from overall cancer registry in 2007(Malaysia National Cancer Registry Report 2007).A cross-sectional design study was conducted in Ampang Hospital in Kuala Lumpur showed that 6out of 105 (5.7%) were diagnosed with multiple myeloma(S A W Fadilah et al,2010).

2.3 Factors associated with multiple myeloma

2.3.1 Sociodemographicfactors with multiple myeloma

Gender,age and race are the known sociodemographic factors associated with multiple myeloma.In United States,the American Cancer Society's estimated 22,350 new cases (12,440 in men and 9,910 in women) will be diagnosed with multiple myelomaand about 10,710 number of deaths (6,070 in men and 4,640 in women) due to multiple myeloma.A study among African Americans also showedthe highest incidence rate among males as compared to females(Parkin DM et al,2002). In Malaysia, the incidence rate of multiple myeloma in 2007 is about 0.7% in males and 0.5% in female(Malaysia National Cancer Registry Report 2007).

A significant association between age and multiple myeloma have reported by cross-sectional study at the tertiary referral centre of Ampang Hospital, Kuala Lumpur, involving 105 patients(SA Fadhilah Wahid,2010).The study found that ages of multiple myeloma patients were significantly higher when compared to other haematological cancer patients which was 65.08 years old versus 49.23 years old ($P = 0.012$).Regarding to the Malaysia Cancer Statistic in 2007, Malay citizenshad higher incidence rate of having multiple myeloma (1.35% in males, 0.8% in females) as compared to others ethnic (Malaysia National Cancer Registry Report 2007).

2.3.2.Personal lifestyle with multiple myeloma.

Obesity,diet and smoke are known personal lifestyle factors associated with multiple myeloma.

Obesity is characterized by World Health Organization as having Body Mass Index (BMI) of ≥ 30 kg/m² while in Asian Body Mass Index (BMI) of ≥ 27 kg/m². Few studies have proved that there was a relative risk increased between obesity with multiple myeloma.For example, a case control study among the Canadian population showed a significant ($p<0.05$) increase risk of having multiple myeloma with increase categories of Body Mass Index (BMI) after adjusting for dietary and lifestyle factor, [BMI < 25 kg/m²

had an odds ratio (OR) = 1.0, a BMI between 25 kg/m² and< 30 kg/m²: OR= 1.49, BMI ≥ 30 kg/m² : OR = 2.0 with 95% confidence interval (CI)](Pan SY el at,2004) .

Alexander DD et al,2007 reported a review few studies that examined the relationship between specific food groups with multiple myeloma(Alexander D.D el at ,2007). This study showed that there was a significant association between multiple myelomaand butter intake among Yugoslavian and Italian . In another study, they discovered that there was an inverse association between eating vegetable with multiple myeloma which means that for those who have less vegetable in his/her diet, they will have a higher risk of getting multiple myeloma(Vlajinac HD el at,2003).

Several studies had shownthat there wasa relation between cigarette smoking and multiple myeloma. Brown LM et al reported that there is a significant positive association for female current smokers with multiple myeloma (OR 5 2.90, 95% CI: 1.14–7.40), but not male (OR>2.37, 95% CI: 0.98–5.74). However, this study reported that there was no association between thenumbers of cigarettes smoked per day or years smoked with multiple myeloma(Brown LM el at,2001).Alexander DD et all also statedthat findings for tobacco use do not support a causal association with multiple myeloma(Alexander D.D el at,2007).

The epidemiologic evidence for alcohol consumption and risk of multiple myeloma is limited. Furthermore, there have been no reports of statistically significant increased risks of multiple myeloma associated with alcohol consumption in general(Baris D el at,2004).

2.3.3 Environmental factors

Pesticides,organic solvent and radiation are the known environmental factors associated with multiple myeloma.

In general category, studies that aimed to evaluatethe risk of multiple myeloma and exposure to pesticides, have been inconsistent result.Some studies reported there was a positive association(Baris D el at,2004) while others reported an inverse associations. In addition, these inconsistency were contributed by the specific classes of pesticides or chemical exposures that there were evaluated(Lee WJ et al ,2004).

The epidemiologic evidence does not support a causal relationship between benzene exposure and multiplemyeloma, where a cohort and case–control studies have showed inconsistently elevated relative risk(Sorahan T,2005).

Early studiesthat evaluated the relationship between ionizing radiation from the atomic bomb and the incidence of multiple myeloma in Hiroshima and Nagasaki atomic bomb survivors found that there was abnormally increased risk(Preston DL et al ,1950-1987).

2.3.4 Genetic factors

Family history and genetic variation are the known genetic factor associated with multiple myeloma.

Several studies have reported that there wasan increment of risk of having multiple myeloma among those who have a family history of multiple myeloma.For example, a study in families of 218 multiple myeloma cases in Iceland reported that there was increased risk of multiple myeloma with 1st degree relative but not in 2nd or 3rd degree of relatives .There wasno association between having a family history of MGUS and multiple myeloma (Ogmundsdottir HM et al,2005) particularly in persons age 65 years and older (Landgren O et al,2006) .

A number of studies suggested that gene mutations, and genetic polymorphisms, may be associated with increased risk of multiple myeloma (Alexander D.D et al,2007). The cytokine growth factors interleukin-6 (IL-6) and interleukin-10 (IL-10), which are involved in inflammatory response, may be important for the pathogenesis of multiple

myeloma(Zaidi et al, 2001) .These interleukins may stimulate the myeloma cell survival and proliferationof myeloma cell lines(Zaidi et al, 2001) . Zheng C et al reported that, these polymorphisms may play a role in the development of the disease (Zheng C et al, 2001) .

2.4 Clinical presentations

Clinical presentationsof patients with Multiple myeloma are anaemia, pancytopenia, pathological fracture, recurrent infection and organ failure. Among all these clinical presentations, pathologicalfracture,anaemia, and recurrent infection are the most common features presented in the clinical setting(C.P. Dancaster et al,1959).

Increased in osteoclastic activity causes the density of the bone to decrease .Low bone density predisposes to pathological fracture and bone pain.Fractures commonly involve the axial skeleton. While bone pain, commonly with lower back pain. The patients may present with haematuria and dysuria(C.P. Dancaster et al,1959).

.In multiple myeloma, proliferation of abnormal plasma cells do secrete dysfunction immunoglobulin which are unable to mediate immune reaction, and this will leads to the disturbance of normal immune reaction.Thus, the patients will be presented with recurrent infection.Increased abnormal immunoglobulin lead to hyperviscosity of the blood and consequently blocking the blood vessel and leads to organ failure such as kidney failure.Suppression of normal blood cells production causes pancytopenia(C.P. Dancaster et al,1959).

2.5Diagnostic criteria

Multiple myeloma is diagnosed based on a combination of clinical presentation and laboratory findings as being describe by International Myeloma Working Group (IMWG) in 2003.The investigation of multiple myeloma includes haematology, biochemistry, and radiology.

International Myeloma Working Group had published a guideline for diagnosis of multiple myeloma in 2003.This diagnosis is based on three criteria which are level of M-protein in serum and/or urine,presence of abnormal plasma cells in bone marrow and impairment of organ related to myeloma.Table 1 showsthe guideline to differentiate diagnosis between MGUS and myeloma (symptomatic, asymptomatic). (Guideline by International Myeloma Working Group ,2003.)

Table 1 : Guideline of diagnosing multiple myeloma by International MyelomaWorking Group in 2003

MGUS	**Asymptomatic Myeloma**	**Symptomatic myeloma**
M-protein in serum <30 g/l	M-protein in serum >30 g/l and/or Bone marrow clonal plasma cells >10 %	M-protein in serum and/or urine**
Bone marrow clonal plasma cells <10 % and low level of plasma cell infiltration in a trephine biopsy (if done)		Bone marrow (clonal) plasma cells or biopsy proven plasmacytoma
No related organ or tissue impairment ((no end organ damage including bone lesions)	No related organ or tissue impairment (no end organ damage including bone lesions) or symptoms	Myeloma-related organ or tissue impairment (including bone lesions)

*If flow cytometry is performed, most plasma cells (> 90%) will show a neoplastic phenotype.

Some patients may have no symptoms but have related organ or tissue impairment.

** No specific concentration required for diagnosis. A small percentage of patients have no detectable M-protein in serum or urine but do have myeloma-related organ impairment (ROTI) and increased bone marrow plasma cells (non-secretory myeloma)

2.6 Laboratory investigation of multiple myeloma and its finding.

2.6.1 Haematological

Full blood count(FBC), full blood picture (FBP) and bone marrow aspirate or trephine are the traditional laboratory investigation but it still use until today. FBC showed pancytopenia and normochromic normocytic or macrocytic anemia. In FBP, the findings are rouleaoux formation, leucoerythroblastosis, and abnormal plasma cell. The abnormal plasma cell presented in 15% of the cases in FBP (Hoffbrand A.V et al,2006) . The presence of red cell rouleaux formation and leucoerythroblastosis in peripheral blood film is highly suggestive of myeloma(Hoffbrand A.V et al,2006).Bone marrow aspiration is mandatory to show and measure marrow involvement by abnormal plasma cells, although the disease may be patchy in nature and sometimes the trephine sample may provide better assessment(Hoffbrand A.V et al,2006) . The bone marrow fragments are usually hypercellular and the cell trails contain numerous abnormal plasma (myeloma) cells.(Hoffbrand A.V et al,2006) Bone marrow immunohistochemistry and flow cytometry studies are useful to confirm presence of monoclonal plasma cell population(SA fadhilah Wahid,2010). Increased plasma cell in bone marrow usually > 20% and plasma cell > 20g/L, with abnormal forms, serum calcium elevation occurs in 45% of patients.(Hoffbrand A.V et al,2006)

2.6.2 Biochemistry analysis

Assessment of the serum calcium,serum immunoglobulin,erythrocyte sedimentation rate (ESR), serum and urine protein electrophoresis, and urine sampleare useful for diagnosis and prognosis assesment .Identification and quantitation of paraprotein or M-protein in serum and urine protein electrophoresis (SPEP and UPEP) is

central in the diagnosis of myeloma. Agarose gel electrophoresis is the method used for screening for M protein, while immunofixation electrophoresis performed to characterize the types of heavy chain and light chain. Electrophoresis and immunofixation of a 24 hour urine specimen is necessary because the mass of the M-protein provides an indirect measurement of the patient's tumour mass (SA fadhilah Wahid,2010). Immunoelectrophoresis shows that the paraprotein is IgG in about 50% of cases, IgA in 25%, IgD in 1% or light chain only M-protein (20%) (Walker R et al. 2007) . Approximately 3% of myeloma is nonsecretory as measured by SPEP and UPEP but approximately two thirds of these patients have clonal free immunoglobulin light chains detectedBox by the serum free light chain (FLC) assay (Walker R et al. 2007) .The other findings are presence of paraprotein,presence of Bence Jones in urine, high ESR and C-reactive protein (CRP).Typically the serum alkaline phosphatase is normal except for patientsthat have pathological fracture.Serum creatinine raised to 20% of case and serum beta 2 microglobulin is often raised and it is useful as prognostic marker in patients with multiple myeloma.(Hoffbrand A.V et al,2006)

2.6.3 Radiological

X-ray is being used to detect the bone lesion and pathological fracture.In multiple myeloma,bone X-ray changes are seen in about 80-90% of patients.(Hoffbrand A.V et al,2006).However,absence of bone lesions does not exclude myeloma.(Hoffbrand A.V et al,2006).Osteolytic lesions occur most frequently in bones containing red marrow, and are common in the skull .

Magnetic resonance imaging (MRI) is more sensitive than a conventional skeletal survey in detecting bone lesions. It is use more frequently as part of the diagnostic work-up of myeloma(S A W Fadhilah ,2010). MRI is recommended to exclude spinal cord compression, soft tissue mass in a localized painful area or for examination of solitary plasmacytoma and smoldering myeloma(Walker R et al. 2007).

2.7 Flow cytometry

2.7.1. Introduction

Flow cytometry is a technique of quantitative single cell analysis(S. Riley and Michael Idowu).Flow cytometer was developed in the 1970's and rapidly became an essential instrument for the biological sciences In addition,use of flow cytometry in the clinical laboratory also has grown in the past decade(S. Riley and Michael Idowu). This is due to the development of user friendly, smaller,cheaper instruments and anincreased in the number of clinical applicationscontinuously(S. Riley and Michael Idowu).

Flow cytometry is defined as the simultaneous measurement of multiple physical characteristics of a single cell as the cell flows in suspension through a measuring device Bakke A.C). A. orfao et al in their study reported that flow cytometry analysis tool will probably be indispensable for a rapid assessment of cell surface and different intracellular characteristic when combined with other clinical data which can help to achieve right diagnosis and patient care (A.Orfao et al,1995).

Multiparametric flow cytometry represents a highly reproducible and objective way of assessing the expression of multiple antigens on a single cell. By comparing patterns of antigen expression on a given cell population with the patterns identified in normal cells of that type, one potentially can identify abnormalities that, if sufficiently great,may substitute for clonality studies in identifying malignancy(Brown M and Wittwer C,2000)

2.7.2 Component of the analyzer

A flow cytometer is made up of three main components which are fluidics, optics, and electronics (S. Riley and Michael Idowu).

- The fluidics system transports particles in a stream of the laser beam forinterrogation.

- The optics system consists of lasers to illuminate the particles in the sample stream and optical filters to direct the resulting light signals to the appropriate detectors.

- The electronics system converts the detected light signals into electronic signals that can be processed by the computer.

2.7.3Principle

The flow cytometry has specifically come to denote the use of fluorescence measurement,usually with a laser light source. In laser flow cytometers, light scatter is used to measure the intrinsic size and granularity of the cell. In addition, fluorescence can be used to measure extrinsic features such as specific protein expression and nucleic acid content using added reagents, such as fluorescent stains and antibodies(S. Riley and Michael Idowu).

2.7.4Understanding of light scatter and fluorescence,gating and 4-colour assay.

2.7.4.1 Light Scatter

Light scattering occurs when a particle deflects incident laser light(S. Riley and Michael Idowu). The extent towhich this occurs depends on the physical properties of a particle,its size and internal complexity(S. Riley and Michael Idowu). Factors that affect light scattering are the cell's membrane, nucleus, and any granular material inside the cell. Cell shape and surface topography also contribute to the total light scatter(S. Riley and Michael Idowu).

Forward-scattered light (FSC) is proportional to cell-surface area or size. FSC is a measurement of mostly diffracted ligh(S. Riley and Michael Idowu)t. It is often used in immunophenotyping to trigger signal processing side-scattered light (SSC) (S. Riley and Michael Idowu).This SSC proportional to cell granularity or internal complexity(S. Riley and Michael Idowu).SSC is a measurement of mostly refracted and reflected light that occurs at anyinterface within the cell where there is a change in refractive index(S. Riley and Michael Idowu).

Correlated measurements of FSC and SSC can allow for differentiation of cell types ina heterogeneous cell population(S. Riley and Michael Idowu). Major leucocyte subpopulations can be differentiatedusing FSC and SSC (S. Riley and Michael Idowu)

2.7.4.2 Fluorescence

A fluorescent compound absorbs light energy over a range of wavelengths that ischaracteristic of that compound(S. Riley and Michael Idowu). This absorption of light

causes an electron in thefluorescent compound to be raised(S. Riley and Michael Idowu). Transition of excess energy from decay electron to its ground state as a photon of light is called fluorescence(S. Riley and Michael Idowu).The range over which a fluorescent compound can be excited called asabsorptionspectrum (S. Riley and Michael Idowu) .

The argon ion laser is commonly used in flow cytometry because the 488-nm lightthat it emits excites more than one fluorochrome(Introduction to Flow Cytometry:A Learning GuideManual Part Number:11-11032-01 April,2000©Becton, Dickinson and Company).Fluorochrome is a fluorescent dye. The amount of fluorescent signal detected isproportional to the number of fluorochrome molecules on the particle(Introduction to Flow Cytometry:A Learning GuideManual Part Number:11-11032-01 April,2000©Becton, Dickinson and Company).When a fluorescent dye is conjugated to a monoclonal antibody, it can be used toidentify a particular cell type based on the individual antigenic surface markers onthe cell .In a mixed population of cells, different fluorochromes can be used to distinguish separate subpopulations.

The staining pattern of each subpopulation, combined with FSC and SSC data, can be used to identify which cells are present in a sample and to count their relative percentages.Examples of the fluorochromes are fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP) and allophycocyanin (APC) (Introduction to Flow Cytometry:A Learning GuideManual Part Number:11-11032-01 April,2000©Becton, Dickinson and Company).

2.7.4.3Gating

A gate is a numerical or graphicalboundary that can be used to define the characteristics of particles(S. Riley and Michael Idowu).. For example, to restrict your analysis to only the lymphocytes in a blood sample containing a mixed population ofcells,based on FSC or cell size, a gate can be set on the FSC vs SSC plot to allow analysis only of cells the size of lymphocytes. The resultalso can display reflect of the fluorescence properties of only the lymphocytes(S. Riley and Michael Idowu).

2.7.4.4 4-colour assay compensation

The staining pattern of each subpopulation, combined with forward scatter(FSC) and side scatter (SSC) data, can be used to identify which cells are present in a sample and to count their relative percentages. The cells can also be sorted if necessarily (S. Riley and Michael Idowu).CD19-FITC, CD138-PE,CD45- PerCP,CD38-APC are combination that areusually used. The 4-colour assay of the screening panel for multiple myeloma inHospital Kuala Lumpur are shown in table 2.

Table 2: Multiple Myeloma panel used in Hospital Kuala Lumpur

F1	F2	F3	F4
CD20	CD138	CD45	CD38
CyKappa	CyLambda	Cd45	CD38
CD138	CD33	CD45	CD38
CD138	CD117	CD20	CD38

2.7.5 Clinical applications of flow cytometry in haematology

2.7.5.1 Diagnosis of haematological maglinancies

Flow cytometry became an essential tool in the diagnosis of haematologic and lymphoid maglinancies(Todd WM,2002).In most cases its help in confirming specific diagnosis, when a classic pattern ispresent(Todd WM,2002).Monoclonalantibodies against leukocyte common antigen (CD45) are often included in the panel to differentiate hematological malignancies from other neoplasms.Its also help to detect populationsof blast cells, since almost all leukemic cell populations exhibit decreased (dim) CD45 expression compared to normal leukocytes(Stetler-Stevenson M et al,2001).The unique capability of flow cytometry to rapidly analyze, even in small samples, multiple cell properties simultaneously such as size, granularity, surface and intracellular antigens and DNA content allow for increased sensitivity in the detection of neoplastic cells .The information should contribute to improve the accuracy and precision in the diagnosis and

classification of leukaemia, lymphomas and lymphoprolferative disorders(Stetler-Stevenson M et al,2001; Ben-Ezra J,2002).

However, flow cytometry is an adjunct to the clinical history and the microscopic examination of cells(Stetler-Stevenson M et al,2001; Ben-Ezra J,2002).

2.7.5.2 Detection of minimal residual

Minimal residual malignant cells are believed the source of disease relapse in many patient(Deptala A and Mayer SP,2001 ; Paietta E,2002).Flow cytometry is used for detection of minimal residual disease (MRD), the persistence of malignant cells in the bone marrow or other tissues after remission(Deptala A and Mayer SP,2001 ; Paietta E,2002).

2.7.5.3 Reticulocyte enumeration

Reticulocytosis occurs in anaemic patients with a functional bone marrow.It is marked by an increased number of peripheral blood reticulocytes,reticulocytopenia occurs in anaemic patients with a dysfunctional bone marrow and is marked by decreased numbers of peripheral blood reticulocyte(Paietta E,2002). Flow cytometry used to obtain information about the functional integrity of the bone marrow(Paietta E,2002). In addition,it is also benificialin monitoring bone marrow regenerative activity after chemotherapy or bone marrow transplantation(Paietta E,2002).

2.7.5.4 Platelet function analysis

The flow cytometer has been essential for the analysis of plateletstructure and function in the research laboratory(Koepke JA,1999).

2.7.6 Specific application in multiple myeloma

2.7.6.1 Multiple Myeloma diagnosis

The use of multiparametric flow cytometry in multiple myeloma diagnostic laboratories is restricted to clinical research studies. It is useful in confirming a

differential diagnosis of uncommon cause of plasma cell disorder. (Perz-Andres M et al,2005).Plasma cell immunophenotyping is best determined by the multiparametrics flowcytometry in at least 3-colour assay which are include CD138,CD38 and CD45 together (Lin P et al,2004).In addition, Rawstron A.C et al,2008 stated that combining of those 3 antigens together with light scatter characteristic would provide the optimal detection rate detection rate of plasma cell disorder.(Rawstron A.C et al,2008). CD138 was reported to be a sensitive marker for identification of plasma cells(Lin P et al,2004).

The monoloclonal CD34 and HLA-DR antigens are markers for hematopoietic stem cells used for the diagnosis haematological maglinancy(Widjenes J et al,1998). CD45 are often included in the panel to differentiate haematological malignancies from other neoplasms (Todd WM,2002).CD38,CD138 and CD45 are the monoclonal antibody usually used for assessment of patient suspected multiple myeloma (Rawstron A.C et al,2008).CD138(syndecan-1) is a trans membrane heparin sulfate proteoglycan that typically expressed by plasma cell not by T or B cell(Widjenes J et al,1998). CD38 is not specific marker for multiple myeloma. It is also can be also detected on haemopoietic stem cells and B and T cells because CD38 neoplastic plasma cell is expressed in low intensity compared to normal plasma cells and might be undistinguish from contaminated T or B cells(Lima M et al,2000).

2.7.6.2 Differential diagnosis plasma cell disorder,multiple myeloma,Monoclonal Gammopathy of Undertermined significance (MGUS) and reactive conditions

Enumeration of plasma cell in bone marrow and demonstration abnormal phenotype together with the presence of monoclonal immunoglobulinis important in assisting of diagnosis multiple myeloma and associated disorder(Rawstron A.C et al,2008).The parameter that can be used are plasma cell as percentage of total leucocyte, plasma cell as expression byimmunophenotype, plasma cell clonality, and abnormal plasma cell percentage as a total plasma cel(Rawstron A.C et al,2008).Enumeration of plasma cell by using flow cytometry more reproducible and reliable in predicting the outcome in multiple myeloma as compared to morphological assessment since lager number of cell are analyzed and less operator bias(Rawstron A.C et al,2008).

2.7.6.3 Predictor of independent prognostic marker in Monoclonal Gammopathy of Undertermined significance (MGUS)

Asymptomatic multiple myeloma and patient with Monoclonal Gammopathy of Undertermined significance (MGUS) may transform to multiple myeloma. Evalutaing of abnormal plasma cell percentage to total plasma cellsin bone marrow is important in differentiating this haemotology disorder(Rawstron A.C et al,2008).

2.7.6.4 Minimal residual disease (MRD) detection in multiple myeloma .

Percentage of the abnormal plasma to total plasma cells or total leucocytes identified by immunophenotyping is used in monitoring minimal residual disease(MRD) in patient with multiple myeloma after treatmen.(Rawstron A.C et al,2008).

2.7.6.5 Prognostic marker in patients with multiple myeloma patient

Diagnosis of multiple myeloma is based on evaluating of the specific antigens by abnormal plasma cell such as CD45,CD117,CD33,CD28 and etc (Rawstron A.C et al,2008). The CD28 is not expressed on normal B cells or plasma cells and according to Shapiro et al, its presence is related to advanced disease.Expression of CD28 was associated with high plasma cells proliferative activity. The Expression of both CD19 and CD28 as well as the absence the CD117 were associated with significantly shorter proression free-survival (PFS) and overall-survival (OS) (B.Paiva et al,2010).

However, the prognostic value of immunophenotype has not been clearly established (San Miguel JF et al,2002).Detection of circulating plasma cells and the CD45 expression pattern was reported to be highly significant prognostic factor (Rawstron A.C et al,2008). Several studies reported multiple myeloma patients with CD56 negative monoclonal plasma cells usually had aggressive disease and a worse prognosis. Lack of CD117 expression was associated with higher level of bone marrow infiltration, greater frequencies of anaemia, renal impairment (creatinine $\geq$ 2mg/dl), elevated beta2-microglobulin, nonhyperiploid cases, Igh translocation including t(11;14) and t(4;14) and del(13q)(B.Paiva et al,2010) .

There are several lists of antigen expression of aberrant or unusual plasma cell in multiple myeloma as reported by Rawstron A.C et al in 2008 which are CD19 negative for 95% of cases, CD56 strongly positive about 75% of cases, CD117 positive in 30% cases, CD20 positive in 30% cases,strongly positive CD28 about 15% - 45% cases,CD81 range from weak to negative and CD200 strongly positive(Rawstron A.C et al,2008).

CD33 is found on myeloid cell surface, but is not detected on haemopoietic stem cell. In multiple myeloma, study had shown that expression of $CD33^+$ patients was noted to have lower survival compared to $CD33^-$ patient group.

2.8Treatment

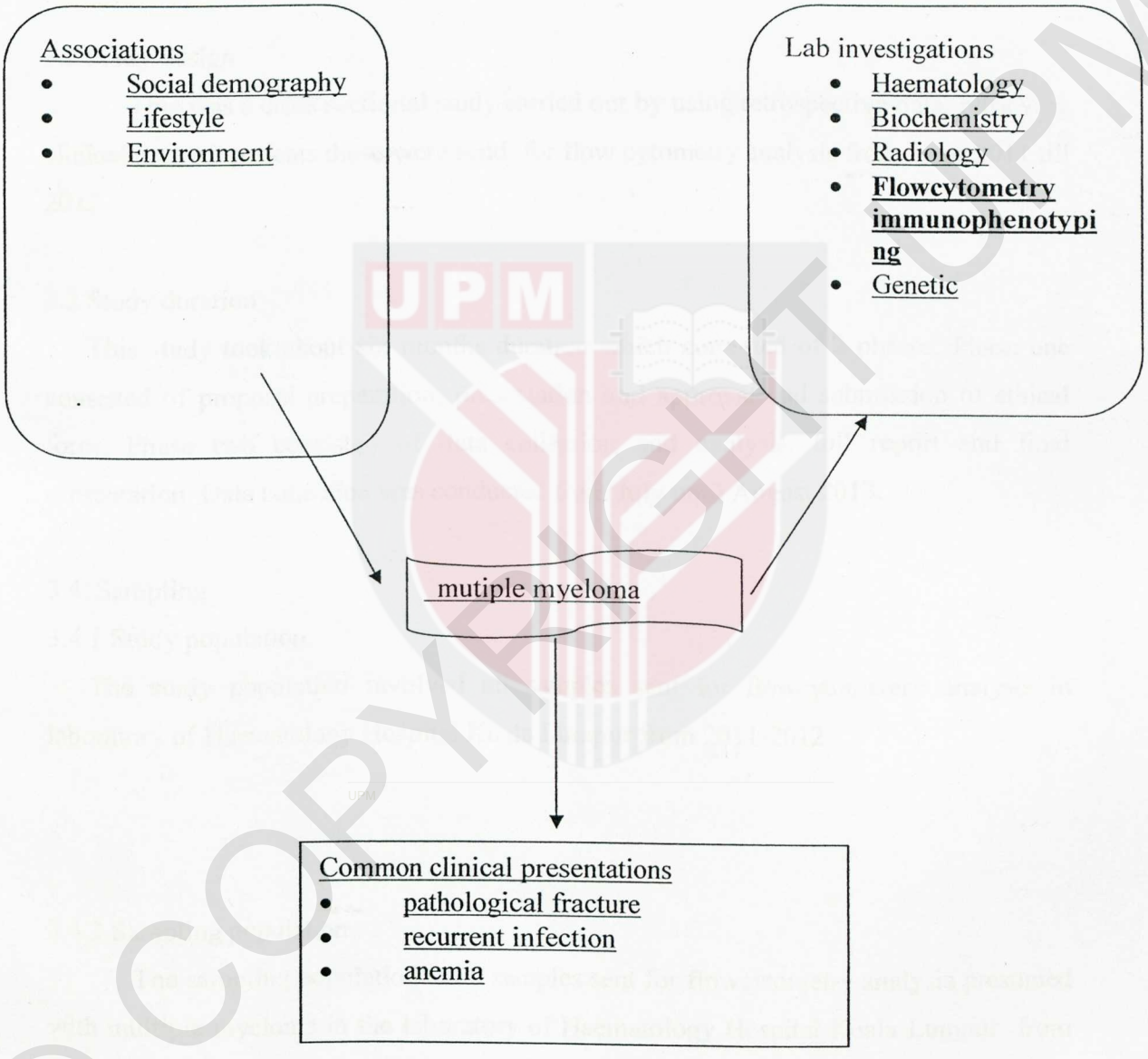
In asymptomatic patients or the early stage of multiple myeloma,no drug is given for asymptomatic patients.The practitioners should follow up their patients counseled them and close monitoring of theirclinal symptom but no treatment is give(S A W Fadilah et al, 2010)

In symptomatic patients for patients with bone disease, bisphosphonate will be given for advanced stage of multiple myeloma.Traditional chemotherapy used for treatment of multiple myeloma which include Melphalan,Vincristine,Cyclophosphamide,Etoposide,Doxorubicin,Liposomal,doxorubicinandBendamustine .Some of these drugs were combinated.Corticosteroid such as prednisone and dexamethasone is given alone or in combination with chemotherapy drugs to reduce the nausea and vomiting experienced by patients.

Newer drugs of choices are immunomodulating agents and protease inhibitors.Immunomodulating agents such as thalidomide,lenalidomide and pamolidomide is used to modulate the immune system of the patient,but the mechanism of the immunomodulating agent is still not well understand.Protease inhibitor such as bortezomib and carfilzomib works by blocking the action of protease so the cell division

will be under normal control.Stem cell transplantation is a standard treatment for younger multiple myeloma patients (S A W Fadilah et al,2010).

2.9Conceptual framework



Chapter 3: Methodology

3.1 Study location

This study was carried out in Haematology laboratory, Department of Pathology, Hospital Kuala Lumpur(HKL).

3.2 Study design

This was a cross sectional study carried out by using retrospective data, retrieving clinical data of patients those were send for flow cytometry analysis from year 2011 till 2012

3.3 Study duration

This study took about six months duration which consisted of 2 phases. Phase one consisted of proposal preparation, presentation and approval and submission of ethical form. Phase two consisted of data collection and analysis, full report and final presentation. Data collection was conducted from July until August 2013.

3.4. Sampling

3.4.1 Study population

The study population involved all samples sent for flowcytometery analysis in laboratory of Haematology Hospital Kuala Lumpur from 2011-2012

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3.4.2 Sampling population

The sampling population is all samples sent for flowcytometry analysis presumed with multiple myeloma in the laboratory of Haematology Hospital Kuala Lumpur from 2011 to 2012

3.4.2 1 Inclusion criteria

The patient who were newly presumed with multiple myeloma based on the flow cytometry analysis record from 2011 to 2012.

3.4.2.2 Exclusion criteria

The patient who were diagnosed with other haematological malignancy or other plasma cell disorders based on flow cytometry analysis record from 2011 to 2012.

3.4.3 Sampling frame

All patients send for flowcytometry analysis in the laboratory of Haematology Hospital Kuala Lumpur from 2011-2012.

3.4.4 Sampling unit

All patients who have been presumed with multiple myeloma based on flowcytometry analysis record in HKL from 2011 to 2012.

3.4.5 Sampling method

An universal sampling method was used to analyze all data collected in our study. Data were gathered from secondary data of flow cytometry analysis analysis.

3.4.6 Sample size

Estimation of sampling size is determined by this formula:

n= sample size

$Z1 - \frac{\alpha}{2}$ = Z statistic for level confidence interval 95% = 0.96

$Z1 - \beta$ = Z statictic for 80% power =0.842

P_1 =Incidence rate multiple myeloma for males =0.7 (Malaysian National Cancer Registry Report 2007)

P_2 = Incidence rate multiple myeloma for females =0.5(Malaysian National Cancer Registry Report 2007)

$$\widetilde{P} = \frac{0.7 + 0.5}{2} = 0.6$$

$$n = \frac{\{z_{1-\frac{\alpha}{2}}\sqrt{2\widetilde{P}(1-\widetilde{P})} + Z_{1-\beta}\sqrt{P_1(1-P_1)+P_2(1-P_2)}\}^2}{(P_1-P_2)^2}$$

$$n = \frac{\{1.96\sqrt{2(\overline{0.6})(1-0.6)} + 0.842\sqrt{0.7(1-0.7)+0.5(1-0.5)}\}^2}{(0.2)^2}$$

≈ 93

3.5 Instrument and data collection

3.5.1 Instrument

Standardized proforma were used to extract data fromimmunophenotyping reports of patients presumed with multiple myeloma from 2011 to 2012 in Hospital Kuala Lumpur.

3.5.2 Data collection technique

Patients demographic data, presenting signs and symptoms, type and frequency of antigen expression was gathered from immunophenotyping reports from haematology laboratory record in Pathology department of Hospital Kuala Lumpur. All the information were filled into the proforma.

3.6 Data Analysis

Univariate analysis was carried out to determine the distribution of the sample population with respect to the socio demographic factor (gender,ethnicity,and age), clinical presentations (pathological fracture, anaemia,recurrent infection and others), and antigen expression (classical and aberrant).

For categorical and nominal variables,bivariate comparisons were conducted using Chi-square test or Fisher's exact test.Data analysis were done using Statistical Package of Social Sciences (SPSS) version 21.0

3.7 Study ethics

This study was conducted after obtained the approval from :

1.Ethical clearance from Medical Ethic Committee of the Faculty of Medicine and Health Sciences,University Putra Malaysia.

2. National Medical Research Register of the Ministry of Health

3. Deputy Dean of Medicine ,Faculty of Medicine and Health Sciences were obtained.

4. Director of Pathology department of Hospital Kuala Lumpur (heamatology unit).

5.All the data obtained will be kept confidential.

3.8 Variables

3.8.1 Dependent variable

Type and frequency antigen expression of patients presumed with multiple myeloma.

3.8.2 Independent variable

The independent variables were the :

- Socio-demographic factors such as gender, age and gender
- Common clinical features such as pathological fracture,anaemia and recurrent infection.

3.9 Limitations

3.9.1 Bias and Confounding

3.9.1.1 Selection bias occurs in this study as the samples were not representative of whole Malaysia population since the study was only restricted to patients in Kuala Lumpur Hospital.

3.9.1.2 Information bias occurs as secondary data was used, so quality control for primary data cannot be assured which will results in missing data in certain records.

3.9.2 Limitations

1. Time limitation
2. Secondary data-retrospective
3. Single centre –Hospital Kuala Lumpur
4. Lack of reference

Chapter 4 : Results

4.1 Percentage

Year 2011

Percentage of multiple myeloma $= \frac{69}{1773} \times 100\%$

$= 3.89\%$

Year 2012

Percentage of multiple myeloma $= \frac{77}{1664} \times 100\%$

$= 4.63\ \%$

4.2 Distribution of study subjects

A total of 3437 immunophenotyping request was send for investigations of suspected haematological malignancies and other plasma cell disorder. Only 141 patients which full filled the inclusion criteria of this study were selected.5.37% of the respondents showed reactive plasma cells were excluded from this study.

4.2.1 Objective 1: Distribution of study subjects by sociodemographic factors (gender, age and ethnic)

Table 3showed that the socio demographic characteristics of study subjects. There were 82(58.2%) male patients as compare to 59 (41.8%)female. Majority of them were Malay (55.3%), followed by Chinese (29.8%), Indian (9.9%) and others ethnic (5.0%) which comprise of 78, 42, 14, and & out of 141 patients respectively. Their age range was 19-55 years old. Patients in the age group of > 64 years old accounted the highest among the age group with the percentage of (29.8%).There were 29.1 % of patients in age group of 60-64 years old. 15.6% and 13.5% of them were in the age group of <50

years and 55-59 years old respectively. Only 12.1 % of them was in the age group of 50-54 years old

Table 3 : Descriptive Statistics of socio demographic (gender,gender and age)

Socio Demographic Factors	Frequency	Percentage (%)
Gender		
Male	82	58.2
Female	59	41.8
Total	141	100.0
Ethnic		
Malay	78	55.3
Chinese	42	29.8
Indian	14	9.9
Others	7	5.0
Total	141	100.0
Age		
<50	22	15.6
50-54	17	12.1
55-59	19	13.5
60-64	41	29.1
>64	42	29.8
Total	141	100.0

4.2.2 Objective 2: Distribution of study subjects by clinical presentation (pathological fracture,anemia,recurrent infection,and others)

Table IV illustrated the distribution of study subjects according to their clinical presentations. Most common presentation was anaemia which accounted for 57.4% then followed by pathological fracture (21.3%). 6.4% of patients presented with recurrent infection. The others clinical presentations of 58.2% patient presented with or without those three clinical features were renal impairment, haematuria, multiple lytic lesion and etc.

Table 4 :Descriptive statistics of clinical presentations

Clinical Presentations	Frequency	Percentage(%)
Pathological fracture		
Yes	30	21.3
No	100	70.9
Not Available	11	7.8
Total	141	100
Anaemia		
Yes	81	57.4
No	48	34.0
Not Available	12	8.5
Total	141	100
Recurrent Infection		
Yes	9	6.4
No	120	85.1
Not Available	12	8.5
Total	141	100
Others		
Yes	82	58.2
No	47	33.3
Not Available	12	8.5

Total	141	100

4.2.3 Objective 3: Distribution of study subject by the antigen expressions (classical and aberrant type)

Table 5 showed distribution of study subject according to the classical and aberrant antigen expression by immunophenotyping. Majority of the patients were expressed the aberrant antigen (67.4%) followed by the classical antigen expression about 32.6% out of 141 patients.

Table 5 : Descriptive Statistics of group antigen expressions.

Antigen Type	Frequency	Percentage(%)
Classical	46	32.6
Aberrant	95	67.4
Total	141	100

Table 6showed that distribution of respondent according to the type and frequency of antigen expression by immunophenotyping. All patients were positive for CD38 (100%). This was followed by CD138(96.5%), CD45(53.9%), CD19 (29.8%) and CD20 (7.1%). Vise versa, most of the patients immunophenotyping results showed was negative for CD20 (92.9%) followed by CD19 (70.2%), CD45 (46.1%), and CD138 (3.5%).

Table 6 :Descriptive statistics of antigen expressions

Classical Antigen	Frequency	Percentage(%)
CD138		
Positive	136	96.5
Negative	5	3.5

Total	141	100
CD38		
Positive	141	100
Negative	0	0
Total	141	100
CD45		
Positive	76	53.9
Negative	65	46.1
Total	141	100
CD19		
Postive	42	29.8
Negative	99	70.2
Total	141	100
CD20		
Positive	10	7.1
Negative	131	92.9
Total	141	100

Table 7 illustrated distribution of respondents according to type and frequency of the aberrant antigen expressions by immunophenotyping analysis Majority of the patients were positive for CD56 (59.6%) followed by CD117 (37.6%) and CD33 (14.2%).However, there were inconclusive results noted for CD33, CD56 and CD117 assessment which comprised of 49,39 and 42 out of 141 patients respectively. Cytoplasmic Kappa and lambda restriction analysis showed (44.7%) of them had cytoplasmic kappa restriction. A total of 95 respondents (53.7%) from the aberrant group, showed the cyKappa restriction. While in the classical group only 26.1% of them had cyKappa restriction. Most of the classical group had inconclusive result for light chain restriction (63.0%).

Table 7 : Descriptive statistics of aberrant antigen expressions.

Aberrant Antigen	Frequency	Percentage(%)
CD33		
Positive	20	14.2
Negative	72	51.1
Inconclusive	49	34.8
Total	141	100
CD56		
Positive	84	59.6
Negative	18	12.8
Inconclusive	39	27.7
Total	141	100
CD117		
Positive	53	37.6
Negative	46	32.6
Inconclusive	42	29.8
Total	141	100
LIGHTCHAIN RESTRICTION		
CyLambda	42	29.8
CyLambda	63	44.7
Inconclusive	36	25.5
Total	141	100.0

4.3 Descriptive and analytical analysis

4.3.1Objective 4: Association between socio demographic factors (gender, age, and ethnic) and aberrant antigen expression (CD33)

Table 8 indicated that nearly 16.9% of female patients had expressed $CD33^+$ followed by male which was 12.2%. Statistically, the result showed there was significant association between gender and CD33 antigen expression in multiple myeloma (p=0.027

Table 8 : Association between socio demographic factors with CD33

	CD33 Expression						
Socio Demographic factors	Total (n=141)	Yes n(%)	No n(%)	Inconclusive n(%)	d.f	X^2 Value	*P*
Gender							
Male	82	10(12.2)	36(43.9)	36(43.9)			
Female	59	10(16.9)	36(61.0)	13(22.0)	2	7.237	0.027
Total	141	20(14.2)	72(51.1)	49(34.8)			
Ethnic							
Malay	77	13(16.9)	34(44.2)	30(39.0)			
Non Malay	64	7(10.9)	38(59.7)	19(29.7)	2	3.321	0.190
Total	141	20(14.2)	72(51.1)	49(34.8)			
Age							
<50	22	2(9.1)	10(45.5)	10(45.5)			
50-54	17	3(17.6)	9(52.9)	5(29.4)			
55-59	19	2(10.5)	11(57.9)	6(31.6)	8	5.499	0.703
60-64	41	9(22.0)	21(51.2)	11(26.8)			
>64	42	4(9.5)	21(50.0)	17(40.5)			
Total	141	20(14.2)	72(51.1)	49(34.8)			

4.3.2Objective 4: Association between socio demographic factors (gender, age, and ethnic) with aberrant antigen expression (CD56)

Table 9 exhibit female patients had of 72.9% which expressed the CD56 while for male patients, the percentage was 50.0%..Our results showed that statistically, there is a significant association between gender and expression of antigen CD56 (p=0.020).

In the other hand, statistically result showed there is no association between age and ethnicity with expression of antigen $CD56^{+}$, $CD56^{-}$.

Table 9 : Association between socio demographic factors with CD56

	CD56 Expression						
Socio Demographic factors	Total (n=141)	Yes n(%)	No n(%)	Inconclusive n(%)	d.f	X^2 Value	*P*
Gender							
Male	82	41(50.0)	14(17.1)	27(32.9)			
Female	59	43(72.9)	4(6.8)	12(20.3)	2	7.829	0.020
Total	141	84(59.6)	18(12.8)	39(27.7)			
Ethnic							
Malay	77	42(54.5)	12(15.6)	23(29.9)			
Non Malay	64	42(65.6)	6(8.2)	16(25.0)	2	2.075	0.354
Total	141	84(59.6)	18(12.8)	39(27.7)			
Age							
<50	22	8(36.4)	5(22.7)	9(40.9)			
50-54	17	12(70.6)	0(00.0)	5(29.4)			
55-59	19	11(57.9)	2(10.5)	6(31.6)	8	12.254	0.140
60-64	41	26(63.4)	8(19.5)	7(17.1)			
>64	42	27(64.3)	3(7.1)	12(28.6)			
Total	141	84(59.6)	18(12.8)	39(27.7)			

4.3.3Objective 4: Association between socio demographic factors (gender, age, and ethnic) with aberrant antigen expression (CD117)

Table 10 indicated that there are no associations between gender, age , and ethnicity with antigen CD117 expression.

Table 10: Association between socio demographic factorrs with CD117

	CD117 Expression						
Socio Demographic factors	Total (n=141)	Yes n(%)	No n(%)	Inconclusive n(%)	d.f	X^2 Value	*p*
Gender							
Male	82	28(34.1)	25(30.5)	29(35.4)			
Female	59	25(42.4)	21(35.6)	13(22.0)	2	2.939	0.230
Total	141	53(37.6)	46(32.6)	42(29.8)			
Ethnic							
Malay	77	28(35.4)	24(31.2)	25(32.5)			
Non Malay	64	25(39.1)	22(34.4)	17(26.6)	2	0.587	0.746
Total	141	53(37.6)	46(32.6)	42(29.8)			
Age							
<50	22	6(27.3)	6(27.3)	10(45.5)			
50-54	17	9(52.9)	3(17.6)	5(29.4)			
55-59	19	6(31.6)	7(36.8)	6(31.6)	8	8.732	0.365
60-64	41	18(43.9)	16(39.0)	7(17.1)			
>64	42	14(33.3)	14(33.3)	14(33.3)			
Total	141	53(37.6)	46(32.6)	42(29.8)			

4.3.4Objective 5: Association between clinical presentation (pathological fracture,anemia,recurrent infection,others) with CD33.

Table 11 exhibit that P value for all clinical presentation were indicated that there is no association between clinical presentation with CD33 antigen expression in multiple myeloma.

Table 11 : Association between clinical presentations with CD33

	CD33 Expression						
Clinical Features	Total (n=141)	Yes n(%)	No n(%)	Inconclusive n(%)	d.f	X^2 Value	*p*
Pathological Fracture							
Yes	30	7(23.3)	14(46.7)	9(30.0)			
No	111	13(11.7)	58(52.3)	40(36.0)	2	2.641	0.267
Total	141	20(14.2)	72(51.1)	49(34.8)			
Anaemia							
Yes	81	10(12.3)	43(53.1)	28(34.6)			
No	60	10(16.7)	29(48.3)	21(35.0)	2	0.608	0.738
Total	141	20(14.2)	72(51.1)	49(34.8)			
Recurrent Infection							
Yes	9	1(11.1)	4(44.4)	4(44.4)			
No	120	19(14.4)	68(51.5)	45(34.1)	2	0.406	0.816
Total	141	20(14.2)	72(51.1)	49(34.8)			
Others							
Yes	82	11(13.4)	37(45.1)	34(41.5)			
No	59	9(15.3)	35(59.3)	15(25.4)	2	3.977	0.137
Total	141	20(14.2)	72(51.1)	49(34.8)			

4.3.5Objective 5: Association between clinical presentation (pathological fracture,anemia,recurrent infection,others) with CD56

Table 12 illustrated that the statistical results showed that there is no association between all the clinical presentations with CD56 antigen expression in multiple myeloma.

Table 12: Association between clinical presentations with CD56

	CD56 Expression						
Clinical Features	Total (n=141)	Yes n(%)	No n(%)	Inconclusive n(%)	d.f	X^2 Value	*P*
Pathological Fracture							
Yes	30	21(70.0)	3(10.0)	6(20.0)			
No	111	62(55.9)	15(13.5)	34(30.6)	2	0.583	0.747
Total	141	84(59.6)	18(12.8)	39(27.7)			
Anaemia							
Yes	81	47(58.0)	12(14.8)	22(27.2)			
No	60	34(56.7)	6(10.0)	20(33.3)	2	1.078	0.583
Total	141	84(59.6)	18(12.8)	39(27.7)			
Recurrent Infection							
Yes	9	4(44.4)	1(11.1)	4(44.4)			
No	132	77(58.3)	17(12.9)	38(28.8)	2	0.997	0.607
Total	141	84(59.6)	18(12.8)	42(29.8)			
Others							
Yes	82	46(56.1)	8(9.8)	28(34.1)			
No	59	35(59.3)	10(16.5)	14(23.7)	2	2.703	0.259
Total	141	81(57.4)	18(12.8)	42(29.8)			

4.3.6Objective 5: Association between clinical presentation (pathological fracture,anemia,recurrent infection,others) with CD117

Table 13 showed that there are no significant association between all clinical presentation studied with CD117 antigen expression in multiple myeloma.

Table 13: Associations between clinical features with CD117

	CD117 Expression						
Clinical Features	Total (n=141)	Yes n(%)	No n(%)	Inconclusive n(%)	d.f	X^2 Value	*P*
Pathological Fracture							
Yes	30	15(50.0)	9(30.0)	6(20.0)			
No	111	38(34.2)	37(33.3)	36(32.4)	2	2.868	0.238
Total	141	53(37.6)	46(32.6)	42(29.8)			
Anaemia							
Yes	81	33(30.4)	24(29.6)	24(29.6)			
No	60	20(33.3)	22(36.7)	18(30.0)	2	1.028	0.598
Total	141	53(37.6)	46(32.6)	42(29.8)			
Recurrent Infection							
Yes	9	3(33.3)	2(22.2)	4(44.4)			
No	132	50(37.6)	44(33.3)	38(28.8)	2	1.059	0.589
Total	141	53(37.6)	46(32.6)	42(29.8)			
Others							
Yes	82	32(39.0)	21(25.6)	29(19.1)			
No	59	21(35.6)	25(42.4)	13(22.0)	2	5.110	0.078
Total	141	53(37.6)	46(32.6)	42(29.8)			

Chapter 5: Discussion, conclusion and recommendation

5.1 Discussion

Although the conventional methods such bone marrow and immunohistochemistry assessments together with demonstration of monoclonal gammapathy were satisfactory for multiple myeloma diagnosis, they were lack of the clinical sensitivity of flow cytometry. Flowcytometric immunophenotyping is a rapid and dispensible tool used to characterize the unique features of biological samples. It allowed studying high number of cells characteristic within a relatively short period of time.Flowcytometry immunophenotyping helped in confirming a diagnosis of plasma cells disorders, risk stratify and prognosticates the patients.

5.1.1 Distribution of respondent by sociodemographic factors (gender,age nad ethnic)

This study aims to determine the type and frequency of antigen expression by flow cytometry in patients presumed with multiple myeloma and correlates its with patient sociodemographic and clinical presentations at diagnosis. Patients' sociodemographic analysis showed that male had a higher risk of getting multiple myeloma as compared to female.This result was similar with report from Malaysia National Cancer Registry Report 2007.Studies by G.Mateon 2008and S hapira R had also shown that male has a higher risk of getting multiple myeloma as compared to female.

Malay was the majority ethnic group which consisted of 55% of the respondents.This findings is similar with report by NCR, 2007 where it stated that the incidence of cancer according to major ethnic group in Malaysia was Malay (54.5%), followed by Chinese (24.9%),Indian (7.5%) and others (13.1%). This findings may also reflect normal Malaysian population distribution,where Malays is the majority ethnic group in the population. D Priscilla et al,2011 also showed Malay was the highest frequency of getting haematological maglinancy.This findings probably reflects of most Malay patients preferred to visit government hospital,meanwhile the non-Malay patients preferred to visit the non-government hospital.

Most of respondents in this study were older than 60 years.Olders group agegroup is one of the known risk factors of multiple myeloma.The result was consistent with the study done by M.Kimman on 2012,which shown that most of the ASEAN cancer patients were from older age group.On top of that,S A Fadhilah Wahid,2010 also reported similar findings where majority of multiple myeloma patients were from the age group 65-70 years old.

5.1.2 Distribution of clinical features in patients presumed with multiple myeloma.

Our results showed the most common clinical presentation was anaemia which accounted for 57.4% then followed by pathological fracture (21.3%), and 6.4% with recurrent infection. This findings is similar with other studies where they reported anaemia also was found in majority of the myeloma patients (Wong K.T et al,1990,Fadhilah S A W ,2010). Fahilah S.A.W also reported that bone related complications such as pathological fractures and spinal cord compression were the major causes of impaired quality of life and performance in multiple myeloma patients(Fadhilah S A W,2010).Our results also showed 58.9% patients had others clinical presentations presented with or without anaemia, pathological fracture and recurrent infection. Moreover,multiple myeloma patients were identified as having more symptoms and problems compared to other haematological cancer(Johnsen AT ,2009).The others clinical features were renal impairment, haematuria, multiple lytic lesion,dysuria, and etc.

5.1.3 Distribution of antigen expressions in patients presumed with multiple myeloma

Flow cytometry analysis had increased the sensitivity of the diagnosis of plasma cells proliferative disorders as it enabled clonality demonstration via expression of cytoplasmic light chain restriction of either kappa or lambda restriction.In this study, the patients were classified as having classical antigen expression if the analysis showed they were positive for both CD 138 and CD38 expression without any of the aberrant antigen markers (CD33, CD56,CD117). The patients were classified as aberrant group when

theywere positive for either one or more of the aberrant markers , even though they were positive for any classical marker (CD138,CD38) (Rawstron A.C et al,2008).

Simultaneous assessment of the antigen expression of the light scatter, CD45, CD38 and CD138 represented the best combination of markers for specific identification of plasma cell . These makers were used to discriminate the abnormal plasma cells from other populationsof leukocytes and other haematopoietic cells.This study revealed the frequencies and patterns of antigen expression found in the 141 patients presumed with multiple myeloma. There was slight difference in the percentage of CD 19 andCD20 expression notedinthis study as compared to previous study by Rawstron A.C et al , 2008. This was probably due to the difference in study population CD138 is an important antigen aids in plasma cell characterization and identification.

CD38 can be detected on haemopoietic stem cells,B and T cells(Rawstron A et al,1999). Neoplastic plasma cells usually express CD38 at lower intensity(Lima M et al,2000) .CD38 primarily showed the first pattern of antigen expression by immunophenotyping.Studied by Lin P et all showed that all multiple myeloma patient showed $CD38^{+}$(Lin P et al,2004).Our study also had shown the same result.CD138 was a specific marker for plasma cells in bone marrow samples(Lin P et al,2004). This antigen also showed first pattern of antigen expression with strong staining clearly separating plasma cells from other cellular components(Lin P et al,2004).Lin P et al showed that 100% of their respondent had CD138 strongly positive(Lin P et al,2004). However, our study result showed 96.5%. This may be cause of technical problems as the CD138 detection sensitivity was wide (60%-100%)(Lin P et al,2004).

CD45 was a key regulator of antigen-mediated signalling and activation in lymphocytes(Kumar S et al,2005). CD45 present in early stages of plasma cells development(Kumar S et al,2005).CD45 population dominates in newly diagnosed and relapsed multiple myeloma(Kumar S et al,2005). CD45,CD38, and CD138, 3-colour assay being used in screening multiple myeloma(Lin P et al,2004). Our study found that 53.9% patient showed $CD45^{+}$. While others studies showed 44% $CD45^{+}$(Kumar S et

al,2005). Recent studies suggesting that the clonal cell population in myeloma resides in the $CD45^+$ fraction of cells indicated the relevance of CD45 biology(Matsui W et al,20014).

CD20 is expressed during maturation process of pre-B cells(Robillard et al,2003). Normally, plasma cell does not express the CD20(Robillard et al,2003). Hayman SR et al showed that expression of CD20 in plasma cells has been associated with shorter survival rate(Hayman et al,1999).In our study result showed that 7.1% patients showed $CD20^+$. Guikenna et al also reported that only some of multiple myeloma patients expressed the CD20(Guikenna et al,2003).

CD19 found in all B cells include lymphoblast, mature B- lymphoid and most of plasma cell normally(Ocqueteau et al,1998). However, in multiple myeloma patients most of patients showed only negative to dim negative(Ocqueteau et al,1998). Our study also found that 70.2% patients had $CD19^-$ and only 29.8% expressed $CD19^+$.

CD33 was a myeloid monocytic cells. This antigen is aberrantly expressed in multiple myeloma. Few studies have reported CD33 expression on plasma cells but the reactivity of the marker has been found in 6.5–12% of myeloma patients (Mateo et al, 2005; Almeida et al, 1999). CD33 expression in myeloma patients correlates with clinical parameters, suggesting its clinicopathological significance. In a study said, patient with $CD33^+$ have higher incidence of having anaemia and thrombocytopenia than $C33^-$ (Kumar S et al,2010).$CD33^+$ also been said significantly had a shorter survival rate as compared to the $CD33^-$(Kumar S et al,2010). The other significant difference observed between the two groups was in the level of serum b2- microglobulin and lactate dehydrogenase (Sahara et al, 2006). These two parameters were higher in the CD33+ group compared to $CD33^-$group.In our study result showed only 14.2% expressed $CD33^+$. Sahara et al also said that only few of myeloma patient express $CD33^+$ (Sahara et al,2006).

CD56 is found in Natural Killer (NK) cells. It is commonly used to identify the abnormal plasma cells (Drach J et al,1991).Typically plasma cell does not express CD56(Drach J et al,1991).Ely and Knowles reported that the expression of CD56 on plasma cells correlated with lytic bone lesions in myeloma patients(Ely and Knowles,2002).Sahara N et al reported that $CD56^+$ associated with poor outcome in multiple myeloma patient treated with conventional therapies(Sahara N et,2002).In one study, patients with $CD56^+$ had level of serum B2- macroglobulin and had higher incidence of extarmedullarydisease,Bence Jones protein,renal insufficiency and thrombocytopenia as compared to $CD56^-$(Sahara N et al,2002).Bataile et al reported that most of multiple myeloma patients expressed $CD56^+$(Bataile et al,2006).One study documented 71% myeloma patients express CD56(Raja K.R.M et al 2010). However, in our study 59.6% express $CD56^+$,12.8% had $CD56^-$ and the last 27.7% was inconclusive.

CD117 is found in progenitors of myeloid, erythroid and megakaryocytic lineage (Bataile et al,2008). Bataile et al also reported that one third of multiple myeloma patients expressed the $CD117^+$(Bataile et al,2008).Our study also showed that 37.6% patients expressed the $CD117^+$.Mateo G et al reported that expression of $CD117^+$ associated with good prognosis in multiple myeloma(Mateo G et al,2008). A study suggested that CD117 is a valuable marker to distinguish abnormal, neoplastic form the normal plasma cells (Almeida J et al,1999). No significant differences were observed between the $CD117^+$and $CD117^-$ groups when comparing clinical and biological parameters, such as M-protein isotype, albumin, B2M, LDH, disease stage, response to chemotherapy and survival time (Kraj et al, 2004).

Clonality of plasma cells can be determined based on the sole presence of either Kappa or Lambda cytoplasmic immunoglobulin light chains(Kumar S et al,2010). In our study showed that 44.7% patients had cyKappa restriction, 29.8% cyLambda restriction and 25.5% was inconclusive. Confirmation of light chain restriction can be difficult when a few of abnormal plasma cells are present and permeability of plasma cells can affect their light scatter properties in some instances may impair the ability to assess the expression of cell surface antigens (Kumar S et al,2010).

5.1.4 Association between sociodemographic factors and clinical presentations with aberrant antigen expressions.

Analysis of the aberrant antigen expressions showed there was no significant association with patients sociodemographic factors; gender, age and ethnic. However, there was a significant association between CD33, CD56 with gender. There was also no significant association of patients presentation at diagnosis i.e pathological fracture ,anaemia ,recurrent infection and other clinical presentations with aberrant antigen expressions .This probably was due to insufficient number of respondents to justify the significance of the results. Literatures search also should there was no study found on the association of antigen expressiona with sociademographic factors and clinical presentations

5.2 CONCLUSION

This study highlights the role of flowcytometry in providing a rapid and accurate result in addition to classical methods used in determining diagnosis of patients with presumed multiple myeloma. From our study, we concluded that the frequency and pattern of antigen expression of patients presumed with multiple myeloma were diverse. Malay, male at their older aged group are at the risk of getting multiple myeloma. Clinical presentations of patients with presumed multiple myeloma was highly heterogeneous. There was no significant association between different antigen expression markers with social demographic factors and clinical presentation at diagnosis. It is important for the laboratory to optimise the usage of immunophenotyping in resolving patients with proliferative plasma cells disorders as it may alter the actual diagnosis, providing the risk stratification value and in future , hopefully it may lead to guidance of treatment for patients.

5.3 RECOMMENDATIONS

A prospective, case control study involving multicentre study should be conducted to obtain more reliable data and to avoid information bias. More variables which include

quantification of monoclonal gammapathy, biochemical profiles (i.e renal profile,serum albumin, serum calcium, serum Beta 2 microglobulin monitoring) , type of treatment given and patient's survival are among the important informations are needed.The clinical presentations should add on back pain and acute renal failure as another 2 categories Combination of those information and their correlation with antigen expression by immunophenotyping will help the clinician and researchers to understand the pathophysiology of multiple myeloma. Further studies which corroborate the relationship between FCM immunophenotype with the underlying genetic alterations also is required for better understanding of disease.

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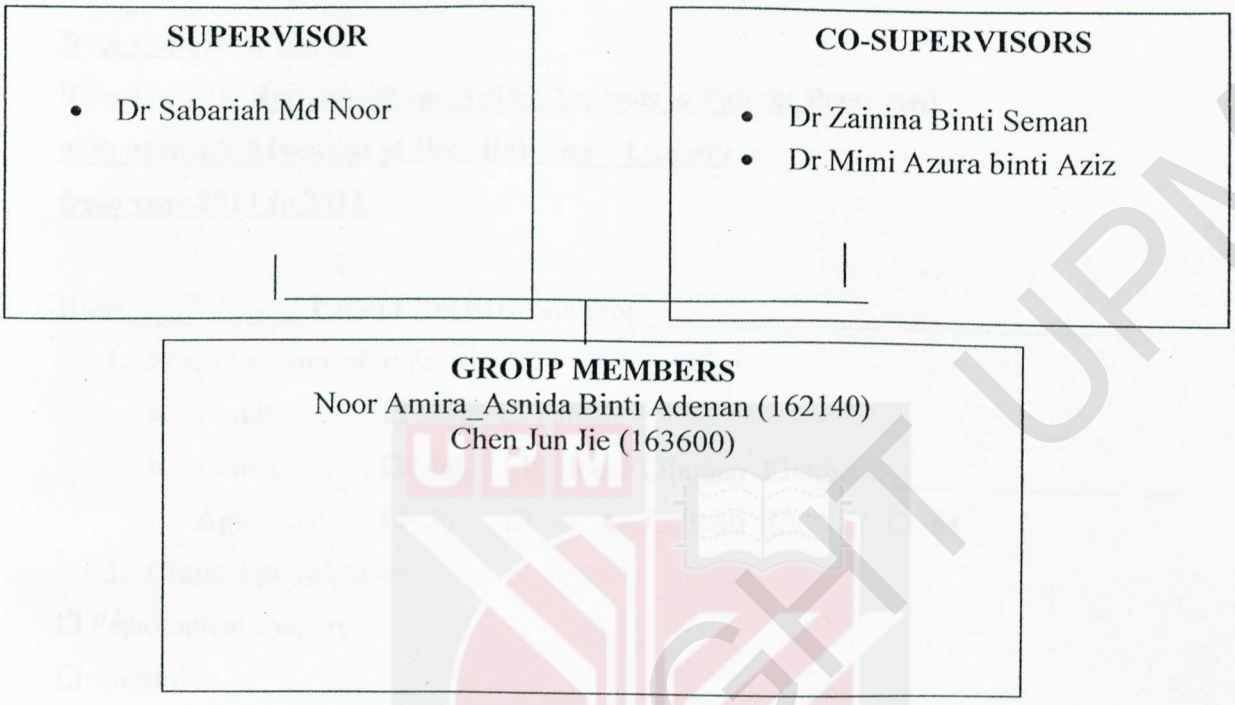
Appendix 1 : Work plan

Topic/Week	1	2	3	4	5	17	18	19	20	21	22	23	24
Proposal preparation	*	*											
Submission of Proposal to Module Coordinator		**											
Proposal Presentation (preparation)		*											
Proposal Presentation concurrent session				**									
Correction of proposal				*									
Preparation of ethical approval / letter				*									
Submission of ethic form & corrected proposal to Dr Suhainizam					**								
Data collection						*	*	*					
Submission of data analysis								**					
Data analysis presentation									**				
Correction of data analysis									*				
Report writing										*	*		
Submission of final report / scientific article											**		
Preparation final presentation											*	*	
Final presentation												**	
Correction of final report /scientific article & Submission of logbook and final report												**	**

Appendix 2 : Research Team



Appendix 3:Budget Planning

No	Items	Estimated cost
1.	Printing	RM 50.00
2.	Hardcover	RM 50.00
3.	Photocopy	RM 100.00
4.	Binding	RM 10.00
5.	Refreshment	RM 50.00
	Total	RM 260.00

Appendix 4 : proforma

Data Collection Form

Research Title :**Immunophenotyping Analysis in Patient Presumed with Multiple Myeloma at Hospital Kuala Lumpur from year 2011 to 2012.**

Date:_____ **Patient Registration No:**_____

1. Socio Demographic Factors
   a. Gender ☐ Male ☐ Female
   b. Ethnic ☐ Malay ☐Chinese ☐Indian ☐Others:_____
   c. Age ☐<50 ☐ 50-54 ☐55-59 ☐60-64 ☐>64
2. Clinical presentation

☐ Pathological fracture
☐ Anaemia
☐ Recurrent infection
☐ Others : _____

3. Antigen expression
   a. CD38 ☐ Positive ☐ Negative
   b. CD138 ☐ Positive ☐ Negative
   c. CD45 ☐ Positive ☐ Negative
   d. CD20 ☐ Positive ☐ Negative
   e. CD33 ☐ Positive ☐ Negative
   f. CD117 ☐ Positive ☐ Negative
   g. CD56 ☐ Positive ☐ Negative
   h. CD19 ☐ Positive ☐ Negative
   i. cyLambda ☐ Positive ☐ Negative
   j. cyKappa ☐ Positive ☐ Negative
   k. Others: _____

----- Forwarded Message -----
From: "nmrr@nmrr.gov.my" <nmrr@nmrr.gov.my>
To: chair_ball92@yahoo.com.my
Sent: Wednesday, 10 July 2013 6:45 AM
Subject: National Medical Research Register - NIH approval for research by Institute for Health Management (IHM) (NMRR ID NMRR-13-523-16066 S2 R0)

Dear Noor Amira_Asnida Binti Adenan (corresponding person) and all investigators,

NMRR ID : NMRR-13-523-16066
Research Title : Immunophenotyping Analysis in Patient Presumed with Multiple Myeloma at Hospital Kuala Lumpur from year 2011 to 2012.
Submission No : S2
RevisionNo : R0

Thank you for submitting your research to the Institute for Health Management (IHM) for MOH National Institute of Health's (NIH) approval.

Your research has been approved subject to MREC's ethics review and approval.

Please keep this email as documentation of approval.

Refer comments below for more details.

Please contact us at nmrr@nmrr.gov.my for enquiries.
Thank you

With warm regards,

Secretariat
Institute for Health Management (IHM)
Secretariat Phone: +6(03)-2296 2800 Ext:8806

National Medical Research Register Secretariat
https://www.nmrr.gov.my
(This is an auto generated email)
(For office use : 16066)

NATIONAL INSTITUTES OF HEALTH (NIH) RECOMMENDATION FOR THE CONDUCT OF RESEARCH IN THE MINISTRY OF HEALTH MALAYSIA
PENGESAHAN INSTITUSI KEBANGSAAN NEGARA UNTUK MENJALANKAN PENYELIDIKAN DI KEMENTERIAN KESIHATAN

This is an auto-generated document. It is issued by one of the research institute under the National Institutes of Health (NIH). The institutes as follows: Institute for Medical Research (IMR), Institute for Public Health (IPH), Clinical research centre (CRC), Institute for health Management (IHM), Institute for Health System Research (IHSR) and Institute for Health Behavioural Research (IHBR).

Dokumen ini adalah cetakan berkomputer. Borang ini dikeluarkan oleh salah satu institusi dibawah National Institutes of Health (NIH) iaitu Institut Penyelidikan Perubatan (IMR), Institut Kesihatan Umum (IKU), Pusat Penyelidikan Klinikal (CRC), Institut Pengurusan Kesihatan (IPK), Institut Pengurusan Sistem Kesihatan (IPSK) dan Institut Penyelidikan Tingkahlaku Kesihatan (IPTK).

Unique NMRR *[Nombor Pendaftaran]*	NMRR-13-523-16066
Research Title *[Tajuk]*	Immunophenotyping Analysis in Patient Presumed with Multiple Myeloma at Hospital Kuala Lumpur from year 2011 to 2012.
Protocol Number if *[Nombor Protokol jika ada]*	

#	**Investigator Name** ***[Nama Penyelidikan]***	**Institution Name** ***[Nama Institusi]***
1	Chen Jun Jie	Kuala Lumpur Hospital
2	Mimi Azura Aziz	Kuala Lumpur Hospital
3	Noor Amira_Asnida Binti Adenan	Kuala Lumpur Hospital
4	Sabariah Bt Md. Noor	Kuala Lumpur Hospital
5	Zainina Seman	Kuala Lumpur Hospital

I have reviewed the above titled research, and has recommended to MREC* for its decision.

Saya telah menyemak penyelidikan yang bertajuk diatas, dan telah disyorkan untuk MREC bagi keputusannya.

Name of Director *[Nama pengarah]*	Dr Roslinah Ali
NIH Institute (IMR, IPH, CRC, IHM, IHSR, IHBR) *[Nama institusi di bawah NIH]*	Institute for Health Management (IHM)
Signature & Official Stamp *[Tandatangan dan Cop Rasmi]*	
Date *[Tarikh]*	10-07-2013

*Final approval is pending MREC decision.

Attachment 2

JKEUPM Ref No. : FPSK_Mei (13)39(undergraduate)

Members of the JKEUPM who reviewed the documents:

Prof. Dr. Zamberi Sekawi

Date of approval: 21/5/2013

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

NAME	DESIGNATION	GENDER	TICK IF PRESENT
Prof. Dr. Norlijah Othman	Paediatrics& Dean, Faculty of Medicine and Health Sciences	Female	√
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	√
Prof. Dr. TengkuAizanAbd Hamid	Gerontologist & Director, Institute of Gerontology	Female	
Prof. Dr. LekhrajRampal	Medical Statistician, Dept of Community Health, Faculty of Medicine and Health Sciences	Male	√
Prof. Dr. Elizabeth George	Pathologist, Dept of Pathology, Faculty of Medicine and Health Sciences	Female	
Prof. Dr. Lim ThiamAun	Anesthesiologist, Dept of Surgery, Faculty of Medicine and Health Sciences	Male	
Prof. Dr. Wan Omar Abdullah	Medical Parasitologist, Dept of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences	Male	√
Prof. Dr. Patimah Ismail	Professor of Biomedicine, Dept of Biomedical Sciences, Faculty of Medicine and Health Sciences	Female	√
Prof. Dr. Azali Mohamed	Professor of Macroeconomics, Dept of Economics, Faculty of Economics and Management	Female	
Assoc. Prof. Dr. Johnson Stanslas	Pharmacologist, Dept of Medicine, Faculty of Medicine and Health Sciences	Male	√
Assoc. Prof Dr. Mansor Abu Talib	Assoc. Professor of Guidance and Counselling, Dept of Human Development and Family Studies, Faculty of Human Ecology	Male	
Assoc. Prof. Dr. Noritah Omar (Lay Person)	Assoc. Professor of English Language, Dept of English Language, Faculty of Communication and Modern Languages	Female	√
Dr. RojanahKahar (Lay Person)	Lecturer of Dept of Human Development and Family Studies, Faculty of Human Ecology	Female	√
Tan Sri Dato' Napsiah Omar (Lay Person)	Chairman, National Population and Family Development Board	Female	√