# DETECTION OF LEUKAEMIA-ASSOCIATED ABERRANT IMMUNOPHENOTYPE (LAIPs) IN ACUTE LEUKAEMIA AND ITS ASSOCIATION WITH HAEMATOLOGICAL PARAMETERS AND TREATMENT OUTCOME

BY

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AML | acute myeloid leukaemia |
| B-ALL | B-acute lymphoblastic leukaemia |
| BCR | B-cell receptor |
| BCR-ABL | breakpoint cluster region-abelson gene |
| CALLA | common acute lymphoblastic leukaemia antigen |
| FLT3 | fms like tyrosine kinase 3 gene |
| LAIPs | leukaemia- associated aberrant immunophenotype |
| MLL | mixed lineage leukaemia gene |
| MPO | myeloperoxidase |
| MRD | minimal residual disease |
| OS | overall survival |
| PCR | polymerase chain reaction |
| RFS | relapse-free survival |
| T-ALL | T acute lymphoblastic leukaemia |
| TCR | T cell receptor |
| WBC | white blood cell |

**PENGESANAN IMUNOFENOTIP ABERAN DALAM LEUKEMIA AKUT DAN PERKAITANNYA DENGAN PARAMETER HEMATOLOGI DAN KESAN RAWATAN**

## ABSTRAK

Leukemia akut merupakan salah satu kanser darah paling kerap ditemui seluruh dunia. Kehadiran antigen sel leukemia yang spesifik dan berbeza pada peringkat awal membantu untuk pendiagnosan dan mengenal pasti jenis sel yang terlibat. Sel – sel leukemia juga boleh menunjukkan imunofenotip aberan pada peringkat diagnosis. Banyak kajian telah dijalankan untuk melihat perkaitannya dengan parameter hematologi dan hasil kajiannya berlainan. Imunofenotip aberan ini biasanya digunakan sebagai penanda untuk penilaian sisa minima penyakit selepas perawatan kemoterapi. Pelbagai modaliti telah diwujudkan untuk menilai sisa penyakit leukemia akut. Salah satu modaliti yang sesuai dan sensitif yang boleh digunakan adalah flositometri. Tujuan kajian ini adalah untuk mengenal pasti kekerapan imunofenotip aberan dalam pesakit leukemia akut untuk setempat dan melihat perkaitannya dengan data sosiodemografik parameter hematologi, dan kesan rawatan selepas kemoterapi fasa induksi.

Kajian retrospektif telah dijalankan dengan mengumpul 134 sampel pesakit leukemia akut yang didiagnos di Hospital USM dalam tempoh masa kajian yang ditetapkan. Sampel-sampel tersebut dianalisa untuk imunofenotip menggunakan flositometri 4-warna dan perkaitannya dengan data sosiodemografik, pengiraan sel darah, ujian sumsum tulang sewaktu diagnosis, keputusan sitogenetik dan status remisi selepas kemoterapi fasa induksi.

Daripada 134 sampel yang dianalisa, seramai 62 pesakit didiagnos sebagai mieloid leukemia akut dan B limfoblastik leukemia akut manakala seramai 10 orang pesakit didiagnos sebagai T limfoblastik leukemia akut. Dalam AML, CD56 merupakan imunofenotip aberan yang paling kerap ditemui diikuti dengan CD7, CD2, CD19 dan cyCD79a. Manakala CD13 merupakan imunofenotip aberan paling kerap dijumpai dalam BALL diikuti dengan CD56, CD2, CD117, CD16, CD11b, and CD4.Bagi TALL pula, kedua-dua CD13 dan CD117 merupakan imunofenotip aberan yang paling kerap diikuti dengan CD33, CD71, CD64, CD11b dan cyCD79a. Daripada kajian ini juga, didapati CD33 mempunyai perkaitan yang signifkan dengan jantina pesakit dalam BALL. Manakala  dalam AML, CD2 mempunyai perkaitan signifikan dengan kumpulan umur dan CD7 mempunyai perkaitan signifikan dengan jantina pesakit dan bilangan platelet semasa diagnosis.

Daripada kajian ini terdapat bilangan pesakit yang signifikan yang  menunjukkan kehadiran imunofenotip aberan sewaktu diagnosis Ini amat berguna untuk menghasilkan panel imunofenotip sendiri bagi tujuan pemantauan sisa penyakit selepas penerimaan kemoterapi. Selain itu, terdapat penanda-penanda yang tertentu menunjukkan perkaitan yang signifikan dengan beberapa parameter hematologi.

**DETECTION OF LEUKAEMIA- ASSOCIATED ABERRANT IMMUNOPHENOTYPE (LAIPs) IN ACUTE LEUKAEMIA AND ITS ASSOCIATION WITH HAEMATOLOGICAL PARAMETERS AND TREAMENT OUTCOME**

## ABSTRACT

Acute leukaemia is one of the commonest haematological malignancies worldwide. Presence of specific and different antigen expressions of leukaemic cells at initial presentation help in establishing the diagnosis and specify the lineage involved. These leukaemic cells may express leukaemia-associated aberrant immunophenotype (LAIPs) at diagnosis. Studies have been done to look for association of these LAIPs with known haematological parameters and the results are various. These LAIPs also has been used as a marker for assessment of residual disease following respective chemotherapy. Many modalities have been developed for its assessment. One of the sensitive and convenient methods is multicolour flow cytometry. The aim of this study was to identify the frequency of LAIPs in acute leukaemia patient in local as well as to look for the association of these markers with sociodemographic data, haematological parameters and remission status (post induction chemotherapy).

A retrospective cohort study was done by collecting 134 samples of acute leukaemia patient diagnosed in HUSM during the study period. The samples were analysed for immunophenotyping using 4-colour flow cytometry and correlate with patients' sociodemographic data, complete blood count, bone marrow aspirate, cytogenetic findings as well as morphologic remission status post induction chemotherapy.

From 134 samples analysed, both acute myeloid leukaemia (AML) and B acute lymphoblastic leukaemia (BALL) were diagnosed in 62 patients each and T acute lymphoblastic leukaemia (TALL) seen in 10 cases. In AML, CD56 is the most common LAIPs marker found followed by CD7, CD2, CD19 and cyCD79a. Whereas, CD 13 is the most common LAIPs found among BALL cases followed by CD56, CD2, CD117, CD16, CD11b and CD4. In TALL, both CD13 and CD117 is the most common LAIPs marker found followed by CD33, CD71,CD64, CD11b and cyCD79a. From this study also, showed CD33 had significant association with gender in BALL. Other than that in AML, CD2 had significant association with age group and CD7 had significant association with gender and platelet count.

From this study, significant number of acute leukaemia patient in local demonstrate presence of LAiPs at diagnosis and this is useful for establishing our own immunophenotyping panels for monitoring residual disease following chemotherapy. Certain markers had significant association with haematological parameters analysed.

# CHAPTER 1

# INTRODUCTION

Acute leukaemia is a type of malignant clonal disorder of immature cells in haemopoietic system. It is a consequence of both proliferative lesion and a failure of differentiation. This will produce leukaemic cells which have abnormal function and failure to progress through normal expected differentiation. This population of cells will dominate the bone marrow activity and consequently causing bone marrow failure.

Identifying the type of acute leukaemia either acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML) primarily are based on morphological assessment as well as detecting patterns of surface antigen expression of leukemic cells by immunophenotyping. Each lineage expressed specific patterns of surface antigen and according to its maturation stages.

Leukaemic cells may demonstrate the leukaemia- associated immunophenotype (LAIPs) which can be detected at diagnosis. It either defined as antigen overexpression, lack of antigen expression asynchronous antigen or cross lineage antigen expression (aberrant). In this study we are focusing more on aberrant expression of immunophenotype in leukaemia. These LAIPs have potential to be used as markers for detection of residual disease. By monitoring the residual disease throughout the course of treatment of acute leukaemia, we can deliver the optimal treatment based on its progression.

Besides, with current World Health Organization's Classification of Tumours of Haemopoietic and Lymphoid Tissues 2008 in which incorporates the molecular, genetic and clinical aspect of the haematological malignancies facilitate the management of the patients. Based on this classification and a number of parameter including age, gender and presenting white blood cell count, current approach for acute leukaemia patient which is acute lymphoblastic leukaemia and acute myeloid leukaemia are basically based on risk adapted intensification

Meanwhile, certain studies showed these LAIPs have association with short survival and increase risk of relapse. It is also associated with lower probabilities to achieve complete remission. However, there are studies shows that these LAIPs have no significant association with prognosis.

The purpose of this study was to determine the presence of LAIPs in acute leukaemia in local by using flow cytometry method at diagnosis. Besides,this study also was to look for association of these LAIPs with sociodemographic data, haematological parameters and treatment outcome (post induction therapy). These data are beneficial in future as it help us to establish the pattern of surface antigen panel to be used in flow cytometry to monitor the leukaemic burden in acute leukaemia patient receiving chemotherapy. Therefore, can deliver effective and adequate chemotherapy agent based on the individual requirement and response to initial treatment.

# CHAPTER 2
# LITERATURE REVIEW

## 2.1 **Introduction of Acute Leukaemia**

### 2.1.1 Epidemiology of Acute Leukaemia

Leukaemia is a disease results from the neoplastic proliferation of haemopoietic or lymphoid cells. It results from mutation of a single stem cell. Usually it involves multiple genetic alterations rather than single event. About 250 000 (2.5%) people are diagnosed with leukaemia yearly worldwide. In Europe, 75 700 of new patients were diagnosed as leukaemia in 2005(Rodriguez-Abreu *et al.*, 2007b). Meanwhile, leukaemia is the commonest childhood cancer in Malaysia. The prevalence was about 35 per million .It was the fourth leading cause of death among cancer patient in Malaysia in 1998, about 311(6.9%) out of 4498 (Lim, 2002).

Geography and ethnic also show variation in leukaemia. The incidence of leukaemia in United States is higher among Caucasians rather than Afro-american and Hispanics (Rodriguez-Abreu *et al.*, 2007b). However, study done by (Shirley *et al.*, 2013) regarding the incidence of acute myeloid leukaemia in ethnic group in England shows no significant between the Whites and other ethnic group. There is also no evidence of intra-ethnic heterogeneity found in this study.

Acute myeloid leukaemia (AML) is a common leukaemia seen in adult population. Small proportion can be seen among early childhood group. It has slightly male predominance in adult acute myeloid leukaemia. The incidence is about 1 to 10 per 100 000 /year between the age of 20 years to 70 years. In acute myeloid leukaemia, the median age at presentation is 70 years old and men is affected more than women,

3:2 (Estey and Döhner, 2006). The United State incidence‘s rate among age in year 2000 to 2003 also quite similar. People age less than 65 years old is 1.8 per 100 000 persons, whereas those age more than 65 years old is 17 per 100 000 persons. In group of patient less than 15 years old, AML comprises 15 to 20% only (Deschler and Lubbert, 2006).

Meanwhile, acute lymphoblastic leukaemia (ALL) further subdivided according to lineage. About three quarter of cases of ALL is of B- acute lymphoblastic leukaemia (B-ALL). And the rest one quarter are of T-acute lymphoblastic leukaemia (T-ALL). In contrast to acute myeloid leukaemia, ALL is most commonly seen among age group less than 20 years old. The incidence rate among 1 to 4 years old group is more than 10 times greater than in the age of 20 to 24 years old. ALL also is slightly more in men than women (Rodriguez-Abreu *et al.*, 2007b). B-ALL is commonly seen among young age group but the disease occurs at all age. T ALL is more commonly seen in male, which is about three quarter of cases. It is common among 20 to 29 year and 30 to 39 year age group (Marks *et al.*, 2009)

### 2.1.2 Classification, Clinical Features and Risk Factor

#### 2.1.2 (a) Classification

In most cases, acute leukaemia can be classified according to lineage, myeloid or lymphoid origin. Acute myeloid leukaemia has been initially categorised based on French-American-British (FAB) Classification which is based on cytomorphology and cytochemistry. However, based on current WHO classification, it incorporates cytogenetic data and defines 4 major categories of AML include acute myeloid leukaemia with recurrent genetic abnormalities, acute myeloid leukaemia with myelodysplasia-related changes, therapy-related myeloid neoplasms and acute myeloid leukaemia, no otherwise specified.(Estey and Döhner, 2006)

**Table 2.1 : WHO 2008 Classification of Acute Myeloid Leukaemia**

| **Acute myeloid leukemia and related neoplasms** |
|---|
| Acute myeloid leukemia with recurrent genetic abnormalities |
| AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1* |
| AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* |
| APL with t(15;17)(q22;q12); *PML-RARA* |
| AML with t(9;11)(p22;q23); *MLLT3-MLL* |
| AML with t(6;9)(p23;q34); *DEK-NUP214* |
| AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1* |
| AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1* |
| *Provisional entity: AML with mutated NPM1* |
| *Provisional entity: AML with mutated CEBPA* |
| Acute myeloid leukemia with myelodysplasia-related changes |
| Therapy-related myeloid neoplasms |
| Acute myeloid leukemia, not otherwise specified |
| AML with minimal differentiation |
| AML without maturation |
| AML with maturation |
| Acute myelomonocytic leukemia |
| Acute monoblastic/monocytic leukemia |
| Acute erythroid leukemia |
| Pure erythroid leukemia |
| Erythroleukemia, erythroid/myeloid |
| Acute megakaryoblastic leukemia |
| Acute basophilic leukemia |
| Acute panmyelosis with myelofibrosis |
| Myeloid sarcoma |
| Myeloid proliferations related to Down syndrome |
| Transient abnormal myelopoiesis |
| Myeloid leukemia associated with Down syndrome |
| Blastic plasmacytoid dendritic cell neoplasm |

Adapted from (Vardiman *et al.*, 2009)

Meanwhile, morphological identification of lymphoblast by microscopy and immunophenotypic assessment of lineage by flow cytometry is important for diagnosis (Inaba *et al.*, 2013). Acute lymphoblastic leukaemia is usually classified as B- acute lymphoblastic leukaemia and T-acute lymphoblastic leukaemia.

**Table 2.2 : WHO 2008 Classification of Acute Lymphoblastic Leukaemia**

| |
|---|
| **B lymphoblastic leukemia/lymphoma** |
| B lymphoblastic leukemia/lymphoma, NOS |
| B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities |
| B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2);*BCR-ABL 1* |
| B lymphoblastic leukemia/lymphoma with t(v;11q23);*MLL* rearranged |
| B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22) *TEL-AML1* (*ETV6-RUNX1*) |
| B lymphoblastic leukemia/lymphoma with hyperdiploidy |
| B lymphoblastic leukemia/lymphoma with hypodiploidy |
| B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32) *IL3-IGH* |
| B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1* |
| **T lymphoblastic leukemia/lymphoma** |

Adapted from (Vardiman *et al.*, 2009)

### 2.1.2 (b) Clinical Features

The first thing to consider in diagnosing acute leukaemia is the initial clinical presentation of the patient. The clinical features of the patient basically results from the replacement of the marrow by the leukaemic cells as well as the infiltration of them to the other organs such as spleen, liver and lymph nodes. The unregulated proliferation the leukaemic cells cause suppression of the normal haemopoietic cells. These may result in anaemia, thrombocytopenia or sometimes pancytopenia. Pallor, fatigue, easily bruising,recurrent infection with unexplained cause and unresolved

fever are possible presentations. Organomegaly specifically liver and spleen as well as lymphadenopathy are secondary to the infiltration of the leukaemic cells.

Acute myeloid leukaemia shares common clinical features for each subtype. However, certain subtype may have particular clinical findings. Hepatosplenomegaly is one of the possible clinical findings in acute myeloid leukaemia particularly those with predominant monocytic component (Barbara *et al.*, 2010a). Lymphadenopathy and infiltration skin, gums and tonsils infiltration are also common in AML with a prominent monocytic component. Profound bleeding tendencies and frequently followed by disseminated intravascular coagulopathy are usually seen in AML with recurrent cytogenetic t(15;17) or known as AML M3 (FAB classification) (Barbara *et al.*, 2010a).

On the other hand, common clinical features of B-ALL are including bruises, pallor, bone pain, lymphadenopathy and hepatospenomegaly. In majority of B-ALL patients, the blasts are present in peripheral blood. As a consequence, the white blood cells are increased with normochromic normocytic anaemia and thrombocytopenia (Barbara *et al.*, 2010b). However, in T-ALL thymic infiltration is very common and maybe associated with pleural and pericardial effusions as well as superior vena cava obstruction. Lymphadenopathy and hepatosplenomegaly also can be seen in TALL cases.(Barbara *et al.*, 2010b)

### 2.1.2 (c) Risk Factors

Most of acute myeloid leukaemia arise de novo with no definite exposure to leukemogenic factors or agents. Only small number of cases is related to known risk factors. These risk factors are include underlying genetic disorder such as Down syndrome, chemical or radiation exposure and also chemotherapy (Deschler and Lubbert, 2006). Meanwhile, infection is known cause of acute leukaemia in childhood acute lymphoblastic leukaemia. Besides, ionising radiation and x-ray pelvimetry during pregnancy also have been postulated as part of the contributing factor in number of cases but some believed that it might be not relevant anymore (Inaba *et al.*, 2013).

### 2.1.3 Diagnosis of Acute Leukaemia

### 2.1.3 (a) Complete Blood Count.

Diagnosis of acute leukaemia is basically started from clinical suspicion when the patients came with clinical features suggestive of acute leukaemia. Based on this suspicion, then complete blood count is analysed. Both AML and ALL may present with leucocytosis, normochromic normocytic anaemia, thrombocytopenia as well as numbers of circulating blast cells. But some AML cases may present with low or normal white cell count especially in classical AML with t (15; 17) (Barbara *et al.*, 2010a). Whereas, in T-ALL the white cell count is usually high but the marrow function is better preserved as compared to B-ALL (J.Bain, 2010).

### 2.1.3(b) Morphology

Blood and marrow smears are examined by using May-Grunwald-Giemsa or Wright Giemsa stain. At least 200 leucocytes on blood smears and 500 nucleated cells on marrow smears should be counted. To diagnose acute leukaemia, about 20% of blast or more is required either in blood or marrow smears. Except in certain case of acute myeloid leukaemia include t(15;17), t(8;21), inv (16) or t(16;16) and some case of erythroleukaemia (Döhner *et al.*, 2010) . On the other hand in acute lymphoblastic leukaemia, there are no definite morphological criteria to differentiate between B and T lineage. And it's quite challenging to appreciate B acute lymphoblastic leukaemia with normal B lineage precursors known as hematogones (Chiaretti *et al.*, 2014). Other than morphologic assessment, cytochemical stain is one of the supplementary tests to identify the lineage of acute leukaemia. Presence of at least 3% blast with positive for myeloperoxidase (MPO) stain or Sudan black B (SBB) defines that the blasts belong to AML. But there are subtypes of AML which are negative for MPO stain include monoblast type (J.Bain, 2010). While, acid phosphatase reaction and periodic-acid Schiff (PAS) stain helps in identifying the ALL (J.Bain, 2010).

**Table 2.3 : Morphologic characteristic of blast cells in acute lymphoblastic leukaemia versus acute myeloid leukaemia**

| | Lymphoblasts | Myeloblasts |
|---|---|---|
| General characteristics | Blast population tends to be homogeneous | Blast population tends to be heterogeneous, with the exception of the undifferentiated form |
| Size | Variable, mainly small | Variable, mainly large |
| Nucleus | Central, mainly round; sometimes indented, particularly in the form in adults<br>Nucleocytoplasmic ratio very high in the form that occurs in children Nucleocytoplasmic ratio lower in the form that occurs in adults | Tending to be eccentric, round, oval or angulated; sometimes convoluted, particularly in the form with a monocytic component<br>Nucleocytoplasmic ratio high in undifferentiated blast cells and in some megakaryoblasts<br>Nucleocytoplasmic ratio mainly low in the form with differentiation |
| Chromatin | Fine, with dispersed condensation<br>Very condensed in small lymphoblasts | Fine, granular, delicately dispersed |
| Nucleoli | Absent in small lymphoblasts<br>Sometimes indistinct | Almost always present, often large and prominent, double or triple |
| Cytoplasm | Scanty, basophilic<br>Sometimes with a single long projection ('hand-mirror cell') | Variable<br>Abundant in monoblasts<br>With protrusions in erythroblasts and megakaryoblasts |
| Granules | Rarely present, azurophilic and always negative for peroxidase, esterases and toluidine blue | Present in forms with differentiation and positive with cytochemical stains<br>– peroxidase in the neutrophil and esoinophil lineages<br>–nonspecific esterase in the monocyte lineage<br>–toluidine blue in the basophil lineage |
| Auer rods | Always absent | Can be present<br>Typically present in the hypergranular promyelocytic form |
| Vacuolation | Can be present | Can be present<br>Almost always present in forms with a monocytic component |

Adapted from (Chiaretti *et al.*, 2014)

### 2.1.3 (c) Immunophenotyping

By using multiparametric flowcytometry at least 3 to 4 colour, we can determine the lineage for newly diagnosed acute leukaemia. There are panel of markers available to identify each lineage consist of combination of various surface,cytoplasmic as well as nuclear antigen markers. No general consensus is provided in determining the cut-off point to be positive for a marker. Most markers take 20% or more leukaemiac cells. Some selected markers take a lower cut off point, 10%. These markers include cytoplasmic CD3, MPO,TdT,CD34 and CD117 (Döhner *et al.*, 2010).

**Table 2.4 : Expression of cell surface and cytoplasmic markers for diagnosis of acute myeloid leukaemia and mixed phenotype acute leukaemia**

| Expression of markers for diagnoses | |
|---|---|
| **Diagnosis of acute myeloid leukemia (AML)*** | |
| Precursor stage | CD34, CD38, CD117, CD133, HLA-DR |
| Granulocytic markers | CD13, CD15, CD16, CD33, CD65, cytoplasmic myeloperoxidase (cMPO) |
| Monocytic markers | Nonspecific esterase (NSE), CD11c, CD14, CD64, lysozyme, CD4, CD11b, CD36, NG2 homologue‡ |
| Megakaryocytic markers | CD41 (glycoprotein IIb/IIIa), CD61 (glycoprotein IIIa), CD42 (glycoprotein 1b) |
| Erythroid marker | CD235a (glycophorin A) |
| **Diagnosis of mixed phenotype acute leukemia (MPAL)†** | |
| Myeloid lineage | MPO or evidence of monocytic differentiation (at least 2 of the following: NSE, CD11c, CD14, CD64, lysozyme) |
| B-lineage | CD19 (strong) with at least one of the following: CD79a, cCD22, CD10, or CD19 (weak) with at least 2 of the following: CD79a, cCD22, CD10 |
| T-lineage | cCD3, or surface CD3 |

*For the diagnosis of AML, the table provides a list of selected markers rather than a mandatory marker panel.
†Requirements for assigning more than one lineage to a single blast population adopted from the WHO classification.[3] Note that the requirement for assigning myeloid lineage in MPAL is more stringent than for establishing a diagnosis of AML. Note also that MPAL can be diagnosed if there are separate populations of lymphoid and myeloid blasts.
‡Most cases with 11q23 abnormalities express the NG2 homologue (encoded by *CSPG4*) reacting with the monoclonal antibody 7.1.

* Adapted from (Döhner *et al.*, 2010)

### 2.1.3 (d) Cytogenetic

Conventional cytogenetic is part of the diagnostic assessment of acute leukaemia. A minimum of 20 metaphase cells analysed from bone marrow is mandatory to determine normal karyotype and recommended to define for abnormal karyotype (Döhner *et al.*, 2010). It is carried out by microscopic analysis of the chromosomes of cells arrested in metaphase. All of the population of leukaemic cells may show the same chromosomal abnormality. Sometimes it has more than one additional abnormality due to further clonal evolution of the cells (J.Bain, 2010). About 70 to 80% of AML cases have clonal cytogenetic abnormalities. The commonest cytogenetic abnormalities is trisomy 8 followed by anomalies of chromosome 7. Some anomalies are found in all morphological subtypes and in both secondary and de novo leukaemia such as trisomy 8 and 21. Both of these anomalies are not related to morphology and clinical features. On the other hand, t(15;17), t(8;21) and

translocation or inv (16) have a strong association with particular morphology subtype (J.Bain, 2010).

### 2.1.4 Treatment of Acute Leukaemia

#### 2.1.4 (a) Treatment of Acute Lymphoblastic Leukaemia

Particularly in adult ALL before giving specific regime for chemotherapy usually corticosteroid is administered as prephase therapy for tumour reduction. Usually drug of choice is prednisolone 20 to 60mg/day or dexamethasone 6 to 16mg/day with or without vincristine, cyclophosphamide as well as allopurinol. In certain cases which very high white cell count requires leucopheresis (Hoelzer *et al.*, 2016). Supportive therapy is required in treating certain condition includes infection and febrile neutropenia. Red cell and platelet transfusion and prophylactic granulocyte colony stimulating factor (GCSF) is required based on need of the patient.

Basically, treatment regimes include remission induction, consolidation, maintenance therapy and also central nervous system (CNS) prophylaxis. In general there are two chemotherapy regimes which are commonly followed. First, is the protocol which patterned after pediatric BFM (Berlin-Frankfurt-Munster). This protocol mostly used in Europian adult ALL. Another approach is by giving alternating intensive chemotherapy identical for induction and consolidation such hyperCVAD. This protocol is commonly used in United States and other parts of the world (Hoelzer *et al.*, 2016).

The purpose for remission induction is to achieve complete remission or molecular complete remission which is evaluated within 6 to 16 weeks from start of chemotherapy. For consolidation usually use systemic high dose therapy includes high dose methotrexate, cytarabine ± asparaginase. Meanwhile, for maintenance therapy usually use daily mercaptopurine and weekly methotrexate within duration 2.5 to 3.0 years (Hoelzer *et al.*, 2016).

### 2.1.4 (b) Treatment of Acute Myeloid Leukaemia

Similar with treatment in ALL, in AML as well supportive treatment is also important in patient receiving chemotherapy. Leucopharesis is required when there is symptomatic hyperleucocytosis but contraindicated in cases suspected acute promyelocytic leukaemia as it can exacerbate coagulopathy (British Committee for Standards in *et al.*, 2006). Hydration and allopurinol is given to prevent tumor lysis syndrome. Red cell transfusion and platelet support as well as antibiotic are given based on patient's need.

Chemotherapy is considered to all patients up to the age of 60 years or in more than 60 years old but fit to receive treatment. The aim for remission induction is to achieve restoration of normal bone marrow function. Complete remission is defined as recovery of normal bone marrow cellularity with fewer than 5% blasts and without detectable cytogenetic abnormality (British Committee for Standards in *et al.*, 2006).

Post remission therapy basically is to prevent relapse with maximal efficiency and minimal toxicity. Option of treatment include consolidation chemotherapy and autologous,allogeneic related or allogeneic unrelated donor transplantation. Maintenance therapy is of benefit in patient with AML who have undergone intensive chemotherapy with possible exception of acute promyelocytic leukaemia (AML M3 subtype) (British Committee for Standards in *et al.*, 2006).

## 2.2 Prognostic Factors in Acute Leukemia

### 2.2.1 Prognostic Factor in Acute Lymphoblastic Leukaemia

In acute lymphoblastic leukaemia, age (infant or ≥ 10 years old), presenting white cell count ≥ 50x $10^9$/L, race (Hispanic or black), male sex and T cell immunophenotype are considered as adverse prognostic factors (Inaba *et al.*, 2013). Review by (Carroll *et al.*, 2003) noted that both age and white blood cells are considered as predictive factors for outcome of the patient in ALL. Based on National Cancer Institute/Rome criteria, they stratify as standard risk for those 1 to 9.99 years old and white blood cell less than or equal to 50 000/µL. Age more than or equal to 10 years old and white cell count more than 50 000/µL is considered as higher risk (Carroll *et al.*, 2003). There is also a study regarding ALL shows that high WBC influence negatively the complete remission duration (Urbano-Ispizua *et al.*, 1990)

Based on the UK National Lymphoblastic Leukaemia (ALL97) childhood acute lymphoblastic leukaemia is stratified into 3 risk groups based on the following criteria:

a) Standard Risk : Age >1<10 years with a highest white cell count before treatment of <50x109/L, not have BCR-ABL, no hypodiploidy (<46 chromosomes) or if 12-24 months old, and no MLL gene rearrangement.
b) Intermediate Risk : All children >10 years old, or with a diagnostic WBC >50x109/L (or both), no BCR-ABL, no hypodiploidy (46 chromosomes), or, if 12-24 months old, and no MLL gene rearrangement.

c) High Risk : All children, irrespective of initial risk category, who have a slow early response (SER) together with those who have BCR-ABL, hypodiploidy (46 chromosomes), or, if 12-24 months old, an MLL gene rearrangement.

**Table 2.5 : Unfavorable Prognostic Features in Acute Lymphoblastic Leukaemia**

| Characteristic | Kantarjian 2004[40] | Hoelzer 1988[39] | Rowe 2005[41] and Lazarus 2006[42] | Le 2006[43] |
|---|---|---|---|---|
| Age, y | >60 | >35 | >35 | Higher vs lower |
| WBC, $\times 10^9$/L | >5 | >30[a] | >30[a] | Higher vs lower |
| LDH | NA | NA | NA | Higher vs lower |
| Time to CR | >1 Course | >4 Wk | – | – |
| Immunophenotype | B | Pro-B, early and mature T | T lineage | – |
| Karyotype | t(9;22) | | t(9;22) | t(9;22); Misc vs normal |
| Molecular | *BCR-ABL* | *BCR-ABL; ALL1-AF4* | NA | NA |
| CNS involvement | Yes | NA | Yes | NA |
| Minimal residual disease | NA | Persistent | NA | NA |

WBC indicates white blood cell count; LDH, lactate dehydrogenase; NA, not available; CR, complete response; Misc, miscellaneous; *BCR-ABL1*, bcr apoptosis facilitator/c-abl oncogene 1 receptor tyrosine kinase gene fusion; *ALL1-AF4*, acute lymphocytic leukemia susceptibility 1/acute mixed-lineage leukemia gene fusion; CNS, central nervous system.

[a]The total was >100 in T-lineage acute lymphoblastic leukemia.

Adapted from (Faderl *et al.*, 2010)

There is a study by (Teuffel *et al.*, 2008) showed that ALL with lower haemoglobin (less than 8g/dL) associated with better event free survival as compared to those with haemoglobin less than 8 g/dL. This study also showed that significant lower haemoglobin in B-ALL with favourable cytgogenetic abnormality which was t(12;21) as compared to B-ALL with positive for t(9;22) who had higher haemoglobin level.

Regarding cytogenetic status, in ALL, best prognosis is associated with hyperdiploidy which defined as 51 to 65 chromosomes or detected by demonstrating

simultaneous trisomy for chromosomes 4,10 and 17 and with t(12;21)(p13;q22). Meanwhile, worst prognosis is associated with t (9;22), MLL gene rearrangement and hypodiploidy (J.Bain, 2010). Only small percentages of ALL in childhood are positive for Philadelphia whereas in adult ALL up to 40% of cases are positive for Philadelphia. This chromosome abnormality are associated with poor outcome with event free survival are 15% or less if treated with chemotherapy alone (Harrison, 2001). High hyperdiploidy is associated with good prognosis in adult ALL as compared in children ALL, 59% had 3-year event free survival (Harrison, 2001).

### 2.2.2 Prognostic Factors in Acute Myeloid Leukaemia

In acute myeloid leukaemia,ECOG performance status and high age are associated with early death rate. Even when adjusting the performance status, high age is still associated with an increased early death rate ($p < 0.001$) (Juliusson *et al.*, 2009). Besides, in acute myeloid leukaemia the percentage of favourable cytogenetic in age more than 75 years old is 4%, lower than patient in age younger than 56 years old, 17%. Whereas, the unfavourable cytogenetic in patient younger than 56 years old is 35%. As compared to those in age more than 75 years old, the percentage is higher, 51% (Appelbaum *et al.*, 2006) . On the other hand, in a study of AML related to treatment outcome, they found that age and total white cell count were not significantly associated with treatment outcome (Al-Mawali *et al.*, 2009b).

Besides, acute myeloid leukaemia also can have abnormal cytogenetic finding at diagnosis which was about 60% (Grimwade and Hills, 2009). In AML with children and adults below age of 55 years, a favourable prognosis is associated with tt(8;21), t

(15;17) and inversion 16. Adverse prognosis is associated with -5,-7, del 5q abnormalities and complex karyotype (J.Bain, 2010). Those patients whom included in favourable cytogenetic risk group, the relapse rate are low if receiving intensive chemotherapy. Meanwhile, in adult AML patient with 3q, deletion of 5q, monosomies of chromosome 5 and 7 or complex karyotype have very poor prognosis. They are candidates for allogenic transplantation (Grimwade and Hills, 2009) .

## 2.3 Immunophenotyping in Acute Leukaemia

### 2.3.1 Flowcytometry

Flowcytometry measures the optical and fluorescence characteristic of single cell. Size and internal complexity can be determined by forward angle light scatter and right-angle scatter respectively. By using multiple fluorochrome, we can assess several cell properties simultaneously (Brown and Wittwer, 2000). In general, fluid based specimens include peripheral blood and bone marrow aspirates are suitable for best result in flowcytometric evaluation. About 500 000 to 1 million cells are stained per tube in order to get the best results. Although as little as 50 000 cells may useful enough if the blast population is the predominant component in the specimen (Peters and Ansari, 2011).

This method is an ideal tool to study the leucocytes, red cells and platelets. There are numbers of clinically relevant aspect of pathophysiology and function that can be measured (Orfao *et al.*, 1995). Blast population in acute leukaemia can better isolated

by using CD45/side scatter gating for more definitive phenotyping (Brown and Wittwer, 2000).

There is numbers of monoclonal antibodies per analysis tube used, most of the clinical laboratories using 3, 4 or 5 colours (using different fluorochromes) (Peters and Ansari, 2011).Several monoclonal antibodies for leucocyte differentiation antigens will be applied to interested blast/leukaemic cells population in one or multicolour staining method. Pattern of expression of the markers is useful in lineage assignment, detect biphenotypic acute leukaemia, to apply current immunophenotypic classification and useful for follow up based on abnormal expression of markers on leukaemic cells (Béné, 2005).

This immunophenotyping is beneficial in distinguishing myeloid and lymphoid in acute leukaemia and minimally differentiated leukaemia (Brown and Wittwer, 2000). Panel using as many as 10 colours are coming into clinical use. This advances will improve the sensitivity in identifying subtle aberrant immunophenotype, low numbers of blast cells in minimal residual diseases and in studies of low cellularity sample in which only one or a few tubes only available for analyse (Peters and Ansari, 2011).

Other than that, detection of certain markers by immunophenotyping particularly in paediatric ALL also can predict the treatment response in future. Children B-ALL with positivity of surface immunoglobulin (Sig) has a very poor prognosis if using

conventional chemotherapy. However, if the cytoplasmic immunoglobulin also present, the prognosis is better even using conventional chemotherapy(Orfao *et al.*, 1995). This review also found that, detection of minimal residual disease in high proportion of ALL cases can be applied by using combination of different panels of monoclonal antibodies as this method able to capture as low as one leukaemic cells among 100 000 cells (Orfao *et al.*, 1995). A review done by (Kern *et al.*, 2010) also showed there was study found that stronger prognostic power of the post induction minimal residual disease assessment by using five-colour flow cytometry. They found that post induction minimal residual disease level influenced both relapse free survival (RFS) and overall survival (OS).

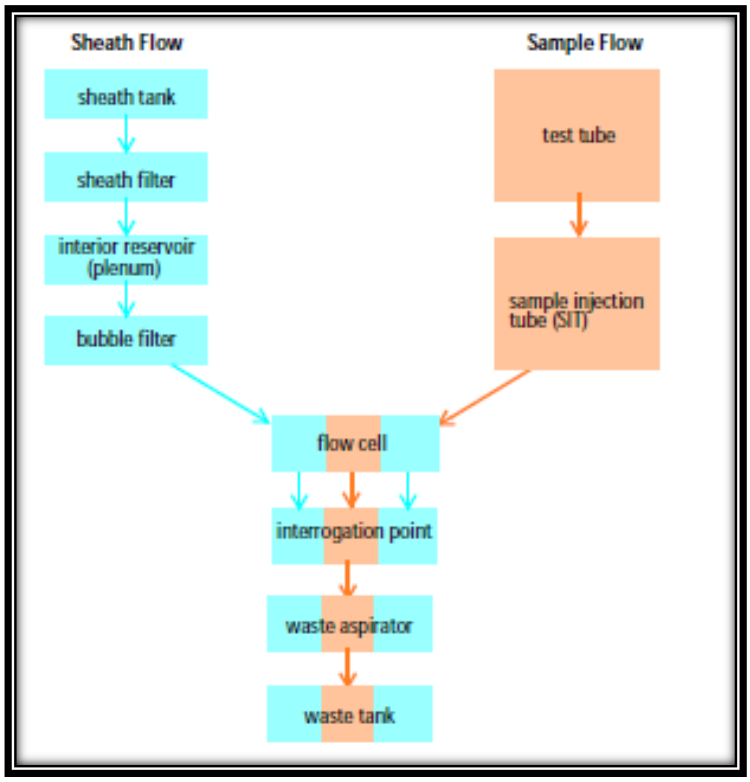


Figure 2.1: Sheath and sample flow pathways for the BD FACSCanto cytometer. Adapted from (FACSCanto, 2004)

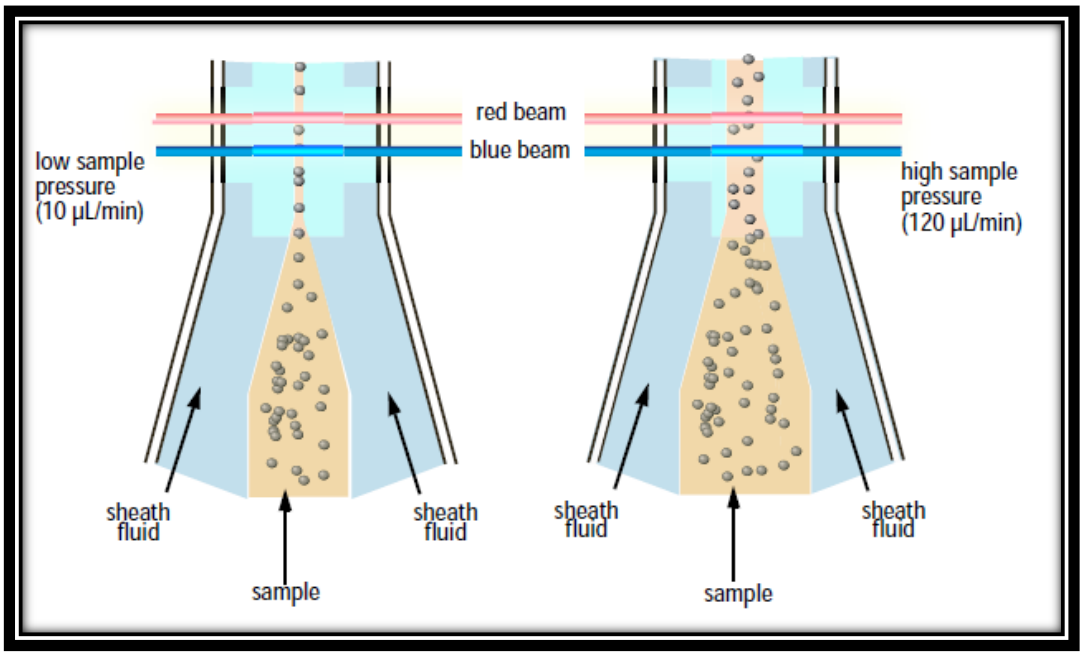


Figure 2.2: Hydrodynamic focusing of the sample are through the flow cells.Adapted from (FACSCanto, 2004)