SURVIVAL
OF CHILDREN WITH ACUTE LEUKAEMIA
IN PAEDIATRIC ONCOLOGY HOSPITAL USM
FROM 1990 to 2010

by

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LIST OFABBREVIATIONS

ACCIS	Automated Childhood Cancer Information System
AIEOP	Italian Association of Paediatric Hematology and Oncology
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloid Leukemia
ANLL	Acute Non Lymphoblastic Leukemia
APL	Acute promyelocytic leukaemia
BFM	Berlin Frankfurt-Munster
BFM-SG	Berlin Frankfurt-Munster Study Group
BM	Bone marrow
BMA/T	Bone marrow aspiration/ trephine
CCG	Children's Cancer Group
CCRP	Childhood Cancer Registry of Piedmont
CI	Confident Interval
CLCG-EORTC	Children Leukemia Oncology Group EORTC
CML	Chronic Myeloid Leukemia
CNS	Central nervous system
COG	Children's Oncology Group
CR	Complete remission
CSF	Cerebrospinal fluid
DIVC	Disseminated intravascular coagulopathy
EFS	Event Free Survival
EGIL	European Group for Immunological Characterization of Leukemia

EORTC	European Organization for Research and Treatment of Cancer
EUROCARE	European co-operative Cancer Registry based Project
FAB	France American British
HR	Hazard Ratio/ High risk
Hospital USM	Hospital Universiti Sains Malaysia
ICP	Intracranial pressure
IQR	Interquartile range
MRC	Medical Research Council
MRD	Minimal residual disease
MTHFR	Methylenetrtrahydrofolate reductase
NCCLS	Northern California Childhood Leukemia Study
OS	Overall Survival
PCR	Polymerase chain reaction
pEFS	predicted Event Free Survival
POG	Paediatric Oncology Group
pOS	predicted Overall Survival
RR	Relative risk
SR	Standard risk
TCCSG	Tokyo Children's Cancer Study Group
TLS	Tumour lysis syndrome
TWC	Total white cell
UKALL	United Kingdom ALL
UKCCS	United Kingdom Childhood Cancer Study
VHR	Very high risk
WHO	World Health Organization

ABSTRAK

Pengenalan

Perbezaan jangka hayat pesakit leukemia akut di negara-negara maju dan negara-negara membangun sudah diketahui umum. Namun, kajian mengenai hasil rawatan dan juga jangka hayat leukemia akut di kalangan kanak-kanak. yang diterbitkan oleh negara-negara membangun termasuk Malaysia masih kurang. Sehingga sekarang, belum ada satu pun kajian sebegini yang diterbitkan dari Hospital USM.

Objektif

Objektif kajian ini adalah untuk menilai kadar jangka hayat menyeluruh (OS) dan jangka hayat tanpa sebarang kejadian (EFS), dan untuk mengenalpasti faktor-faktor jangka hayat tanpa sebarang kejadian untuk pesakit Leukaemia Lymphoblastic Akut (ALL) dan Leukemia Myeloid Akut (AML).

Kaedah Kajian

Kajian ini dijalankan secara retrospektif melibatkan kanak-kanak berumur antara bayi baru lahir hingga 12 tahun yang disahkan menghidap penyakit ALL dan AML serta menerima kemoterapi di Unit Onkologi Kanak-Kanak Hospital USM dari tahun 1990 hingga 2010. Kanak-kanak dipilih dan dikenalpasti daripada senarai pendaftaran di unit onkologi dan unit rekod perubatan Hospital USM. Model Kepelbagaian Regresi Cox digunakan untuk meramal faktor-faktor berisiko tinggi untuk kematian atau penyakit berulang bagi penyakit ALL dan AML secara berasingan.

Keputusan

Terdapat sebanyak 334 kanak-kanak menghidap penyakit leukemia akut (257 ALL dan 77 AML) yang didaftarkan di dalam senarai pendaftaran. Sebanyak 224 kanak-kanak ALL dan 59 AML yang menyertai kajian, memberikan kadar penyertaan sebanyak 87% untuk ALL dan 77% untuk AML. Purata jangkamasa rawatan susulan adalah 73 bulan. Kadar jangka hayat menyeluruh (OS) untuk ALL pada 1, 3 dan 5 tahun adalah 77.7%, 66.9% dan 63.5% dan untuk AML masing-masing 59.3%, 41.4% dan 39.1%. Kadar kehidupan tanpa sebarang kejadian (EFS) untuk ALL pada 1, 3 dan 5 tahun adalah 69.6%, 54.1% dan 47.8%, manakala untuk AML masing-masing 52.0%, 40.6% dan 38.1%.

Analisa Multivariat menunjukkan faktor-faktor berisiko tinggi untuk ALL adalah umur kanak-kanak melebihi 10 tahun semasa penyakit disahkan (HR 3.6; 95% CI: 1.9-6.7), dan umur antara 5 hingga 9.9 tahun (HR 1.6; 95% CI 1.1-2.4).

Analisa yang sama untuk AML menunjukkan saiz limpa > 5cm (HR 4.1; 95% CI: 1.4-11.1), dan protokol rawatan AML BFM-87 (HR 2.9; 95% CI: 1.3-6.3) adalah lebih berisiko berbanding saiz limpa ≤ 5cm dan protokol rawatan AML 12 UK.

Kesimpulan

Jangka hayat untuk ALL dan AML dalam kajian ini adalah setanding dengan negara-negara membangun tetapi masih rendah berbanding dengan negara-negara maju. Kajian ini juga telah dapat mengenalpasti faktor-faktor peningkatan risiko kematian dan penyakit berulang untuk penyakit ALL dan AML di tempat kajian.

ABSTRACT

Introduction

It is well known that there is difference in survival of children with acute leukaemia between developed and developing countries. However, there are lack of reports in the treatment outcome and the survival of children with acute leukaemia in developing countries including Malaysia. To date, there is no published study predicting the treatment outcome in Hospital USM.

Objectives

The objectives of this study were to evaluate the overall survival (OS) and event free survival (EFS) rate and to identify factors that determine the EFS of childhood Acute Lymphoblastic Leukaemia (ALL) and Acute Myeloid Leukaemia (AML) in Hospital USM.

Methodology

This study was a retrospective record review of children from newborn to 12 years old who were diagnosed with ALL and AML and received chemotherapy in Paediatric Oncology Unit Hospital USM from year 1990 to 2010. Children who were recruited were identified from registry in paediatric oncology unit and medical records in Hospital USM. Multiple Cox Regression model was used to predict the poor prognostic factors for increased risk of death or relapsed for ALL and AML separately.

Results

There were 334 acute leukaemia children (257 ALL and 77 AML) in the registry. Out of these, 224 ALL and 59 AML were enrolled, giving a response rate of 87% for ALL and 77% for AML. The mean duration of follow up was 73 months. The OS rate for ALL at 1, 3 and 5 years were 77.7%, 66.9% and 63.5% respectively and for AML were 59.3%, 41.4% and 39.1% respectively. The EFS rate for ALL at 1, 3 and 5 years were 69.6%, 54.1% and 47.8%, for AML were 52.0%, 40.6% and 38.1% respectively.

Multivariate analysis showed that the independent poor prognostic factor for ALL are presenting age at diagnosis; age above 10 years old (HR 3.6; 95% CI: 1.9-6.7), and aged between 5 to 9.9 years old (HR 1.6; 95% CI: 1.1–2.4).

Similar analysis showed that spleen size > 5cm (HR 4.1; 95% CI: 1.4-11.1), and treatment protocol with AML BFM-87 (HR 2.9; 95% CI: 1.3-6.3) were independent poor prognostic factors for AML compared to spleen size ≤ 5cm and AML 12 UK protocol.

Conclusion

Survival rate in this study was comparable to developing countries but remained low compared to developed countries. The study had also identified a few prognostic factors for increased risk of death and relapsed in ALL and AML for the local set up.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Hospital Universiti Sains Malaysia (Hospital USM) is the only tertiary centre for Paediatric Oncology in the East-Coast of Peninsular Malaysia, and is the referral centre for childhood malignancies for the state of Kelantan, Terengganu and Pahang. Paediatric Oncology Unit Hospital USM officially started in 1989. It is a 23 bedded ward specifically design for the treatment of oncology children. Most of the children are warded and treated for haematological malignancies which include acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML). Other cases being treated in this ward are lymphoma and multiple solid tumours.

The aim of this study was to evaluate the survival of childhood ALL and AML who received chemotherapy in Paediatric Oncology Unit Hospital USM from year 1990 to 2010 as well as to identify the factors that determine the survival of childhood acute leukaemia.

In Malaysia, only a few studies have been published on the treatment outcome of acute leukaemia. Ng *et al.* (2000) published a study regarding treatment outcome of childhood ALL from Hospital University Kuala Lumpur, while a similar study done in Hospital USM by Nik Nasiruddin *et al.* (2007) remained unpublished.

1.2 SURVIVAL

ALL represents approximately 80% of all childhood leukaemias, followed by AML (15%), and CML (5%). The worldwide incidence has increased and it becomes an important reason for childhood mortality. Fortunately with modern treatment regime, the treatment responses are encouraging leading to better overall survival (OS). The OS and event free survival (EFS) can be looked at different time frames, such as at 1 year, 2 years, 3 years, 5 years, 10 years, 15 years and 20 years.

There are no single established criteria for the definition of OS and EFS. Review of other studies showed that there were various definitions of OS and EFS. OS is defined as the time from the diagnosis until death from any causes or until the last follow up (Tang *et al.* 2008; Yeoh *et al.* 2012).

In Malaysia, EFS is defined as the interval between the date of diagnosis and the earliest occurrence of the following events: induction failure, leukaemic relapse, death from any cause, last contact, development of a second malignant neoplasm and bone marrow transplant (Ng *et al.*, 2000).

Bonilla *et al.* (2010) in El Salvador defined EFS as the time from diagnosis to the first event such as resistant disease, relapse, and second malignant neoplasm, abandonment of treatment or death or last contact.

In an unpublished study in Hospital USM by Nik Nasiruddin *et al.* (2007), EFS was defined as the time from initiation of therapy to the date of failure (any relapse, death or secondary cancer) or to the date when the patient was confirmed to be well at the time of recruitment, whichever occurred first.

In France, Sirvent *et al.* (2011) calculated the EFS from the date of complete remission until the first event (relapse, death of any cause) or until the last follow up.

In this study, EFS was considered the time from diagnosis to the date of any event or to the date when patient was confirmed to be well, or at the date of census, whichever occurred first, (the event was considered as relapse, defaultment, death or any major complications).

1.2.1 Survival in Developed Countries

In developed countries, many studies had been published regarding survival and treatment outcome of childhood acute leukaemia. A study conducted in France between the year 1990 to 2000, involving 4807 children with acute leukaemia found that the survival rate for ALL at 1 year was 94% (95% CI: 93, 95) and at 5 years was 82% (95% CI: 80, 83). On the other hand, AML survival was consistently lower than ALL, it was 95% (95% CI: 91, 95) at 3 months and 60% (95% CI: 57, 64) at 3 years (Goubin *et al.*, 2006).

There was a longer study duration completed in Italy that showed the trend of ALL survival in between the year 1972 to 2001. In this study, a total of 967 leukaemia children were recruited and out of this, 754 were children with ALL. The results showed that 10 years survival for ALL has improved from 86% (95% CI: 83, 88) to 97% (95% CI: 95, 97) for children diagnosed from 1972 to 1976 and from the year 1997 to 2001. ALL showed stable survival rates after 10 years following diagnosis (Zuccolo *et al.*, 2006).

The difference in improvement of survival between ALL and AML was observed in the European studies as well. This was supported by data collected from the Automated Childhood Cancer Information System (ACCIS) project from the year 1978 to 1997. The improvements were attributed to better intensive care treatment protocol and adherence to treatment, (Coebergh *et al.*, 2006). The study also emphasized on survival

of 25623 children with acute leukaemia. The 5 years survival went from 51% (95% CI: 50, 53) in children diagnosed from 1978 to 1982, to 77% (95% CI: 76, 78) for those diagnosed from the year 1993 to 1997. Overall survival in North Europe for ALL was 83% (95% CI: 80, 85), whereas for AML it was 54% (95% CI: 46, 62) (Coebergh *et al.*, 2006).

United Kingdom Childhood Cancer Study (UKCCS) reported survival rate of 1899 children with leukaemia diagnosed from 1991 to 1996. In this report, 1573 were children with ALL and 326 were AML. The 5, 10 and 15 years survival rate for ALL were 81.1% (95% CI: 79, 82), 75.3% (95% CI: 73, 77) and 74.2% (95% CI: 71, 76) respectively. Whereas for AML, 5, 10 and 15 years survival were 55.9% (95% CI: 50, 61), 53.8% (95% CI: 47, 59) and 53.5% (95% CI: 47, 59) respectively.

The most recent paper that was published in the Journal of Clinical Oncology in 2012 showed that ALL survival rates had improved significantly. The study conducted by the University of Colorado Cancer Centre Aurora, Colorado, which included 21000 children and more than half of the population were from the United States who were diagnosed from 1990 to 2005. The author found that 5 year survival rates improved from 83.7% to 90.4% in the year 1990 to 1994 and in the year 2000 to 2005 respectively. The survival rates increased for boys and girls in all races and ages except in infants as most of the infants died due to the side effects of treatment. Furthermore, infant had different biological feature of leukaemia which carries poorer prognosis (Hunger *et al.*, 2012).

In a study conducted in Brazil among ALL children aged from 2 months to 89 months in 1995 to 2004. Total of 84 children were treated with Brazilian Group for the Treatment of Childhood Leukaemia Protocol with their 5 years EFS was 74.1% (Scrideli *et al.*, 2006).

A similar study was conducted in Czech Republic looking at survival of childhood AML. The study enrolled 61 AML children who were treated with AML BFM 93 Protocol. It was reported that 73.8% of them achieved complete remission, OS and EFS rates at 5 years were 45.3% and 42.3% respectively. As expected, the survival was better for standard risk group compared to high risk group with EFS of 62.5% versus 29.7% respectively ($p = 0.030$) (Stary *et al.*, 2004).

In addition to that, a study published in the United Kingdom that was conducted from 1995 to 2002 involving children less than 16 years old showed the 10 years OS and EFS to be 63% and 54% respectively (Gibson *et al.*, 2011).

1.2.2 Survival in Developing and Non-developed Countries

There was a difference in terms of treatment outcome between developed and developing countries. The survival was found to be lower in developing countries compared to the developed countries (Nik Nasiruddin *et al.*, 2007).

In our country, there were not many studies conducted on this topic. A study done in University Hospital Kuala Lumpur involving 575 children aged 12 years old and below from 1980 to 1995 reported 2 years survival rate for ALL was 67% (Ng *et al.*, 2000).

In Hospital USM Kelantan, unpublished study by Nik Nasiruddin *et al.* (2007) described the treatment outcome of children with ALL. In this study, a total of 102 ALL children were recruited from the year 1990 to 2003. They found that overall EFS rate was 81.4% (SE ± 3.9) at 1 year, 59.8% (SE ± 4.9) at 3 years, 55.3% (SE ± 5) at 5 years and 52% (SE ± 5.2) at 10 years.

In a recent study conducted among Malaysian and Singaporean population from 2002 to 2011 by using risk adapted treatment modified BFM ALL protocol. They studied 556

children aged 1 month to 18 years old using minimal residual disease (MRD) as treatment stratification. They found that 5 and 6 years OS and EFS rate were 88% and 80% respectively (Yeoh *et al.*, 2012).

Another study carried out by Chan *et al.* (2004) demonstrated an increased in 5 years EFS rate among AML children who were treated with AML BFM 83 regimes in University of Malaya Medical Centre. In this study, a total of 174 paediatric children were enrolled from 1985 to 1999. The 5 years EFS rate for children diagnosed from 1985 to 1993 was 30.7% and from 1993 to 1999 was 41.3% respectively.

The increased in survival rate of childhood leukaemia was also demonstrated in a study published in our neighbouring country, Singapore. This study was conducted from the year 1988 to 1994. A total of 66 children were recruited, in which 53 children were ALL and 13 were AML. A two year disease free survival (time from completed treatment until remission or no disease) for ALL was 62% and 30% for AML (Quah *et al.*, 1996).

In addition to this, there were also a few studies in China regarding the survival of acute leukaemia. Tang *et al.* (2008) in their study from the year 1998 to 2004 involving 346 ALL children aged less than 16 years old revealed that 5 years overall EFS rates as 38.5%. They found high rate of abandon treatment among the children (48%).

Another study on AML involving 185 children aged less than 16 years old from year 1997 to 2005 found that 32% of the children refused chemotherapy. For those who were treated, the 5 years OS and EFS rates were 50% and 46% respectively (Xu *et al.*, 2010).

In conclusion, the overall findings from these studies showed improvement of survival rate over the years and it was higher in developed countries. Advancement in current

chemotherapy currently showed an increase in cure rate and better survival rate in acute leukaemia. In all childhood malignancies, ALL achieved the most favourable prognosis.

1.3 EPIDEMIOLOGY AND INCIDENCE

The incidence of acute leukaemia has arisen over the years. This had been reported in many studies and it was largely attributed by many factors such as higher diagnostic awareness, changing in environment and pollutant. The increased in incidence of acute leukaemia was found to be irrespective of age, gender or staying in developed or developing countries. For ALL, the incidence was more prevalent in male compared to female and the most affected age group was 1 to 4 years old with peak age between 2 to 4 years old. However, for AML the incidence was equal between both genders. The highest incidence occurred in those aged 1 to 4 years old with a peak incidence at 2 years old.

To date, there are many large studies (trials) looking at the incidence of childhood leukaemia according to age and gender. One such study was done in Europe between 1978 and 1997 which involved 17065 leukaemia children aged from 0 to 14 years old. Majority of these children 8043 (47%) were within age group 1 to 4, 4831 (28%) age 5 to 9, 3293 (19%) between the age 10 to 14, while only 898 (5%) belongs to age < 1 years old (Coebergh *et al.*, 2006).

Another study in France which was conducted in 1990 to 2000, recruited 3995 of ALL children aged between 0 to 14 years old and this study also demonstrated similar incidence of acute leukaemia according to age group. For AML, a total number of 812 children were recruited in the study and 132 (16%) aged less than 1 year old, 259 (32%) aged 1 to 4 years old, 197 (24%) aged 5 to 9 years old and 224 (28%) aged 10 to 14 years old (Goubin *et al.*, 2006).

Interestingly, a study by Coebergh *et al.* in 2006 showed a male predominance in incidence of childhood ALL. Out of the 17065 children with ALL recruited in this study, 9551 (56%) were boys while 7514 (44%) were girls.

The overall increase in rate of childhood acute leukaemia over the years was observed in a study conducted in Ibn Ghazwan Women and Children Hospital, Paediatric Oncology Ward in Basrah, Iraq between 1993 and 2007. Total of 698 leukaemia children were registered from 1993 to 2007, comprising the age from 0 to 14 years old. The incidence of acute leukaemia was increased with the rate of 2.7 every 3 years until 2007 (Hagopian *et al.*, 2010).

Another study conducted in Thailand between 1985 and 2002 reported that the incidence has increased by 2.4% per year in boys (95% CI: -0.5 to 5.3) and 4.1% per year in girls (95% CI: 1.1 to 7.2) (Kamsa-ard *et al.*, 2006).

1.4 ETIOLOGY

The cause of acute leukaemia is multifactorial. The leukaemic transformation involves complex interactions within host (chromosomal damage) either through in utero gene abnormality or damage due to chemical exposure or infective agents. From studies among twins as well as neonatal blood spots, it has been possible to track the first initiating genetic events in fetal hematopoietic stem cells in utero for most precursors B-cell ALL and AML. In Basrah, there are many environmental factors that have been implicated as the causative agents for leukaemia such as ionizing irradiation and certain chemicals like benzene and cytotoxics (Hagopian *et al.*, 2010).

Tim Eden, (2010) in his article review stated that folate deficiency and alterations in folate metabolism resulting from polymorphic variants of the enzyme

methylenetetrahydrofolate reductase (MTHFR) had been associated with cancer, neural tube defect and heart disease. Lower activity of MTHFR has been reported to be protective for ALL.

A study was conducted by Malaysian and Singaporean ALL Study Group, showed the various genetic susceptibility to childhood ALL. In this study, cord blood sample of 756 healthy newborns (346 Chinese and 410 Malays) and blood of 531 children with ALL (321 Chinese and 210 Malays) from the year 1998 to 2008 were tested for genetic variability. They found that Malay population predominantly carries (Quinine oxyreductase) NQO1 609CT genotype. A comparison was made between gender, this gene showed significant protective effect on leukaemia risk in Malay boys (OR = 0.4; 95% CI: 0.22, 0.66; p = 0.001). In the Chinese cohort, instead of NQO1 609CT genotype, they carry MTHFR 1298 c-allele; and this is significantly associated with increased risk of leukaemia among Chinese boys (OR = 1.7; 95% CI: 1.17, 2.44; p = 0.005) (Yeoh *et al.*, 2010).

On the other hand, several inherited genetic abnormalities has been showed as predisposing factor to leukaemia including Down's syndrome, Fanconi's anemia, Bloom syndrome and ataxia-telengiectasia. In cancer treatment review, an exposure to pesticide also had been found to increase risk of leukaemia (OR = 1.5; 95% CI: 1.0, 2.2). One example was parental exposure to hydrocarbons (OR = 1.8, CI: 1.3, 2.5). Benzene had also been implicated as a potential leukaemogenic agent as well (Eden, 2010).

1.5 CLINICAL FEATURES

The clinical features of childhood acute leukaemia are mainly attributed by ineffective haematopoiesis. Thus, it results in anaemia, thrombocytopenia, abnormal white cell, infiltration of leukemic cell into organs and constitutional symptoms. In general, the presentation for ALL and AML could be similar. The usual presentations for this childhood malignancy are pallor and lethargic, prolonged fever, bruises or bleeding tendency. Disseminated intravascular coagulopathy (DIVC) might be observed at initial presentation of AML especially in acute promyelocytic leukaemia (APL). Other features are due to organ infiltration by leukaemic cells such as hepatosplenomegaly, lymphadenopathy, testicular swelling, arthritis, acute renal failure, meningeal syndrome (features of increase ICP, papilloedema, retinal haemorrhage) or anterior mediastinal mass (T-ALL) and chloromatous tumour (AML).

1.6 DIAGNOSIS

The presence of blast cell in peripheral blood film is suggestive of leukaemia. However, gold standard for the diagnosis is by morphological FAB criteria and cytochemistry analyses with > 25% of blast cells present in BMA.

1.7 CLASSIFICATION

1.7.1 Classification of ALL

Blast cells are the leukemic cells. There are two methods of classification of this disease. The best classification is the FAB classification (French-American–British) which is based on the morphological of the cell. This classification subdivided ALL into

3 subgroups:

i) L1 : small lymphoblast, little cytoplasm (good prognosis)

ii) L2 : larger and pleomorphic lymphoblast, more cytoplasm

iii) L3 : large lymphoblast, cytoplasmic vacuoles, fine stippled nuclear chromatin

This classification has a prognostic value. The L1 subtype is more frequent in younger children, whereas L3 is more common in older children. The L1 subtype is associated with good prognosis (Schrappe *et al.*, 2000).

The other classification is based on immunophenotypes classification, which can be divided into B-lineage and T-lineage cells. It is based on the differentiation stages of lymphoid progenitors during haematopoiesis (Plasschaert *et al.* 2004).

European Group for the immunological characterization of Leukaemia (EGIL) classified ALL into B-cell, T-cell or biphenotypic acute leukaemia (mixed type). B lineage markers are CD 10, CD 19, CD 22 and CD 79a, whereas T-cell markers are CD 1a, CD 2, CD 3, CD 4, CD 5, CD 7, and CD 8. All of the B-cell and T-cell can additionally express myeloid antigens or stem-cell antigen CD 34. Biphenotypic acute leukaemia (BAL) is a rare lineage and it co-expresses markers of two different lineages. It is defined when scores are more than 2 for myeloid lineage and more than 1 for the lymphoid lineage. It also shows much higher incidence of CD 34 antigen expression, extramedullary infiltration, relapse and resistance to therapy after relapse.

1.7.2 Classification of AML

For AML classification, two systems have been used; The French American British (FAB) classification and newer World Health Organization (WHO) classification.

The older FAB system divides AML into subtypes, based on the type of cell involved and the stage of maturation. It is classified mainly based on morphology using cytochemical stains. There are eight subtypes of AML; from M0 to M7, (as shown in Table 1).

The WHO classification system looks into several broad groups, cytogenetics and early response to treatment and it will take into account the prognostic factors, (as shown in Table 2) (Gertjan *et al.*, 2007).

Table 1: FAB Classification of AML

FAB Classification of AML	
M0	This subtype of AML is made up of very immature cells. This type of cells can be distinguished from ALL by flow cytometry. It is very rare in paediatric age group.
M1	This subtype is made up of immature myeloblasts. It can be recognized by the way the cells look under the microscope by using cytochemical stains.
M2	This subtype is composed of slightly more mature form of myeloblasts. It is the most common AML subtype in children.
M3	It also known as APL; it is made up of promyelocytes, which are more mature forms of myeloblasts. Treatment of APL is different from other subtypes of AML, as it prone to have DIVC.
M4	This is also known as acute myelomonocytic leukaemia. The cells are early form of monoblast. It is more common in children less than two
M5	years old. It is known as acute monocytic leukaemia and made up of monoblasts. Like M4 subtypes, it is more common in children younger than 2 years of age.
M6	This subtype is known as acute erythroblastic leukaemia. It starts in erythroblasts, the cells that normally mature into red blood cells. It is very rare in paediatric.
M7	This is known as acute megakaryoblastic leukaemia. The cells are megakaryoblasts, which normally mature into megakaryocytes (the cells that make platelets).

Table 2: WHO Classification of AML

WHO Classification of AML	
AML with recurrent cytogenetic translocations	AML with t(8;12)(q22;q22), AML1(CBF-α)/ETO Acute promylocytic leukaemia AML with t(15;17) and variant; PML/RARα AML with abnormal bone marrow eusinophils AML with 11q23 (MLL gene) abnormalities
AML with multilineage dysplasia	With prior myelodysplastic syndrome Without prior myelodysplastic syndrome
AML with myelodysplactic syndrome, therapy related	Alkylating agent related Epipodophyllotoxin related Other types
AML not otherwise categorized	AML minimally differentiated AML without maturation AML with maturation Acute myelomonocytic leukaemia Acute monocytic leukaemia Acute eryhtroid leukaemia Acute megakaryocytic leukaemia Acute basophilic leukaemia Acute panmyelosis with myelofibrosis

1.8 PROGNOSTIC FACTORS

1.8.1 Prognostic Factors for ALL

Few prognostic factors had been identified for childhood ALL. The risk of relapse mostly depends on the risk factor themselves. Different institutions applied prognostic factor differently to define risk categories, based on differences in biologic and socioeconomic background. Biologic variables included demographic features such as age and sex, disease related features such as initial TWC, uric acid level, DNA index, immunophenotype, presence of mediastinal mass at diagnosis and CNS status.

Risk category is further divided into standard risk (SR) or high risk (HR). SR is defined when age at presentation is between 1 to 10 years old, initial TWC $< 50 \times 10^9$/L, absence of high risk features such as CNS and testicular involvement, T cell immunophenotype, M3 marrow on D15 or M2/ M3 marrow on D36 and DNA index 1.16 to 1.6 (Bonilla *et al.*, 2010). M2 marrow is defined as the presence of 5 to 25% of blast cells in bone marrow (partial remission) while M3 marrow is defined as the presence of more than 25% blast cells in bone marrow (not in remission).

Age at diagnosis is one of the prognostic factors for ALL. Infants and children older than 10 years old have poorer prognosis and this had been shown in many previous studies. In France, 5 years EFS rate was 87% (95% CI: 85, 88) for children aged 1 to 4 years, followed by children 5 to 9 years old with EFS rate of 83% (95% CI: 81, 85). The 5 years EFS for children aged 10 to 14 years was 72% (95% CI: 69, 76) and infant less than 1 year old was only 48% (95% CI: 39, 57), it was statistically significant ($p = 0.001$) (Goubin *et al.*, 2006).

A similar pattern was seen in a recent study in the United Kingdom by Lightfoot *et al.* (2012) involving 1559 ALL children less than 14 years old. Five years EFS rate were 85%, 80%, 70% and 45% in children age 1 to 5, 6 to 9, more than 9 years and less than 1 year old respectively. Another study conducted in Pakistan showed children aged 1 to 9 years old had a better outcome compared to infants < 1 year or children > 10 years old ($p < 0.040$) (Khalid *et al.*, 2010).

Another prognostic factor for ALL is gender. Previous studies have shown that female have better outcome compared to male, and this differences appeared after 2 to 3 years old. In France a study was carried out by Goubin *et al.* (2006) showed that 5 years EFS rate in female was 83% (95% CI: 81, 85) and 81% (95% CI: 79, 82) in male, however the statistically insignificant ($p = 0.070$). This finding is similarly shown in a study in United Kingdom involving 1573 children aged 0 to 14 years old from 1991 to 1996. They found 5 years EFS rate in female as 82.6% and in male as 79.8% ($p = 0.024$) (Johnston *et al.*, 2010).

In Malaysia, Ng *et al.* (2000) found that 5 years EFS rate in male was 70% while in female it was 75% ($p = 0.100$). Another study in India revealed gender had a significant influence on the outcome of survival with a higher failure rate in boys ($p = 0.003$), (Kulkani *et al.*, 2004). They postulated that this was due to the testes acting as a 'sanctuary site' and blood testes barrier protected the leukemic cells from the anticancer drugs. This may be due to lack of stratification of treatment and hence high risk patient may have been under treated.

Majority of children with malignancies live in lower socioeconomic country or come from lower socioeconomic background (Bonilla *et al.*, 2010). The cure rate of ALL is higher in developed compared to developing countries. A few contributing factors have been identified such as lower educational background, lack of knowledge about the disease as well as effect of the treatment. Therefore, they are prone to default or abandon the treatment and this subsequently will lead to relapse.

A study done in El Selvador, a low socioeconomic country from the year 2000 to 2007 looked at the predictors of EFS in children with ALL from lower income countries. There were 443 with 260 children in standard risk and 180 children in high risk group. Five years EFS was 56% (SE ± 4.5) for standard risk group and 48.6% (SE ± 5.5) for high risk group. The 5 years OS rate was 77.7% (SE ± 3.8) for standard risk and 61.9% (SE ± 5.8) for high risk group. Parental educational level was measured in relation to the treatment outcome and the findings from this study suggest that parental secondary education gives better outcome compared to the illiterate and primary education level (HR 0.49; 95% CI: 0.29, 0.84; $p < 0.001$). This was mainly due to better treatment adherence in this group. Apart from socioeconomic status, other useful predictors of EFS include the level of income, number of siblings and nutritional status among these children (Bonilla *et al.*, 2010).

Treatment outcome associated with high total white cell count (TWC) at diagnosis has been proven as a risk factor for relapse. This represents a significant tumour burden.
A local published study by Ng *et al.* (2000) showed that high TWC and low haemoglobin level were significantly related to poorer prognosis. Out of 567 children, 126 of them had TWC above 50 x $10^3/\mu L$ at presentation and 70 children with high

TWC had failure rate (lack of response to induction therapy, relapsed during first complete remission or death due to any causes), ($p = 0.003$).

In a Brazillian study, total of 84 ALL children involved, 59 children with TWC < 50 x $10^3/\mu L$ had 5 years EFS rate of 79.3%, compared to 25 children with TWC > 50 $10^3/\mu L$ had 5 years EFS rate of 62.0% ($p = 0.030$) (Scrideli *et al.*, 2006). A similar outcome in children presented with TWC of more than 100 x $10^3/\mu L$, it was highly associated with CNS relapse rate (HR 2.9; $p = 0.002$) (Sirvent *et al.*, 2011).

In another study using multivariate analysis among Indian children showed that high TWC was a significant predictor for relapse ($p= 0.001$) (Kulkarni *et al.*, 2009).

Other than TWC at presentation, the size of liver and spleen also provide the measurement of leukemic cell burden. The size of liver or spleen (more than 3 to 5 cm), it has a positive correlation as poor prognostic factors (Ng *et al.*, 2000). Hepatosplenomegaly was present in about 50 to 65% of childhood ALL (Plasschaert *et al.*, 2004). A large study completed in United States involving 8447 childhood ALL from year 1983 to 1995. They found that children with splenomegaly had a relative risk of relapse of 1.3 times (95% CI: 1.2, 1.4) while children with hepatomegaly had a relative risk of relapse of 1.1 times (95% CI: 0.9, 1.2) (Bhatia *et al.*, 2002).

ALL is classified based on differentiation stages of development T-lineage and B-lineage lymphoid progenitors. T-cell immunophenotype has poorer prognosis even though it is less common compared to B-cell ALL as T-cell was associated with high TWC at presentation, mediastinal mass and it occurs in older children. Children who did not go into remission were in the older age group, diagnosed with T-cell ALL, and this result is statistically significant ($p = 0.020$) (Sazawal *et al.*, 2001).

Supriyadi *et al.* (2012) conducted a study in Indonesia involving 239 ALL children from year 2006 to 2011. They were treated with Indonesian 2006 Protocol. Seventy seven percent of them were B–cell lineage and 23% were T-cell lineage. Majority of T-cell children were in high risk group with TWC more than 50 x $10^3/\mu L$. Children with T-cell lineage with myeloid expression had significant higher adverse prognostic factors compared to myeloid negative ($p = 0.028$). Three years EFS rate was 40% in myeloid positive compared to 77% in myeloid negative. B-cell lineage carried good prognosis because it was associated with lower TWC.

CNS infiltration or meningeal leukaemia is one of the frequent manifestations of extramedullary leukaemia. Children may present with signs and symptoms of increased in intracranial pressure such as vomiting, headache, blurring of vision, seizure or neurological deficit. CNS infiltration at diagnosis is known to be one of the poor prognostic factor and children must be stratified in high risk group.

CNS infiltration is defined as finding of more than 5 cell/ uL^{60} in cytocentrifuge CSF blast cell (Plaschaert *et al.*, 2004). It is divided into CNS 1, CNS 2 and CNS 3.

CNS 1 is defined as less than 5 WBCs/uL with no CSF blast cells. CNS 2 is when there is less than 5 WBCs/uL with CSF blast cells and CNS 3 is presence of 5 or more WBCs/uL with the presence of CSF blast cells or cranial nerve palsy.

Ng *et al.*, 2000 showed among Malaysian population, CNS involvement at diagnosis was 2% (Ng *et al.*, 2000).

Similar finding by Plaschaert who described in Netherland population, the CNS involvement was about 2.6 to 3.6% (Plaschaert *et al.*, 2004).

Kulkarni *et al.* (2004) concluded in their study that there was high incidence of relapse especially CNS due to more children with high risk group compared to developed

country. Their 5 year survival rate in CNS involvement was 38.7% whereas in non CNS involvement was 72%.

In EORTC Children Leukaemia Group study, they conducted a study on survival rate in children with initial CSF involvement treated without cranial irradiation. This study involved ALL children aged less than 18 years from year 1989 to 1998 in France, Belgium and Portugal. They found that OS rate among children with CNS 1 at 8 years was 80.9% (SE ± 0.9) whereas in children with CNS 3 was 67.4% (SE ± 6.8) (Sirvent *et al.,* 2011). The evidence support the need for additional CNS directed therapy, intrathecal and radiotherapy and prophylactic CNS therapy to increase survival rate.

Relapse of disease is known to be one of the major events in acute leukaemia. It is a major complication which caused mortality and morbidity. Relapse can occur at many sites and majority occurred in the BM followed by CNS, testis or combination. An Indian study showed typical pattern of relapsed in childhood ALL. The study found 24% (n = 127/532) of their children developed relapse. Majority of relapse occurred while children were still on chemotherapy. Bone marrow relapse occurred in 40.8% (n = 51) and CNS relapse in 18.9% (n = 24). Isolated testicular relapse were seen in 15.3% (n = 17/111). Significant predictors for relapse were male gender (p = 0.030) and high TWC at presentation (p = 0.001) (Kulkarni *et al.*, 2009).

In recent paper published by Yeoh *et al.* (2012), out of 556 children, 6% (n=36) children developed relapse and 61% of them had isolated BM relapse.

Early treatment response to steroid also give significant prognostication for childhood ALL. It indicates the rate of clearance of leukaemic cells from the blood or BM during early phase of therapy. Each centre has different definition of early response. Good

early response is defined as TWC in FBP at day 8 of induction with steroid of less than 1000/ μL or absence of blast, whereas poor early response is defined as presence of TWC in FBP more than 1000/ μL or still presence of blast cells at day 8 of induction with steroid. Undetermined early response is defined when there was uncertainty of blast cells in FBP at day 8 of induction. Uncertainty of blast is described by haematologist as 'suspicious cells'. Five years EFS of good early response was 82.8% (SE ± 0.9) compared to poor early response was only 34.3% (SE ± 3.4) (Schrappe *et al.*, 2000).

Some other centres used the definition of good early response as blast cells less than 25% in BM at Day 7 of four chemotherapy agents during induction therapy or blast cells less than 1000/ μL in FBP after 7 days of initial prednisolone therapy, or less than 25% of blast cells in BM at day 14 following three chemotherapy agents during induction therapy (Sackmann-Muriel *et al.*, 1999).

In Malaysia, Jiang *et al.* (2011) defined good prednisolone response as children with less than 1000/ μL peripheral blast after 7 days of prednisolone and poor prednisolone response when peripheral blast counts more than 1000/ μL after 7 days of prednisolone therapy. This study involved 472 children, 422 children who had good response to steroid and the rest of 50 children had prednisolone poor response. They found significantly lower EFS rate in prednisolone poor response group ($p = 0.001$). Another study by Yeoh *et al.* (2012) documented prednisolone response status from 4 hospitals in Malaysia and Singapore, their 6 years EFS rate were 63% and 83% in children with poor prednisolone response and good prednisolone response group respectively.

In Indonesia, a study looking at early response to dexamethasone as a prognostic factor for ALL was carried out as well. They have involved 165 children aged less than 14

years old from 1999 to 2006. Out of 165 children , 133 of them with good early response had 26.3% (SE ± 3.8) 5 years EFS rate compared to 33 children with poor early response had 9.7% (SE ± 5.3) 5 years EFS rate ($p < 0.001$). The HR for poor early response was 1.9 times (95% CI: 1.25, 1.91) with statistically significant ($p = 0.030$) (Widjajanto *et al.*, 2012).

L-asparaginase is an effective anticancer drug. The outcome of paediatric ALL has improved dramatically over 40 years through a large trial including the use of asparaginase. It has been used for the past 30 years. The drug has improved EFS rate in children with ALL when given during the induction and maintenance phase. A paediatric study in relapsed ALL using pegaspargase showed patient with lower serum asparaginase enzyme activity did not achieve complete remission (Dan Douer, 2008).

Asparaginase acts as a bacterial enzyme that catalyzed the hydrolysis of asparagine to Aspartic acid. Depletion in asparagine from the serum leads to inhibition of protein synthesis and subsequently causing apoptotic cell death of leukemic cells. There are 3 forms of preparation as shown in Table 3.

Asparaginase has a distinct toxicity profile. The side effects range from hypersensitivity, hyperglycaemia, hepatocellular dysfunction, neurotoxicity and pancreatitis (Narta *et al.*, 2007). The most common side effect is hypersensitivity reaction manifested as urticaria, local erythema, indurations, skin rashes and anaphylaxis. Mild reaction can be treated with anti-histamine but in severe form, the drug needs to be terminated. If the reaction is moderate, the children will be subjected to rapid desensitization programme.

There was a study done in Ankara, Turkey from 2004 to 2008 involving 19 children with systemic anaphylactic reaction. Prior to intravenous E.*coli* asparaginase, the children were given premedication using systemic corticosteroid and antihistamine 1 hour before asparaginase. Subsequently intravenous E.*coli* asparaginase will be administered 1 IU and it was doubled every 10 minutes until desired total dose was reached. The medication was discontinued if children developed anaphylactic reaction (Soyer *et al.*, 2008)

A National Cancer Institute Common Toxicity Criteria (CTC) graded the hypersensitivity reaction ranging from 0 to 4. Grade 0 is no reaction, grade 1 is mild local skin induration or oedema less than 10 cm, grade 2 is urticaria, grade 3 is when the children have severe local reaction of more than 10 cm with bronchospasm and grade 4 when the children developed systemic anaphylaxis which can lead to death.

Table 3: Types of preparation of asparaginase

Form of asparaginase	Notes	Half-life(days)-IM administration
E. *coli*	Original form, can induce hypersensitivity reactions	1.28 ± 0.35
Erwinia	Minimal cross-reactivity with E. *coli* preparation	0.65 ± 0.13
Pegylated E. *coli* (pegaspargase or PEG)	Decrease immunogenicity	5.73 ± 3.24

Other than that, treatment response is one of the prognostic factor for childhood ALL. There were many techniques to detect early treatment response in childhood ALL, especially in developed countries. The clearance of leukemic cells from the blood or

BM is an independent prognostic factor for ALL. Previously, detection of minimal residual disease (MRD) has been performed based on morphological findings. However since 1990's, MRD can be detected using PCR.

With advanced molecular technique such as PCR for measurement of leukemic cells in BM, this may explain the MRD. MRD has been a powerful tool for assessing relapse in ALL and it becomes a risk factor for relapse (Sawada *et al.,* 2009). The study considered negative MRD if level of blast cells in the BM < 1.5×10^{-4}. The relative risk of relapse in children with MRD compared to children with absent residual disease was almost 6 times. In the absence of MRD determination, good early treatment response is defined as the absence of blast in peripheral blood film at D8 of induction (Schrappe *et al.*, 2000), or blast cells < 5% in bone marrow at D15 of induction.

Looking at the genetic aspect, both chromosome number (ploidy) and structural abnormality have a powerful prognostic indicator for the treatment outcome. The number of chromosome in leukemic clone is subdivided into high hyperploids (more than 50 chromosomes), low hyperploids with 47-50 chromosomes, pseudodiploid in which 46 chromosomes with structural abnormality, diploid (normal 46 chromosomes), and hypodiploid with less than 46 chromosome (Plasschaert *et al.,* 2004). High hyperploids are associated with good risk factors such as low TWC and common type of ALL. Incidence of high hyperploids in children was 20-32%, whereas adult was about 5-12%. Hypodiploidy had poor prognosis and they were stratified in high risk group. There were 2-4% of children with B-cell ALL had t (9, 22) translocation; Philadelphia and 1-2% had t (4, 11) translocation. Both were associated with poor outcome. Children with t (4, 11) translocation has is the worst prognosis. Translocation t (12, 21) is the most common genetic lesion in childhood ALL that is associated with