AN EXPLORATIVE STUDY ON FACTORS ASSOCIATED WITH NEUTROPENIA ONSET AND SEVERITY AMONG CANCER PATIENTS

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by

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LIST OF ABBREVIATIONS

µl	Microliter
1st Cycle	First Cycle
2nd Cycle	Second Cycle
3rd Cycle	Third Cycle
5-FU	5-Fluorouracil
AML	Acute Myeloid Leukemia
ANC	Absolute Neutrophil Count
Ara-C	Cytosine Arabinoside
ASCO	American Society of Clinical Oncology
CBC	Complete Blood Count
CHOP	Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone
CIN	Chemotherapy Induce Neutropenia
CMF	Cyclophosphamide, Methotrexate and 5-Fluorouracil
CMV	*Cytomegalovirus*
CNS	Central Nervous System
CRC	Clinical Research Center
CSF's	Colony Stimulating Factors
CVC	Central Venous Catheter
CVSC	Central Venous Sectional Catheter
DNA	Deoxyribonucleic Acid
EC	Epirubicin and Cyclophosphamide

FAD	Food and Drug Administration
FBC	Full Blood Count
FEC	Fluorouracil, Epirubicin and Cyclophosphamide
FN	Febrile Neutropenia
G+ve	Gram-Positive
G-CSF	Granulocyte-Colony Stimulation Factor
GIT	Gastro Intestinal Tract
GM-CSF	Granulocyte-Macrophage Colony Stimulation Factors
GNB	Gram-Negative Bacteria
G-ve	Gram-Negative
HIV	Human Immunodeficiency Virus
HSCT	Hematopoietic Stem Cell Transplantation
HSV	Herpes Simplex Virus
I.M.	Intramuscular
I.V.	Intravenous
IDSA	Infectious Diseases Society of America
KSA	Kingdom of Saudi Arabia
L	Liter
LON	Late Onset Neutropenia
NCCN	National Comprehensive Cancer Network
NHL	Non-Hodgkin Lymphoma
NSCLC	Non Small Cell Lung Cancer
QOL	Quality of Life
RBC	Red Blood Cell

R-CHOP	Rituximab, Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone
RNA	Ribonucleic Acid
SLE	Systemic Lupus Erythromatosis
SPSS	Statistical Package for Social Sciences
TNF	Tumor Necrosis Factor
UK	United Kingdome
USA	United State of America
USM	Universiti Sains Malaysia
UTI	Urinary Tract Infection
VZV	*Varicella Zoster* Virus
WBC	White Blood Cell

KAJIAN EXPLORATIF MENGKAJI FAKTOR-FAKTOR BERKAITAN DENGAN MASA MULA DAN KETERUKAN NEUTROPENIA DI KALANGAN PESAKIT KANSER

ABSTRAK

Neutropenia ialah satu keadaan apabila kandungan neutrophil dalam darah menjadi kurarg daripada 1500 sel/ µl dan ianya merupakan satu kesan sampingan biasa akibat kemoterapi kanser. Selain dikaitkan dengan mobiditi dan mortaliti yang agak tinggi, neutropenia juga dapat menyebabkan pengurangan dos atau penangguhan rawatan kemoterapi yang mungkin mempunyai kesan tidak baik keatas kualiti kehidupan pesakit kanser dan dapat menyebabkan peningkatan beban ekonomi. Masa mula neutropenia sering berlaku selepas kitaran pertama kemoterapi kanser kerana kebanyakan kemusnahan sum-sum tulang berlaku selepas kitaran pertama iaitu pada dua minggu pertama pemberian kemoterapi. Keterukan neutropenia diklaskan sebagai neutropenia ringan apabila kiraan neutrofil absolut (ANC) turun antara 1500 dan 1000 sel/ µl; neutropenia sederhana apabila ANC antara 1000 dan 500 sel/ µl dan neutropenia teruk apabila ANC kurang daripada 500 sel/ µl. Objektif utama kajian ini ialah untuk mengenalpasti faktor-faktor risiko utama yang mempunyai hubungan dengan masa mula dan keterukan neutropenia serta untuk menentukan rawatan neutropenia yang sering digunakan. Satu kajian pemerhatian retrospektif yang menggunakan persampelan mudah telah dijalankan ke atas pesakit tumor pejal yang dimasukan ke Hospital Pulau Pinang di antara tahun 2003 hingga 2006. Sebanyak 4503 rekod pesakit dalam tempoh ini telah diperiksa dan didapati hanya 117 sahaja yang memenuhi kriteria penyertaan. Data demografik pesakit, jenis diagnosis kanser, data pemberian kemoterapi, data

neutropenia, data makmal dan data rawatan telah dikumpulkan dengan menggunakan borang pengumpulan data. Ujian-ujian statistik inferensial bersesuaian iaitu ujian Chi-square dan ujian Fisher's Exact telah digunakan bagi menganalisakan data-data yang dikumpul. Bagi variabel-variabel yang menunjukkan hubungan signifikan, Logistic Regression (Multinomial Logistic Regression) telah digunakan bagi menentukan faktor utama yarg mempunyai hubungan dengan masa mula dan keterukan neutropeneia. Keputusan kajian ini menunjukkan kebanyakan neutropenia berlaku di kalangan kuam wanita (73.5%) berbangsa Cina (70.1%) dan berumur di antara lingkungan 50 hingga 59 tahun (39.3%). Walau bagaimanapun, data-data demografik ini bukan merupakan faktor risiko bagi masa mula dan keterukan neutropeneia. Neutropenia sering berlaku di kalangan pesakit kanser payu dara (64.1%) tetapi tiada hubungan signifikan diperolehi antara jenis kanser yang di diagnosiskan dengan masa mula dan keterukan neutropenia. Gabungan siklofosfamid, epirubisin dan 5-flurourasil (40.2%) adalah regimen yang paling banyak digunakan tetapi masih terdapat hubungan yang tidak signifikan antara kemoterapi dengan masa mula dan keterukan neutropenia. Didapati kebanyakan pasakit mula mengalami neutropenia selepas dua minggu administrasi kemoterapi (84, 71.8%) yang bermaksud bahawa neutropenia berlaku selepas kitaran pertama kemoterapi. Juga didapati kebanyakan pesakit neutropenia mengalami neutropenia ringan (43, 36.8%) dan neutropenia sederhana (41, 35%). Terdapat hubungan signifikan antara pusingan pertama kemoterapi dengan masa mula neutropenia tetapi hubungan adalah tidak signifikan dengan keterukan neutropenia. Hubungan signifikan wujud antara demam dengan keterukan neutropenia. Tambahan lagi, hipotensi dan pening kepala adalah dua tanda klinikal utama yang menunjukkan hubungan signifikan dengan keterukan neutropenia. Bakteria gram-negatif adalah penyebab utama jangkitan di kalangan pesakit

neutropenia yang dikaji. Filgrastim adalah rawatan utama dan diikuti oleh gabungan filgrastim dan ceftazidime bagi mengatasi keterukan neutropenia serta merawat jangkitan yang berkaitan. Oleh demikian, kajian ini menunjukkan pusingan pertama kemoterapi adalah faktor risiko yang mempunyai hubungan dengan masa mula neutropenia. Manakala demam, sakit kepala dan hipotensi adalah faktor-faktor risiko yang mempunyai hubungan dengan keterukan neutropenia. Filgrastim adalah rawatan yang paling biasa digunakan.

Katakunci: Masa mula neutropenia, Keterukan neutropenia, Kajian pemerhatian retrospektif, Kemoterapi, Demam, Tanda klinikal, Rawatan neutropenia.

AN EXPLORATIVE STUDY ON FACTORS ASSOCIATED WITH NEUTROPENIA ONSET AND SEVERITY AMONG CANCER PATIENTS

ABSTRACT

Neutropenia is a decreased in the absolute number of neutrophils in the blood to less than 1500 cell/ µl and is a common side effect of cancer chemotherapy. Apart from being associated with considerable morbidity and mortality, neutropenia could also result in the dose reduction or a delay in chemotherapy courses that could have a detrimental effect on cancer patients quality of lives and could cause a considerable economic burden. Neutropenia onset mostly occurred after the first cycle of cancer chemotherapy since the major bone marrow destruction happened after the first cycle i.e. within the first two weeks of chemotherapy administration. Neutropenia severity is classified as mild neutropenia when the absolute neutrophil count (ANC) drops between 1500 and 1000 cell/ µl, moderate neutropenia when ANC falls between 1000 and 500 cell/ µl and severe neutropenia when ANC falls below 500 cell/ µl. Thus the main objective of this study is to identify the main risk factors associated with the onset and severity of neutropenia and to determine the most common treatment used for neutropenia. An observational retrospective study using convenient sampling was carried out on solid tumor patients admitted to Penang Hospital between 2003 and 2006. A total number of 4503 patients files during this period were reviewed and 117 were found to fulfilled the inclusion criteria. Patient's demographic data, type of cancer diagnosis, chemotherapy administrated data, neutropenia data, laboratory data and treatment data were collected into data collection form. Appropriate inferential statistics tests were used to analysed the data collected which were Chi-square test and Fisher's

Exact test. As for the variables that showed a significant association, Logistic Regression (Multinomial Logistic Regression) was used to find the main risk factor associated with onset and severity of neutropenia. The result of this study showed that neutropenia occurred more in the female (73.5%), in the Chinese (70.1%) and between the age of 50-59 years old (39.3%). However, the demographic data was found not to be a risk factor for neutropenia onset and severity. Neutropenia seems to occur mostly in breast cancer patients (64.1%) but there was no significant association between types of cancer diagnosed and neutropenia onset and severity. Combination of cyclophosphamide, epirubicin and 5-flurouracil (40.2%) was the most frequent regimen used but again there was no significant association between the chemotherapy and neutropenia onset and severity. It was found that most of the patients started to develop neutropenia after two weeks of chemotherapy administration (84; 71.8%) which means that neutropenia occurred after the first cycle of chemotherapy. Also it was found that most of the neutropenic patients suffered mild (43; 36.8%) to moderate neutropenia (41; 35%). There was a significant association between the first cycle of chemotherapy and neutropenia onset but an insignificant association was seen with neutropenia severity. Significant association existed between fever and severity of neutropenia. In addition the two major clinical signs that showed significant association with neutropenia severity were hypotension and headache. Gram-negative bacteria was found to be the predominant cause of infection among the neutropenic patients studied. Filgrastim is the major type of treatment used followed by filgrastim plus ceftazidime combination to overcome neutropenia severity and infection associated with it. Thus this study showed that the first chemotherapy cycle is a risk factor associated with neutropenia onset. Where as fever, headache and hypotension are complication and risk factors associated

with neutropenia severity. Filgrastim was the most common treatment used. So it is a very important to take special supervision for cancer patients especially after first cycle of chemotherapy which consider the most dangerous risk factor for neutropenia onset. The uses of filgrastim (Neupogen®) should be emphasized beside accurate calculation for chemotherapy doses. Emphasize on doing laboratory tests (i.e., for neutrophil count), keep monitoring for body temperature before and after chemotherapy cycle. Emphasize on doing culture and sensitivity tests and there must be no delay in administration of empirical antibiotic therapy especially with a wide range antibiotic therapy like beta-lactam antibiotic until the culture and sensitivity test appear.

Keywords: Neutropenia onset, Neutropenia severity, Observational retrospective study, Chemotherapy, Fever, Clinical signs, Neutropenia treatment.

CHAPTER 1

INTRODUCTION

1.1 White Blood Cells and Neutrophil

Neutrophil is the major type of white blood cell (WBC) which form part of the whole blood. They are also called polymorphonucleat leucocytes or granulocytes and represent about 45 % - 70 % of the total WBC (Walker and Edwards, 2003; Dale, 2005). They are synthesized and produced from the hematopoietic stem cell that is found in the bone marrow and it required 10-14 days for hematopoietic stem cell to produce mature neutrophils (Dale, 2004; Dale, 2005). Neutrophils are considered as the main defense mechanism of the body innate host defense system since they will protect the body from invasion of micro-organisms (Dale, 2004). Neutrophils act as phagocytes and get rid of bacterial, fungal, germs or any foreign body in the blood (Frey, 1999). Neutrophil's cytoplasm also contains granules, for which they are also called granulocytes. These granules contain glycogen and antibacterial substances (Dale, 2005). Neutrophils have a very short life span that is only for 6-10 hours but during inflammation or infection neutrophils will migrate to the inflamed tissues, exert their phagocyte action and remain active there for about 2-6 days (Dale, 2005; Frey, 1999). Neutrophils have receptors on their surfaces that will help them to contact and bind to the tissues and vascular endothelial that lie beside or near the problem or infection or inflammation sites (Dale, 2005).

1.2 Definition of Neutropenia

Neutropenia is defined as a decrease in the absolute number of neutrophils in the blood. Clinically, neutropenia is defined as a decrease in the absolute neutrophil count (ANC) of more than two standard deviations below the normal range. So the patient is considered as neutropenic when the ANC is lower than the normal level which is 1500 cell/ µl (Dale, 2005).

So generally this dangerous condition called neutropenia will occur when the neutrophils cells counts is lower than the normal count which is 1500 cell/ µl (Dale, 2005; Walker and Edwards, 2003; Frey, 1999; Frey and Granger, 2002; Linker, 2000). Even so when the neutrophil count is more than 1000 cell/ µl there will still be normal protection against infection. When the level of neutrophils falls to 500-1000 cell/ µl it is classified as mild neutropenia and moderate neutropenia happened when the count falls between 200-500 cell/ µl (Dale, 2005; Frey, 1999; Frey and Granger, 2002). When the neutrophil count falls lower than 200 cell/ µl it is considered as a very serious condition that requires the patient to be admitted to the hospital and treated with antibiotics (Frey, 1999; Frey and Granger, 2002). It has been reported that the normal neutrophil count in the white man is 1500 cell/ µl while in the black man the normal is lower (i.e., 1200 cell/ µl) (Linker, 2000).

While febrile neutropenia (i.e., when patient developed fever) is mostly associated with chemotherapy regimen it may also occur after irradiation of most parts of the bone marrow. Here the term febrile neutropenia is usually used to describe the occurrence of neutropenia and fever that is when the body temperature is $\geq 38.3^{\circ}C$ or oral temperature $\geq 38^{\circ}C$ for more than one hour and the neutrophil count is ≤ 500 cell/ µl or it will reduced

to ≤ 500 cell/ µl within the next 48 hours (Bledsoe, *et al*., 2005; Dale, 2005; AL-Ahwal, 2005; Lyman and Wilmot, 2006).

1.3 Overview of Neutropenia

There are many studies on neutropenia which looked at the mechanism of neutropenia occurrence, the problems that may occur due to neutropenia, the treatment of neutropenia and the management of neutropenia in order to save patients life.

The study by Nwakoby *et al*. (2001) showed that neutrophilia is most commonly seen in patients who suffered from either infection or inflammation. The neutrophil cells will be the first cells arriving at the sites of damage or problem. Approximately 100 billion neutrophils could be produced during one day. Thus neutrophils are considered as the major defense mechanism. Figure 1.1 shows the action of neutrophil as a phagocyte.

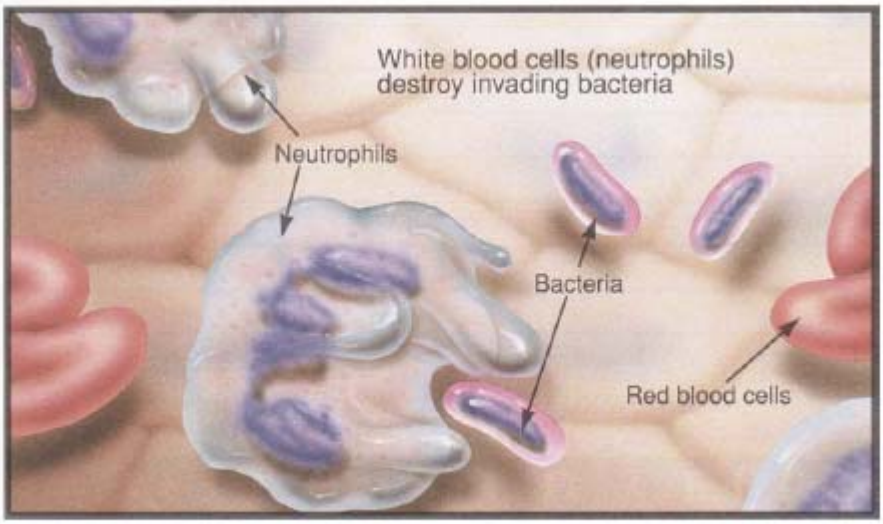

Figure 1.1: The neutrophil cell act as phagocyte which is the major defense mechanism (Bolyard *et al.*, 2001).

So when an inflammation occurs or take place this will lead to a direct or indirect stimulation of the bone marrow which will lead to an increase in the number of neutrophils in the blood. The mature neutrophils will live for a short period of time (i.e., 6-10 hours) and then they will die by a process called apoptosis. But there are some factors that will lead to an increase in the life span of the neutrophil cells which include granulocyte-colony stimulation factor (G-CSF), granulocyte-macrophage colony stimulation factor (GM-CSF), interleukine-2, gamma interferon, tumor necrosis factor (TNF) and glucocorticoids. While on the other hand there are some materials like nitric oxide generations both endogenous and exogenous will destroy the neutrophils or stimulate neutrophil apoptosis (Nwakoby *et al*., 2001). This is shown in Figure 1.2 below:

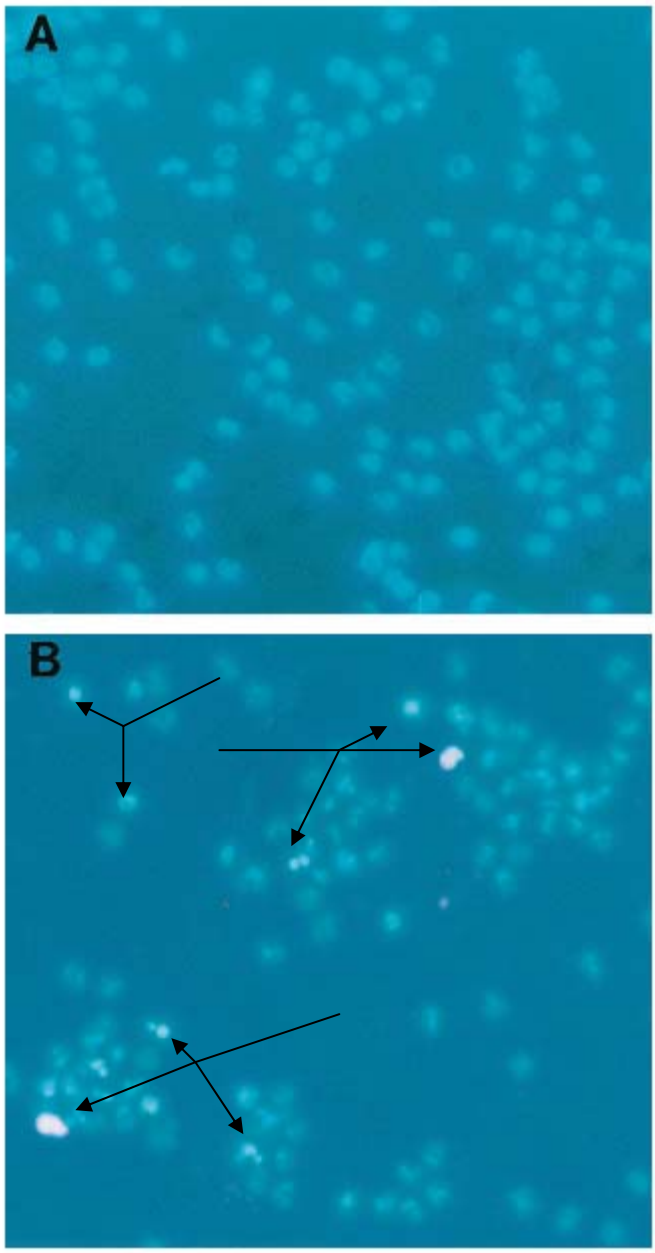

Figure 1.2: The apoptosis process: picture A shows normal blood while picture B shows apoptosis which lead to neutropenia (Nwakoby *et al*., 2001).

1.4 Adverse Effect of Neutropenia

The incidence of neutropenia has been shown to have negative effects on the patient. The percentage of cancer patients who did not receive their total amount of chemotherapy drug doses has increased. Fifty percent (50%) of these cancer patients received less than 85% of the actual doses because of neutropenia. Breast cancer seems to be the type of cancer most associated with neutropenia complication. About 25% of the breast cancer patients suffered from neutropenia resulting in reduction of their chemotherapy doses (Lyman and Wilmot, 2006).

The study by Ashley *et al*. (2004) in USA clearly described the effect of neutropenia on quality of life. Thirty-four (34) neutropenic patients developed grade 4 neutropenia after being administered myelosuppressive chemotherapy at days 21 or 28 of the first cycle. Their neutrophil counts were assessed on days 7, 10, 14, 21 and 28 of the chemotherapy cycle. Each patient with neutrophil counts lower than 1.5×10^9 cell/ L was interviewed by the researchers. The questions were concerned with the effects of neutropenia which include physical feelings and sensations, interactions with others, daily activities, financial impact, sex life, ability to work, satisfaction with medical care, emotions and many others aspects of quality of life. The results of this study showed that neutropenia has a dramatic effect on the patient life and fatigue was the predominant characteristic. Patients described fatigue as feeling weak, exhausted and tired. Psychological problems were also reported by the patients, such as sadness, anxiety, reduce self-worth and inability of fulfilling normal roles. These results hence showed that neutropenia as a result of chemotherapy do effect patient's quality of life.

Another study conducted also in USA by Ropka and Faan (2007) examined the effect of neutropenia on patient's life. The result of this study also showed that the

occurrence of neutropenia has a detrimental effect on the patient's life. These detrimental effects like fatigue considered as the most common side effect, that lead to a decreased in their ability to perform the daily life activities. Also decreased and delayed in chemotherapy treatment had lead to increased in cancer cell growth and tumor size. This will lead to serious side effects on patients lives that may lead to their death.

1.5 Neutropenia Incidence and Prevalence

Neutropenia is a common side effect of cancer chemotherapy and will occur or take place in one out of three patients who received chemotherapy. Once neutropenia happens chemotherapy should be delayed in order to give the body a chance for producing new neutrophils (Neutropenia Association Inc. 1993). There are no or very few studies that give an exact number of neutropenia prevalence but some studies only mentioned it as a common side effect. It has also been considered by some as a common and major complication which will threaten the patients lives and reduced or delay the chemotherapy doses (Di Maio *et al.,* 2005; Fortner *et al.*, 2005; Gabrilove, 2005; Timmer-Bonte *et al.*, 2005). While studies by the Neutropenia Association Inc and European Council for Rare Diseases considered severe chronic neutropenia, cyclic neutropenia, idiopathic neutropenia and autoimmune neutropenia as rare diseases especially the severe chronic neutropenia. However, there was no accurate number or percentage of neutropenia prevalence or occurrence as a side effect of chemotherapy was reported (Neutropenia Association Inc. 1993 and The European Parliament and the Council of the European Union, 1999). While Munshi (2000) mentioned in his study that neutropenia is a common side effect most often due to chemotherapy treatment but on the other hand severe neutropenia occurrence is uncommon, but it can cause serious

mortality and morbidity as a result of infection associated with it. He also mentioned that epidemiological studies showed a wide variation in the occurrence of neutropenia according to geographical regions such as the average occurrence of neutropenia in the USA is 56.4 case per million people.

1.6 Neutropenia Diagnosis

The diagnosis of neutropenia is made by performing the following tests:

1.6.1 Blood Count Monitoring

The first test to investigate the suspicion of neutropenia presence is to perform complete blood count (CBC) or full blood count (FBC) where by them neutrophil numbers will be measured. If the results showed a low neutrophil count then these tests will be repeated in order to be certain that neutropenia is actually present (Bolyard *et al*., 2001).

1.6.2 Bone Marrow Aspirate / Trephine Biopsy

After performing blood test a bone examination could be carried out to confirm the results. Bone marrow biopsy is done by obtaining bone marrow aspirates from 2 sites that is from the middle of the bone and also from solid bonier part of the bone. This bone biopsy is performed with patient under general anesthesia or local anesthesia with sedation. These bone marrow samples are usually taken from the large bones such as large pelvic bone, the ilium, or sometimes from the flat breastbone (i.e., the sternum) (Bolyard *et al*., 2001).

1.7 Neutropenia Causes

Factors that play a major role in causing neutropenia are demographic factors, hematological disorder, autoimmune diseases, infection, drugs reactions and chemotherapy or radiotherapy (Dale, 2005; Frey and Granger, 2002; Lyman and Wilmot, 2006).

Neutrophils production decrease in the elderly since they have a lower ability to produce mature neutrophils than younger people. Besides that the neutrophil count in white man is higher than that in the black man. Also neutropenia incidence seems to be more in women than men (Dale, 2005; Frey and Granger, 2002; Linker, 2000).

Hematological diseases like leukemia, myelodysplastic syndrome, Hodgkin's and non-Hodgkin's diseases and multiple myloma have been shown to cause neutropenia (Dale, 2005). In these hematological diseases severe destruction of bone marrow leads to destruction of stem cells. This will result in the prevention or decrease in neutrophils production and thus causing neutropenia (Dale, 2005). Also there is an effect on red blood cells (RBC) and platelets production which will lead to severe anemia and thrombocytopenia. Usually the patient will suffer from fever $\geq 38.5^{\circ}C$ as well as gingivitis, bleeding, stomatitis, bone chills and patient might also collapse (Verstraete *et al.*, 1997).

Neutropenia has also been associated with autoimmune disease like systemic lupus erythromatosis (SLE). The neutropenia in SLE is usually mild and the patient may not suffer from serious bacterial infection. However patients with Sjören syndrome and rheumatoid arthritis may have severe neutropenia so they are at higher risk of bacterial or fungal infection. Neutropenia may also occur with aplastic anemia (Dale, 2005).

Many drugs such as diuretics, chlorpromazine, and allopurinol have been shown to cause neutropenia. Two mechanisms have been suggested to cause neutropenia. Firstly, the drug produce dose dependant toxicity on cell production, protein synthesis, bone marrow and cell survival. Secondly, the mechanism may involve the drug inducing immunological reactions. For an example the binding of drugs with the surface of the neutrophil cell will lead to cell destruction and cause production of neutropenia. These two mechanisms are not always seen but happen only in a small percentage of the patients. Also these two mechanisms need a long duration of use that is from starting the administration of the drug to the appearance of neutropenia (Dale, 2005).

While in the case of solid tumor as the focused of this present study, most of the patients have normal neutrophils but due to the intensive chemotherapeutics drugs and regimens neutropenia developed (Rolston, 2001).

Neutropenia has been seen to be mostly associated with cancer chemotherapy and radiotherapy. These chemotherapeutic drugs will effect the production of folic acid as well as the synthesis of DNA, RNA and protein by acting as anti metabolite which will lead to bone marrow destruction (Frey, 1999; Dale, 2005; Frey and Granger, 2002; Linker, 2000; Verstraete *et al*., 1997). Bone marrow destruction will then lead to a decrease in neutrophil cell production. Due to this, the chemotherapy and radiotherapy are considered as the main causes for neutropenia or febrile neutropenia (i.e., neutropenia when associated with fever and ANC $\leq$ 500 cell/ µl). Also these chemotherapeutic drugs will kill and suppress every cell that is highly dividing or actively growing like cancer cell but unfortunately they also have an effect on the blood cells especially bone cells and neutrophil cells (Fortner *et al*., 2005).

Examples of chemotherapeutic drugs which are highly associated with neutropenia are actinomycin, asparaginase, cytarabine, busulfan, cisplatin, daunorubicin, etoposide, fluorouracil, ifosfamide and methotrexate (Dale, 2005; Linker, 2000; kimble-koda, *et al*., 2002).

1.7.1 Neutropenia and Demographic Factors

There are studies indicating that there is a relationship between age, ethnic group and gender of the patient with neutropenia induced as a side effect of chemotherapy drug use (Dale, 2004; Frey and Granger, 2002).

A study that was done by Wolff *et al*. (2005) in the USA looked at the risk factors including gender associated with neutropenia and the development of its complication. The study was a prospective study on 2,222 patients. Eight hundred and eighty six (886) or 40% of these patients were 65 years old or older. The results of the study showed that there was a significant association relationship between severity of neutropenia and gender (P= 0.001). A significant relationship between neutropenia complications and gender (P= 0.004) was also obtained. In their study, 66.5% of the total patients were female and 33.5% were male. Thus it seems that female suffered from neutropenia much more than male.

1.7.2 Neutropenia and Solid Cancer

Neutropenia has been shown to be associated with solid tumor especially breast cancer where by about 25% of them developed neutropenia. However this association seems to happen with the use of specific chemotherapeutic drugs or regimens (Dale,

2004). There are very few studies which focused on the causative factors of neutropenia in solid cancer.

A study by Koasak *et al*. (2002) in USA looked at the differences between the occurrence of fever and neutropenia in pediatric patients with solid cancer and leukemia. Two hundred and eighty three (283) pediatric febrile neutropenic pediatric patients were enrolled in the retrospective study. Thirty eight percent (38%) of the patients had leukemia and 62% had solid tumors. The results showed that fever of unexplained origin developed in 73% of the leukemic patients and 74% of the solid tumor patients. The median duration for neutropenia in leukemic patients was 9 days while in solid tumor patients was 7.5 days. Both were significant with P <0.0002. Severe neutropenia with ANC < 100 cell/ µl was present in 75% of leukemic patients verses 70% of patients with solid tumor. But there was no significant association between severity with both solid tumor and leukemia. So Koasak et al found that there was a significant association between cancer type and neutropenia duration, but there was no significant association between the cancer type and severity of neutropenia.

A second study looking at the relationship between solid tumors and neutropenia severity and risk factors was done by AL-Ahwal (2005) in Kingdom Saudi Arabia (KSA). A total number of 56 solid cancer patients suffering from febrile neutropenia were studied in order to obtain the association. Those patients consisted of 38.2% male and 67.2% female. Looking at the age, 61.2% of them were less than 50 years old while 38.8% were more than 50 years old. The duration of neutropenia was less than 7days in 92.5% and 7-14 days in 7.5% of the patients. None of the patients experienced neutropenia more than 14 days. The results of the study showed that the association of neutropenia duration with solid tumor was insignificant (P= 0.081). The results also

showed that only 16.4% of patients suffered from severe neutropenia with ANC < 100 cell/ µl. The association between the severity of neutropenia both with ANC higher than or lower than 100 cell/ µl and solid tumor were insignificant since *P*= 0.692 for Chi-square test and Fisher's Exact test. Looking at the risks factors, the results showed that the association between severity of neutropenia and solid tumor was not significant but the association of co-morbidity of chemotherapy and solid cancer was significant.

1.7.3 Neutropenia and Chemotherapy Drugs and Cycles

Many studies showed neutropenia as the negative result of the use of chemotherapeutics drugs. Neutropenia occurrence or onset is mainly and highly associated with the first cycle of the chemotherapy more than the others or subsequent cycles. Chemotherapeutic drugs will cause the depletion of the bone marrow which will lead to the reduction in the production of the neutrophils and consequently lead to neutropenia. In addition neutropenia severity will also increase because of these chemotherapeutics drugs (Kern, 2001).

Figure 1.3 shows the highly dividing cells that could be depleted because of the effect of chemotherapy.

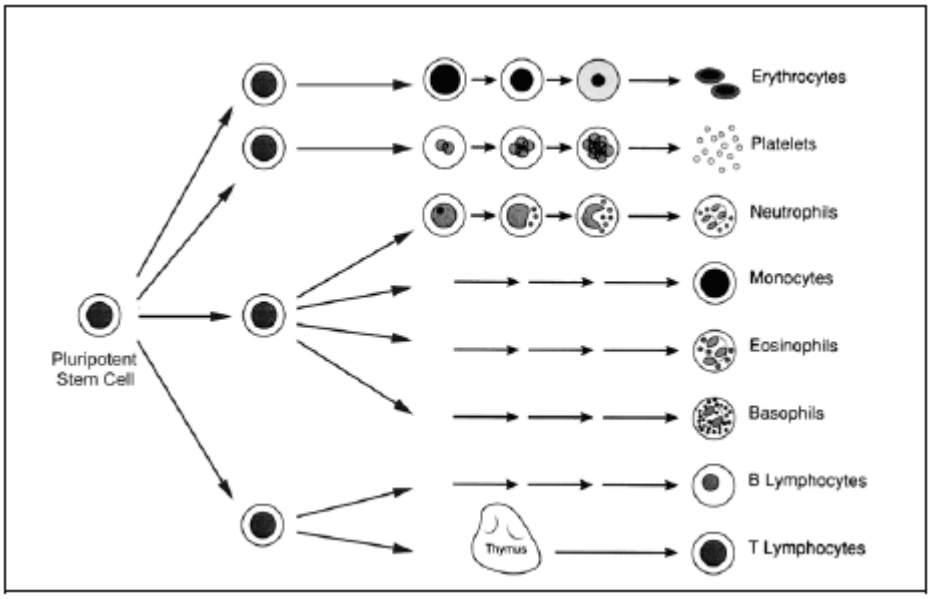

Figure 1.3: The highly dividing stem cell that all the blood cells are derived from (Bolyard *et al*., 2001).

The study by Di Maio *et al*. (2005) produced an interesting result where by the effect of the chemotherapeutic drugs were clearly shown. The total number of patients involved in this study was 1265. The patients were classified into two groups. The first group consisted of 436 patients and were given all the chemotherapeutics drugs used in the experiment. The types of chemotherapeutics drugs used were gemcitabine, cisplatin and vinorelbine and these drugs were used in different trials. These patients were called “inland markers” since they were still living after receiving all the different types of chemotherapies over 180 days. The second group of 829 patients did not receive any of the chemotherapeutics drugs and were called “out of land markers”. The main result produced by this study was the ratio of the living for those inland markers patients who suffered from mild neutropenia was 0.74 of the whole cases. While for those patients who suffered from severe neutropenia the ratio of death was 0.65 of the total cases. Thus this

study emphasized that the correct doses of chemotherapeutics drugs must be used in order to prevent the incidence of lethal neutropenia.

Chemotherapy as an important risk factor for neutropenia occurrence and severity was also supported by Friese (2006) who reported that neutropenia was the major hematological toxicity induced by the cancer chemotherapy. This would thus lead to dangerous conditions such as life threatening infection.

While in USA a study conducted by Wolf *et al*. (2005) on 2,222 cancer patients showed that 1/3 of them (733) received fewer than 4 cycles of the chemotherapy because of chemotherapy complications. The most important result obtained was that half of the neutropenia events occurred within the first cycle of chemotherapy especially among the breast cancer patients.

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1.8 Neutropenia Stages and Source of Infection

Neutropenia stages encompass the period of neutropenia as well as its association with infections. These infections are the results of immunosuppression which could involve bacterial, fungal or even viral infections (Greene, 2004). Neutropenia levels and stages that developed in cancer patients depend on the chemotherapeutics drugs they received (Agaliotis, 2004). Neutropenia stages and its main source of infection will be emphasized below.

1.8.1 Neutropenia From Day 0-7

This period of neutropenia involve the appearance of bacterial infection arising from the gastro-intestinal track (GIT) or the skin. The most important factor that will lead to this bacterial infection is the high use of improper antibiotics which will lead to the

production or occurrence of bacteremia. This happens because of the suppression of gram-positive bacteria which would lead to the occurrence of gram-negative infection and this later lead to bacteremia (Greene, 2004).

1.8.2 Neutropenia From Day 7-14

In the second week of neutropenia the occurrence of bacterial infection is still mainly originate from the GIT which is again the primary source of bacteria. Also with the improper use of antibiotics, this would cause a lack of the aerobic gram-positive and gram-negative bacteria. This would thus result in the anaerobes and the yeast to become the main cause of infection. All of these will lead to the increase in the growth of candida in the GIT and will result in candidemia which is a new risk for the patient. The main signs of candida infection are fever, headache and chills within 2 hours. After 3 hours the candida infection will move from the GIT to the blood stream as well as to the urinary tract. This type of candida infection will colonize the GIT and is considered as a common infection in neutropenic patients especially after two weeks of neutropenia occurrence (Greene, 2004).

1.8.3 Neutropenia After Two Weeks or More

This period is the most dangerous and serious period for both clinicians and patients. At this time the clinicians would be trying to protect and prevent the patient from being infected. As for the patients, this is a critical and dangerous period because they are very prone to infections. During this period, beside GIT and the skin the endogenous infection sources now involved the respiratory tract. Also the hospital environment and the hospital staff are considered as an exogenous source of infection and

play an important role in patients infections. Viral infection caused by *BK virus, Herpes simplex and Varicella zoster* are now becoming a dangerous factor. An important characteristic of this period is that occurrence of bacterial resistance towards antibiotics. This is especially seen among gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa* which are mostly resistance to various types of antibiotics. The main causes for anaerobic infection at this stage are *Clostridium species* and *Fusobacterium specie*. While for the fungal infection they are also of the resistant types such as *Candida albicans* species which is resistant to fluconazole (Greene, 2004).

1.9 Neutropenia, Fever, Clinical Signs and Infection Types

1.9.1 Neutropenia and Fever

The occurrence of fever in neutropenic patients is an indication of the presence of infection. When a neutropenic patient has ANC ≤ 500 cell/ µl and the oral temperature ≥ 38.3˚C or ≥ 38˚C over one hour minimally then the patient is suffering from febrile neutropenia. When fever is present then 15%-20% of these fever are associated with bacteremia primarily and fungal infection as the secondary infection (Flaherty, 1999).

In case of severe neutropenia the patients will show two main characteristics which are firstly fever and secondly one of the signs of infection such as gingivitis, abdominal pain which resulted because of liver infection, kidney infection and pneumonia (Flaherty, 1999; Frey, 1999).

There are many studies which focused on fever and neutropenia such as the study by Zia Rahman *et al.* (1997) in USA on 174 breast cancer patients. The median age of the patients was 54 years (29-74 years) and they received a total of 610 chemotherapy

courses. The median number of the chemotherapeutics courses received was 3 (range 1-17 courses). One of the aims of his study was to look for the relationship between fever and ANC. The results showed that the association of fever with neutropenia was not significant until ANC is less than 500 cell/ µl ($P< 0.01$).

Klastersky (2004) in Belgium showed that 10%-50% of fever occurred with solid tumor and 80% with blood malignancy. In this study it was mentioned that the fever happened as a complication of the chemotherapy drug used and it required 7 to 12 days of treatment.

Another retrospective study by Ammann *et al*. (2004) in Switzerland was carried out on 132 febrile neutropenic patients. In their study, fever associated with neutropenia was a result of chemotherapeutics drugs used and 20%-55% of the fever was because of bacterial infection. Thus empirical antibiotic used was important. The main conclusion of their study was that the increased in the occurrence of febrile neutropenia is associated with the increased in the chemotherapy intensity. They also recommended that modification of the first line antibiotics treatment was not necessary because this will lead to change in the resistance pattern of bacteria.

Another study by Jenson (2003) in USA highlighted that fever and neutropenia were the major complications of the use of chemotherapeutics drugs. Fifty years ago mortality due to these complications was very high (80%) however at present the mortality rate has reduced to lower than 20%. They also reported that the incidence of fever and neutropenia was very high in patients who received intensive chemotherapy. Apart from infection (bacterial), fever in neutropenic patients can be caused by pyrogenic medications, blood transfusion as well as allergic reactions.

1.9.2 Neutropenia and Clinical Signs

Signs and symptoms associated with neutropenia are usually subtle, but fever remains the main and the most important sign considered as an indication of early infection (Talcott and Rubenstein, 2001). The presence of these clinical signs usually occurs in those patients who were treated with chemotherapy drugs that could lead to severe neutropenia. These signs are considered as a result of severe neutropenia since patients with severe neutropenia have been shown to develop problem and infections of the liver, kidney and chest (pneumonia) as well as oral mucositis and rectal ulcer. The occurrence of bacterial, fungal or even viral infections among severe neutropenic patient is very high thus this would lead to the clinical signs and fever (Dale, 2004; Frey and Granger, 2002; Schimpff, 2001).

One study conducted by Munschi (2000) in the USA suggested that it is very important that the physician must focus on the signs and symptoms associated with severe neutropenia. Also this study indicated that any neutropenic patients with ANC < 500 cell/ µl and with the presence of fever and signs of infection need immediate hospitalization. While those who were classified as mild neutropenic patients without fever and signs of infection just need monitoring of the blood count several times per week for 6 to 8 weeks. So this study demonstrated that signs and symptoms of infection or inflammation were mainly associated with severe neutropenia.

Also the National Comprehensive Cancer Network (NCCN®) highlighted that when cancer treatment caused neutropenia, this is considered as a serious condition since it could lead to infection especially when associated with fever. The usual conditions associated with severe neutropenia are hypotension, liver infection, kidney infection and

pneumonia. So NCCN guideline wrote that the signs associated with severe neutropenia were a result of infection (NCCN, 2006).

1.9.3 Neutropenia and Infection Types

When neutropenia or febrile neutropenia occur the patient will be at risk of infection either by gram-positive (G+ve) bacteria, gram-negative (G-ve) bacteria, fungal or even viral infection (Linker, 2000). About 60 % of the patients are infected with G+ve organisms which include *Coagulaes-negative staphylococcus* and *Staphylococcus epidermis* and 30% are infected with G-ve organisms such as *Escherichia coli, Klebsiella* spp. *and Pseudomonas aeruginosa*. While 10% of febrile neutropenic patients are infected by fungal infection such as *Candida* and *Aspergillus* (Flaherty, 1999). Fungal infection is considered as a secondary infection however it could also become a primary infection if neutropenia persist for more than 10 days (Mitchell, 1999). Thus two blood cultures are needed for investigation that is one for bacteria and the other for fungal. These blood cultures should be taken one from the central venous catheter and the other from the peripheral vein (Mitchell, 1999). Bone aspiration and biopsy should also be taken in order to ascertain the main cause of the infection (Mitchell, 1999). Viral infection happens with neutropenic patient especially with recipients of bone marrow transplant. Usually these viruses include *Herpes simplex*, *Cytomegalovirus virus* and *Varicella-zoster* which is the most dangerous infection in neutropenic patient (Mitchell, 1999).

1.9.3.1 Neutropenia and Bacterial Infection

The gram-negative (G-ve) bacterial infection has been the major and the predominant infection in the last 3 decades. During 1970s the G-ve infection represented 60%-70% of the infections, while starting from the middle 1980s gram-positive (G+ve) became the major and predominant bacterial infection (Linker, 2000; Kimble-koda, *et al*., 2002; Zinner, 1999; Sylvester, 2003).

Table 1.1 show the types of bacteria that may cause infection in neutropenic patients.

Table 1.1: The types of bacteria that may cause infection in neutropenic patient (Baskaran *et al.*, 2007).

Gram positive bacteria	Gram negative bacteria
1- *Streptococcus species* 2- *Coagulase-negative staphylococcus* 3- *Enterococcus species* 4- *Bacillus species* 5- *Corynebacterium species*	1- *Enterobacter species* 2- *Escherichia coli* 3- *Pseudomonas aeruginosa* 4- *Klebsiella species* 5- *Proteus mirabilis*

There are many studies that have looked at bacterial infection in neutropenic patients which will be discussed below.

Firstly, a study was performed in Turkey by Gencer *et al*. (2003) on a total number of 177 neutropenic patients with median age of 55 years old (14 to 93 years). Ninety eight (98, 55%) of them were men and 79 (45%) of them were women. All of the

patients were suffering from neutropenia in different stages of severity and 67 of them were suffering from infection. Among the 67 patients with infection, 38 were diagnosed microbiologically while 29 were not. Most of the patients suffered from lower respiratory tract infection followed by urinary tract infection. The types of bacteria isolated were *Escherichia coli* (31%), *Klebsiella pneumonia* (18%), *Pseudomonas aeruginosa* (13%) and *Streptococcus pneumoniae* (13%). So this study showed that infection among the neutropenic patients was mostly caused by G –ve bacteria.

A second study was done by Maschmeyer and Hass (2007) in USA. This study suggested that the main factor that leads to mortality and morbidity among neutropenic patients was infection. They reported that since 1995 the predominant organism causing blood infection was gram-positive cocci bacteria, which increased from 62% to 72%. While the gram-negative bacilli infection decreased from 21.5% to 14% and the fungal infection decreased from 15% to 8%. They also suggested several factors that could lead to the increased in the gram-positive bacterial infection such as the high doses of chemotherapy (cytarabine), the used of oral nonabsorbable antibiotics and the used of proton pump inhibitors. Beside that severe mucosal damage of the gastrointestinal tract also put the neutropenic patients under the risk of gram-negative, gram-positive and candida infections.

Another study by Rolston (2005) focused on the factors that may increase the risk of bacterial infection. This study highlighted seven factors that could increase the risk of infection among neutropenic patients which are:

1- The neutropenia duration and severity.

2- The chemotherapeutic drug type and intensity.

3- The patient’s condition.

4- The antibiotics effect.

5- The central venous section catheter (CVSC) or other external device used.

6- The characteristic of the surrounding environment for the patient.

7- The prolonged period of hospital stay.

This study mentioned a very unique and important point which is the site of blood sampling taken for diagnosis of bacteria causing the infection. Since most of the studies depend only on blood stream culture this could lead to incorrect or incomplete picture for the type of bacterial infection. This is because only 15%-25% of the neutropenic patients develop bloodstream infections. While the other major sites for infections are the respiratory tract, skin, gastro intestinal tract (GIT) and the urinary tract. The major type of bacteria at these sites that could cause infection is the gram-negative bacteria, while in the blood stream the major type of bacterial causing infection is the gram-positive type. Another important focused of this study was the incidence of poly microbial infection. Neutropenic patient suffered from more than one infection and more than one bacterial type. Approximately 80% of the poly microbial infections contain a gram-negative bacteria. It was also reported that the percentage of gram-positive infection associated with mono microbial infection has declined from 75% to about 50%. Thus a new guideline was proposed whereby the entire bacterial infection spectrum must be obtained before starting empirical antibiotic treatment. Also in selecting the suitable empirical antibiotics, both blood stream culture test and biopsy results are needed.

1.9.3.2 Neutropenia and Fungal Infection

The fungi kingdom is very large and contain more than 100,000 species but only 200 species are responsible for infection in human (Dale, 2005). But unfortunately in the

last few years there are changes in the kind of the fungal infection and it has became dangerous and is considered as a life-threatening infection. This is especially of concern in patients who are really under continues danger of possibility being infected. These are patients with immunodeficiency virus (HIV), cancer patients (i.e., neutropenic patients), transplant recipients and patients on immune suppressive treatment (Richardson and Warnock, 2003). Fungal infection is usually slower than bacterial infection and usually occur by inhalation of spores or direct inoculation of the skin. Fungal infection usually occurs or takes place because of the overwhelming growth of the resident fungal population (Dale, 2005).

1.9.3.3 Neutropenia and Viral Infection

Viral infection occurs frequently in people during their lives. It will usually cause an unpleasant feeling but has no serious dangerous effect on their lives except for cancer patients especially those who suffer from neutropenia. This is because of the weakness or defect in their immune system. The most predominant virus infecting cancer patients is *Cytomegalovirus*, while *Herpes simplex virus* (HSV) and *Herpes zoster virus* (HZV) are less frequent (Frey and Granger, 2005).

1.10 Neutropenia and Its Treatment

1.10.1 Granulocyte Colony Stimulating Factors (G-CSF)

Colony stimulating factors (CSF's) are glycoproteins which regulate the proliferation, differentiation, function activity and survival of myeloid cells. This will help in reducing the duration and severity of neutropenia as a result of chemotherapy use (Dale, 2004).