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 **Clinical features, investigations and outcome of protocol CCG 1961 with high-risk ACUTE LYMPHOBLASTIC LEUKEMIA in children**

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# Background

Leukemia is one of the most common types of cancer among children around the world. This blood-related disease is caused by the uncontrolled growth of one or many lines of malignant hematopoietic stem cell. Acute lymphoblastic leukemia (ALL) accounts for approximately 75% of all cases of childhood leukemia. In Asia, ALL makes up 51% of leukemia cases in children under 15 year of age. Children suffer from this disease, if not receive proper and timely diagnosis and treatment, would die very quickly. Approximately 4900 children are diagnosed with ALL each year in the United States, with an incidence of 29 per million of all US children. The peak incidence of ALL occurs between 2 to 5 years of age and maybe trending downward in both the United Kingdom and United States. The incidence of ALL is higher among boys than girls, and this difference is greatest among pubertal children.

In recent years, pediatric ALL is often cited as one of the true success stories of modern medicine, with the cure rate improving from zero prior to the advent of modern chemotherapy and radiation therapy to current overall event- free survival (EFS) rate of about 80%. This success has been due to the development of classifications, active chemotherapeutic agents, immunology, genetics and bio-molecules into diagnosis, treatment, monitoring the disease and understanding the prognosis factors. In Vietnam, the National Hospital of Pediatrics (NHP) has made initiative in researches regarding clinical and pre-clinical high-risk ALL on 164 patients in 2006 by Dr Nguyen Hoang Nam; another research is on treatment outcome of standard risk ALL on 98 patients, with EFS is 68.1% by Dr Bui Ngoc Lan. Other researches in different children and cancer hospitals also have rough assessment regarding ALL treatment results of different clinical protocol such as FRALLE (France), ALL-BFM 90. So far, no research on high-risk childhood ALL with complete assessment and proper clinical protocol applicable to Vietnam has been conducted. Thus, we carried out our research on the topic “**Clinical features, investigations and outcome of protocol CCG 1961 with high-risk ALL in children”**. Two targets of the research are:

*1. Describe the clinical features and investigations of high-risk ALL in children at National Hospital of Pediatrics.*

*2. Assess the treatment results of high-risk ALL in children using modified protocol CGG 1961 at National Hospital of Pediatrics.*

Content: This article consists of 116 pages – Background (2 pages), Chapter I: Overview (36 pages), Chapter II: Patients and methodology (17 pages), Chapter III: Results (28 pages), Chapter IV: Discussion (29 pages), Conclusion (2 pages), Acknowledgement (1 page), Feedback (1 page). The result includes 45 tables, 8 graphs. The research uses 99 references (in both Vietnamese and English).

**Chapter I: OVERVIEW**

* 1. **EPIDEMIOLOGY**

According to statistics, ALL remains the most common malignancy in children, both in Vietnam and in the world. It occurred initially in Great Britain in the 1920s, in the USA in the 1940s and Japan in the 1960s. The appearance of this peaks correspond to major periods of industrialization in this countries, suggesting that they may reflect different periods of exposure to new environmental leukemogens. The incidence of ALL in children across the globe is between 1 to 4 cases/100.000 children below age 15. The geographic variation may reflect, in part, the distribution of different immunologic ALL subtypes. There appears to be lower incidence of common ALL developing countries and higher incidence of T cell ALL in the more industrialized countries. In Vietnam, the annual rate of occurrence of cancer in children is 52 cases/million children. By 2013, the average number of children with cancer per year is 1405. In NHP, leukemia accounts for 45.2% of childhood cancer, with ALL makes up to 67.5% of these patients. Currently, the Department of Oncology is using a CCG’s protocol of American, with modifications for realistic application.

* 1. **CLINACAL FEATURES AND INVESTIGATIONS**

High-risk ALL in children has some clinical presentations and investigations similar to other types of ALL such as signs and symptoms reflect the impact of bone marrow infiltration with leukemic cells and extent of extramedullary disease spread: the lymphatic leukemia, central nervous system (CNS) leukemia and other organs. Hematologic abnormalities include: full blood count, blast cells dominate over other types of cells in the marrow. Confirmation of ALL diagnosis is made more than 25% lymphoblast in a bone marrow. Bone marrow samples will undergo immunophenotyping and karyotyping to determine whether it is B or pre-B cells ALL, T or AML. The result of karyotype will be used for prognosis, thus to choose the appropriate protocol. Other tests include chest X- ray to detect mediastinal tumors, coagulation test, abdominal ultrasound, cerebrospinal fluid cells: central nervous system penetrated when CSF has more than 5 WBC/mm3, lymphoblast are found.

* 1. **PROGNOSTIC FACTORS AND RISK FACTORS**

**1.3.1. Classification by risk factors:** Categorization by the National Cancer Institute (America) divided ALL into 2 types:

- Standard risk: when patient is between 1 and 10 years old and the initial leukocyte count is below 50G/L.

- High risk: Patients aged below 1 year old or ≥ 10 years old or the initial leukocyte count greater 50G/L. ALL patients aged below 1 year old normally have bad prognosis, thus a separate protocol is needed.

**1.3.2 Prognostic factors:**

- White blood cells count at diagnosis

- Age at diagnosis, gender, race, disadvantage such as: hepatoslenomegaly and enlarge peripheral lymph node, central nervous system infiltration, testicular leukemia, chromosome abnormalities.

- Factors involved treatment: response to treatment in bone marrow on day 7, day 14 and day 28. Lymphoblast <5% indicates a good prognosis, 5-25%: incomplete remission and over 25%: no remission.

**1.4. TREATMENT**

High-risk ALL protocols all follow the principles based on BFM protocol. The aim of initial ALL treatment is induction of remission with agents. After complete remission has been achieved, subsequent therapy is required to maintain the stability of the remission: consolidation phase, intensification therapy. The maintenance therapy with the total duration of 2 to 3 years is suitable for pre B cell ALL and T cell ALL. The CCG 1961 protocol was applied to patients in NHP since 2005. Patients are assessed on the 7th day of the induction phase, if lymphoblast of marrow is equal to or below 25% (M1 and M2), the patients are rapid early response (RER) and will follow arm B of the protocol. If lymphoblast is more than 25% (M3), patients are slow early response (SER) and will follow the slow response of protocol CCG 1961. The outcome of this protocol in America has an OS rate of 80.4% and an EFS rate of 71.3%.

**Chapter II: PATIENTS AND METHODOLOGY**

* 1. **PATIENTS**

**2.1.1. Clinical features and laboratory findings:**

Observe on 129 patients diagnosed with high-risk ALL and admitted to Department of Oncology in NHP between the period 1/6/2008 to 31/12/2012.

**Selection Criteria:**

* + - 1. ***Leukemia diagnosis:***

Clinical: Signs and symptoms: fever, fatigue, loss of appetite. Anemia, petechie or bleeding. Extramedullary disease spread: mediastinal mass, testicular leukemia, the degree of hepatoslenomegaly, lymphadenopathy, nervous central symptoms disease, pain of the bone.

- Full blood count: Hemoglobin (Hb), WBC count may be increased, normal or decreased but usually there is a substantial decrease in neutrophils number, lymphoblast may be found in blood capillaries, platelets count usually decreases.

- Bone marrow smear: if lymphoblast ≥ 25% of blood cells in bone marrow, the patient would be diagnosed with leukemia. This is the golden standard to determine leukemia and morphologic classification via FAB. In the bone marrow, lymphoblast will dominate over other types of blood cells such as leukocyte, red blood cells and platelets.

***2.1.1.2. ALL diagnosis:***

- POX (Peroxidase) negative on bone marrow cells.

- Calssification immunophenotype: Undergo flow cytometry, MPO (Myelo Peroxydase) negative. Three immunologic subsets were delineated: T cells, B cells and non T/non B cells.

***2.1.1.3. High-risk ALL diagnosis:***

- Patients aged > 1 and ≤ 10 with WBC count ≥ 50 G/L,

- Patients aged > 10 at time of diagnosis,

Criteria based on unfavorable prognosis of ALL:

- Patients with biphenotype (immunophenotype) .

- Patients with translocation t(9;22), t(4;11)

- Patients with hypodiploidy (<45 chromosomes)

* + 1. **Outcome of CCG 1961 protocol treatment:**

The target patients for research are 102 ALL patients admitted to Department of Oncology of NHP between the period 1/6/2008 to 31/12/2012.

Patients are treated and monitored according to CCG 1961 protocol. The time of observation is until 31/5/2015.

* 1. **METHODOLOGY**
		1. **Planning:** Experimental study without case-control groups, consisting of 2 parts: - Part 1: Describe study, with cross-sectional description clinical features and investigations of children diagnosed with high-risk ALL admitted to NHP.

- Part 2: Prospective study the results of high-risk ALL patients according to modified CCG 1961 protocol.

* + 1. **Content:**

***2.2.2.1. Content for goal 1***: Research on clinical features and investigations:

- Categorization based on aged at time of admittance, gender, clinical presentations and laboratory findings.

- Bone marrow aspiration are collected to assess classification: Morphology (FAB), Immunophenotype of lymphoblast: pre B cells, T cells, biphenotype. Chromosomal abnormalities: Structure and number.

- Biochemical charaterization: liver and kidney function, calcium, glucose level, tumor lysis syndrome, coagulation test: Fibrinogen, Prothrombine, APTT, CRP to determine the infection.

- Prognostic factors: age, gender, hepatosplenomegaly, lymphadenopathy, Hemoglobine level, WBC count, platelets count at the time of diagnosis and comparision the other unfavorable factors.

***2.2.2.2 Research content for goal 2:*** Assessment on the results of ALL treatment according to modified CCG 1961 protocol.

The protocol being used for treatment is the US CCG 1961 arm B. This is a protocol for high-risk ALL patients, with some modifications for better application in Vietnam context such as: L-Asparaginase is a form of E. Coli ASP from Kyowa (Japan), 6 thioguanin is replaced by 6MP; intrathecal by cyratabine day 0 is replaced by MTX.

* Assessment on induction phase, bone marrow aspiration day 28.
* Assessment post- induction phase.

+ Total number of patients complete therapy

+ Number of patients relapse

+ Number of patients undergoing treatment

+ Number of patients dies during treatment.

+ Hematologic abnormalities, other abnormal laboratory findings during induction phase.

+ OS based on Kaplan-Meier

+ EFS based on Kaplan-Meier

+ OS and EFS by ages based on Kaplan-Meier

+ OS and EFS by gender based on Kaplan-Meier

+ OS and EFS by RER and SER based on Kaplan-Meier

+ Prognostic factors involved OS analysis based on Cox’s proportional hazard model.

* + 1. **Assessment criteria:**

- Complete remission: no clinical signs and symptoms, full blood test and bone marrow test results are M1. Incomplete remission: bone marrow result is M2, clinical symptoms are mitigated compared to before treatment. Failure remission: bone marrow result is M3, clinical symptoms are not mitigated.

- Relapse: Lymphoblast rate in bone marrow is ≥ 25%. Testicular relapse is when testicle swells and hurts, when poked with small needles, lymphoblast is found. Central nervous system relapse: when patients show signs of headache, vomiting, damage in cerebral nerves and CSF contain lymphoblast at a rate of > 5 cells/mm3.

- Assessment of side effects: on coagulation, on peripheral blood cells and bone marrow according to the standard in CCG 1961 protocol. Infection assessment, anemia and tumor lysis syndrome,

- Close follow up patients during and out of resident time, and during regular check-up time according to CCG 1961 protocol.

**Chapter III: RESULTS**

**3.1 CLINICAL FEATURES AND INVESTIGATIONS:**

The research is conducted on 129 patients with 87 boys (67.4%) and 43 girls (32.6%), boy: girl ratio is 2.07. Average age: 7.0 ± 4.4. Aged 1-5: 45.7%, aged ≥ 10: 31.8%, aged 5-10: 22.5%.

**3.1.1 Clinical presentations:**

 Table 3.1: Clinical features usually found in ALL

|  |  |  |
| --- | --- | --- |
| **Clinical features** | **Number of patients** | **Percentage**  |
| Fever | 117 | 90,7% |
| Hepatomegaly | 95 | 73,6% |
| Hemorrhage | 84 | 65,1% |
| Spleenomegaly | 83 | 64,3% |
| Lymphadenopathy | 54 | 41,9% |
| Pain of the bone | 39 | 30,2% |

**Comments:** Most patients admitted to NHP show signs of fever, sporadic or continuous fever makes up 90.7%. 65.1% of patients have hemorrhage. High-risk ALL patients usually have these symptoms: hepatomegaly, sleenomegaly and lymphadenopathy at the percentage of 73.6%, 64.3% and 41.9% respectively. Pain in the bone is less common at 30.2%.

**3.1.2 Full blood test characteristics:**

Table 3.2: Full blood test characteristics

|  |  |  |  |
| --- | --- | --- | --- |
| **Blood index** | **Patients** | **Percentage** | **Average** |
| Hb: < 60 g/L  60- 90 g/L  90- 110 g/L > 110 g/L | 2672229 | 20,2%55,8%17%7% | 76,5 ± 20,69 (31-140 g/L) |
| WBC: < 10 G/L 10 - < 50 G/L ≥ 50 G/L | 243174 | 18,6%24,0%57,4% | 110,8± 136,14(0,7- 686,5 G/L) |
| Platelets: < 20 G/L 20- 99 G/L ≥ 100 G/L | 386823 | 29,5%52,7%17,8% | 62,4 ± 93.46(4- 544 G/L) |

**Comments**: Blood tests show that more than half of patients suffer from mild anemia (55.8%); 7% have normal Hb. The average Hb level is 76,5± 20,69 g/L. 57,4% of the patients has WBC count ≥ 50 G/L, while patients with WBC count < 10 G/L only makes up 18.6%, average WBC count is 110,8± 136,14 G/L. 82,2% of the patients have reduced platelets count < 100 G/L, 17.8% has normal platelets.

**3.1.3 Bone marrow characteristics of high-risk ALL**

Elevated number of bone marrow cells during diagnosis accounts for 60.4% of the patients, while decreased number of bone marrow cells during diagnosis is only 7%. The average bone marrow cells count is 196,9 ± 155,8 (between 2,9 G/L & 729,2 G/L). Lymphoblast percentage is within the range of 29% to 99%, the average is 82.6 ± 14.7%. Among high-risk ALL, morphologic classification (FAB): L1 makes up the majority: 55%, L2 is less common with 40.3%. Based on morphology alone, there is a small number of patients, 6/129, wrongly diagnosed with AML (4.7%).

Pre B cell ALL has 105 cases (81.4%), T cell has 17 cases (13.18%). There are 3 cases (2.32%) in which it is not possible to identify the type of ALL. Among 129 patients there are 105 cases of CD10 (+), accounting for 81.4% and 24 cases of CD10(-), which is 18.4%. There are 29/129 patients (22.48%) are biphenotype or preB, T doimanted with traces of cell-mediated immunity from other types of cells.

Cytogenetic of high-risk ALL: there are only 97 patients shows positive results for chromosomal culture from lymphoblast.

Table 3.3 Results of chromosomal culture from lymphoblast

|  |  |  |
| --- | --- | --- |
| **Chromosomal culture** | **Patients** | **Percentage** |
| Normal | 58 | 59,8% |
| Hypodiploidy | 23 | 23,7% |
| Hyperdiploidy | 4 | 4,1% |
| Abnormal chromosome | 12 | 12,4% |
| Total | 97 | 100% |

**Comments:** 59.8% of patients have normal karyotype (46 XX or 46XY). 23.7% of patients have hypodiploid (<45). 12.4% of patients have abnormal chromosomes. There are only 4 cases (4.1%) in which patients have hyperdiploid (>47 XX or >47 XY), these factors have good prognosis.

Among the 12 patients with abnormal chromosomes, translocation is the most common mutation – 6/12, followed by deletion (4/12) and addition (3/12).

Translocations found are t(9;22)(q34;q11.2), t(3;12)(q26;p13), t(9;12)(p24;q36), t(1;2)(p36;q36) and t(1;19(q23;p13). Deletions found are del(6)(q15), -6, -16; del(4)(q32;q34); del(3p), del(12q) and del 11q. Additions include add (8)(q23), +14, +20. Some patients’ chromosome shows both deletion and addition and translocation.

**3.1.4 ALL-related prognosis factors**

Comparisons between unfavorable factors such as WBC count during diagnosis, age, gender, biphenotype, hypodiploid between boys and girls, between the age groups above and below 10 shows that WBC ≥ 50 G/L is more common among children under age 10 than among children above age 10 (p < 0.01).

**3.2 OUTCOME OF TREATMENT BASE ON CCG 1961 PROTOCOL**

Among the 129 high risks ALL patients there are 102 patients who are treated according to CCG 1961 protocol. The patients are followed up from the start of treatment until death or until the end of treatment and regular check-up afterwards. The end of monitoring time is 31/5/2015. The results are 12 patients died during the induction phase (11.76%) while the other 90 got into complete remission (88.24%). 77 patients were treated according to arm B of CCG 1961 protocol due to RER and 13 followed SER protocol. 5 patients were still undergoing treatment (4.9%) and 42 completed treatment (41.18%).

**3.2.1 Induction phase results:**

Among 102 patients treated according to the CCG 1961 protocol, 3 died before day 7 of the induction phase, 95 others undergo bone marrow aspiration to examine the responsiveness to the treatment. Results are as follow:

Table 3.4. Bone marrow on day 7 of induction phase

|  |  |  |
| --- | --- | --- |
| **On day 7** | **Patients** | **Percentage %** |
| M1 | 75 | 75,8 |
| M2 | 8 | 8,1 |
| M3 | 16 | 16,1 |
| Total | 99 | 100 |

Comments: Percentage of patients who reach RER is 82.9% (75.8% M1 and 8.1% M2), only 16.1% have SER (M3). Patients with M2 and M3 will have their bone marrow aspirate on day 14 of induction phase. Results show that 8 M2 patients reach M1 on day 14, 11 M3 patients reach M1 on day 14 (68.75%), 2 patients died before day 14 and 3 reach M2 (18.75%).

Side effects occurred during induction phase are: fever (59.8%), stomachache (27.5%), vomiting and nausea (41.2%), diarrhea (18.6%), constipation (11.8%), mouth ulcer (50%), pneumonia and broncho-alveolitis (11.8%).

During the induction phase, patients undergo many rounds of blood test, coagulation test and biochemical test. Hb, WBC and platelets count usually undergo substantial drop (level III and IV), along with bone marrow cells. Prothrombine ratio, fibrinogen ratio and liver function (SGOT, SGPT) usually have less changes (level I and II). Glucose level increases substantially, there are 7 cases (6.86%) with > 10 mmol/L due to side effects of L-Asparaginase and dexamethasone, 21 patients (20.59%) has decreased sodium level (<130 mEq/L), 12 (11.7%) has decreased potassium level (< 3 mEq/L) and 36 (35.29%) has decreased calcium level (< 2 mEq/L). Elevated glucose will stop when L-Asparaginase and dexamethasone are no longer used.

Table 3.5 Results of induction phase

|  |  |  |
| --- | --- | --- |
| **Results** | **Number of patients** | **Percentage** |
| Complete remission | 90 | 88,24% |
| Fatality | 12 | 11,76% |
| Total | 102 | 100% |

**Comments**: 88.24% of patients reach complete remission by the end of induction phase. 12 patients (11.76%) died during treatment.

80 patients undergo blood culture during induction phase, among them 21 cases (26.25%) has positive. Bacteria that can be found are mostly nosocomial infection. Bacteria that can be isolated are: K. Pneumoniae, Staphylococcus Aerius, Staphylococcus group F, E. Coli, Pseudomonas Aeruginosa, Acinetobacter Sp, Serratia Marcescens and Candida fungus.

**3.2.3 CCG 1961 protocol results based on Kaplan-Meyer**

90 patients continue post- induction and follow up (77 RER and 13 SER). Results show that 17 suffer from relapse (16.67%) and 26/90 patients died post- induction. Total number of patients under observation by the end of research is 47.



Graph 3.1 OS ratio based on Kaplan-Meier.

Percentage of OS patients for 5 years is 48.6 ± 5.0%, survival for 1 year is 72.5% and survival for 2 years is 54.7%.



Graph 3.2 EFS ratio based on Kaplan-Meier.

EFS ratio for 5 years is 46.0 ± 5.0%. Survival after 1 year rate is 68.6% ± 4.6%, survival after 2 years rate is 53.7% ± 5.0%.

Table 3.6. OS and EFS by gender

|  |  |  |
| --- | --- | --- |
| **Gender** | **5 years OS** | **5 years EFS** |
|  | % | SD | 95% CI | % | SD | 95% CI |
| Boys | 54,8 | 4,6 | 45,8 – 63,7 | 52,9 | 4,6 | 43,9 – 61,9 |
| Girls | 30,5 | 4,5 | 21,7 – 39,4 | 29,6 | 4,6 | 20,6 – 38,6 |
|  | p = 0.006 | p = 0.01 |

**Comments:** Boys have higher OS ratio than girls, 54.8% ± 4.6% and 30.5% ± 4.5% respectively. EFS ratio of boys is also higher than that of girls (52.9 ± 4.6% and 29.6 ± 4.6%). Statistical significance p < 0.05.

Table 3.7 OS and EFS by age group

|  |  |  |
| --- | --- | --- |
| **Age**  | **5 years OS**  | **5 years EFS** |
|  | % | SD | 95% CI | % | SD | 95% CI |
| < 10  | 46,8 | 6,2 | 34,7 – 59,0 | 45,1 | 4,5 | 36,3 – 54,0 |
| ≥ 10 | 47,1 | 4,5 | 38,3 – 55,9 | 46,1 | 6,3 | 33,7 – 58,5 |
|  | p = 0.97 | p = 0.905 |

**Comments:** OS and EFS for children aged above and below 10 is 47.1±4.5% and 46.8±6.2%, 45.1±4.5% and 46.1±6.3% respectively. There is no difference between the two age groups (p>0.05).

Table 3.8. OS and EFS by bone marrow response on day 7.

|  |  |  |
| --- | --- | --- |
| **Day 7 response** | **OS by day 7 response** | **EFS by day 7 response** |
| % | SD | 95% CI | % | SD | 95% CI |
| RER | 49,6 | 3,9 | 41,9 – 57,3 | 47,8 | 3,9 | 40,1 – 55,6 |
| SER | 31,1 | 8,1 | 15,1 – 39,8 | 30,4 | 8,3 | 14,2 – 46,6 |
|  | p = 0.069 | p = 0.09 |

**Comments:** OS and EFS ratio of the RER on day 7 of induction phase are higher than that of the SER group (49.6% and 47%, 31.1% and 30.4%). However, this difference has no statistical significance (p>0.05).

Univariate analysis based on Cox’s proportional hazard model on some prognostic factors such as age, gender, WBC count at diagnosis, lymphoblast on day 7 of induction phase, hypodiploid, translocation t(9;22), biphenotype, renal insufficiency, CD 10 (-) show that: gender factor and renal insufficiency affect patients’ OS (p<0.05). Multivariate analysis on prognostic factors that affect OS indicates: 4 factors: gender, tumor lysis syndrome, lymphoblast day 7 and hypodiploid or translocation t(9;22) affect patients’ OS ratio (p<0.05).

**Chapter IV: DISCUSSION**

**4.1 CLINICAL PRESENTATIONS AND LABORATORY FINDINGS**

**4.1.1 Epidemiology and clinical presentations:**

In our research, 129 patients are categorized into the high-risk ALL group. Among them, 102 undergo treatment and follow up according to the CCG 1961 protocol until the end of the research. In the high-risk ALL group, child patients aged above 10 are 31.8%, the majority of patients aged between 1 to 5: 45.7%. NH Nam shows that children aged ≥ 10 accounts for 46.3% while children aged 1-5 accounts for only 29.8%. This indicates that children are more likely to be categorized into high-risk group based on other poor conditions such as high WBC, hypodiploidy, biphenotyping. America with CCG 1961 protocol there are 1299 patients, age above 10 group takes up 63.2% (821 patients), age under 10 takes up 36.8% (478 patients). Boy: girl ratio is 2.07. This result is similar to that of local and foreign researchers – more commonly found in boys than in girls. Table 3.1 shows the clinical features on admission: fever is the most common (90.7%), followed by hepatomegaly, spleenomegaly and lymphadenopathy, petechie and bone pain. These are similar to what is found by NH Nam and Judith FM. There is no difference in terms of clinical characteristics between T and B cells ALL.

**4.1.2 Hematologic characteristics:**

Table 3.2 shows hematologic abnormalities at presentation. We usually visit cases in which upon diagnosis at NHP, lymphoblast have been found, for some children, lymphoblast take up 90%, other types of blood cells decrease substantially. Children ALL admission are usually afflicted with anemia by WHO standards (average Hb is 76.5g/L), very few patients do not have anemia (7%). WBC ≥ 50G/L is 57.4% while Judith FM only has 17% and patients without anemia is 12%, NH Nam has WBC at diagnosis ≥ 50G/L of 70.7% and 11% of patients have no anemia. Patients with the highest WBC is 686.51 G/L. Platelets < 100G/L is 82.2%, similar to the 2 other local and foreign researchers. This shows that children ALL usually go to the hospital late. Since lymphoblast dominates over other types in bone marrow, all patients have decreased number of neutrophils, which is also the reason for fever due to bacterial infection, children will be treated with antibiotics and chemotherapy together.

**4.1.3 Lymphoblast in bone marrow:**

Classification FAB is still applied in Vietnam, this is the initial categorization that helps researchers to plan other tests such as immunophenotypic, cytogenetic, FISH. The FAB system defines L2 usually has worse prognosis than L1 in ALL although this characteristic no longer holds prognosis value recently. However, in some cases, morphologic classification is not adequate to make an accurate diagnosis and small number of patients was wrongly diagnosed with AML (4.7%), hence determination of the immunophenotype classification is needed. The most common of immunophenotype in childhood ALL is B cell (81.4%), T cell is 13.18%. The percentage of lymphoblast found in this research is similar to that of other overseas researches: 80-85% of childhood ALL cases have predominant B cell precursors, T-cell is 15-20% of the cases across different researches. In our research, there are 29 cases (22.48%) of biphenotype (T or B cells or AML) but 25 cases have either B or T cell as the dominant phenotype, 4 cases in which both phenotypes are equally represented. Other researched in the world shows that ALL children with AML markers is 7-25%. Patients with ALL antigen CD 10 (+) or *common* ALL has better prognosis than those with CD10 (-), this results confirmed that 18.6% of patients has CD10 (-) and 81.4% has CD10 (+), patients with CD10 (-) usually have other unfavorable prognosis such as elevated WBC count, hypodiploid.

**Cytogenetic:** Since 2007, the Department of Bio- Molecular Genetics of NHP has started chromosomal culture for patients who are assumed to have leukemia. Until now, this test has been done frequently. However, we have limited results since the normal results takes up 59.8% whereas only 40.2% has abnormal chromosome, this result in overseas centre discovers 80% chromosomal abnormality among ALL patients. Nowadays, thanks to modern technology, in almost 100% of the cases, chromosomal abnormality is detected. Table 3.3 shows that the results of chromosomal culture from lymphoblast include: structural chromosomal abnormalities 12.3% (12/97 patients) and number chromosomal abnormalities: hypodiploidy 23.7% and hyperdiploidy is 4.1%. About structure: 6/12 is translocations, deletion is 4/12 and addition is 3/12. We only encounter 2/12 cases of unfavorable prognosis translocation t(9;22). High- risk ALL chromosomal abnormalities in our research with that of PNT Van 2013 shows lower results. When doing research on chromosomal abnormalities in both childhood and adult ALL, this author discover 4 abnormal genetic combinations of 4 translocations t(12;21), t(9;22), t(4;11), t(1;19) takes up 25.7%. Nita LS has the percentage of patients with hypodiploid in 2 patient groups of 7.2% and 12.3%. Thus, NHP needs to apply more advanced technology to detect chromosomal abnormalities in ALL to help doctors choose the appropriate protocol. The 2 patients whose harmful translocation t(9;22) are detected and treated with CCG 1961 protocol have unfavorable prognosis such as elevate WBC count (181 G/L and 82.55G/L), hepatosplenomegaly, no response to treatment (M3 on day 7 of induction phase), one child aged above 10 and early relapse after consolidation phase, 1 child died after 4 months of treatment due to severe infection. In another patient 11q deletion is detected, which is a poor prognosis, this patient relapse early after 5 months of treatment. Hence, in our research, chromosomal abnormalities with unfavorable prognosis [hypodiploid, t(9;22) and 11q deletion] takes up 63.4% (26/41) of all the cases of chromosomal culture with abnormal results.

**4.3 OUTCOME BASE ON CCG 1961 PROTOCOL:**

**4.3.1 Induction phase results:**

According to CCG 1961 protocol, bone marrow aspirate must be checked on day 7 of induction phase to assess the response to treatment. Our research indicates that RER percentage is 83.9% (75.8% M1 and 8.1% M2), SER (M3) is 16.1% (table 3.4). When compared this result with that of the CCG 1961 research group (RER is 71.4% & SER is 28.6%), our SER percentage is higher but the death ratio before day 7 is higher (3/102) than that of CCG 1961 research (3/2057), showing that supportive care with ALL chemotherapy is an important factor leading to patients passing the induction phase. Depends on each protocol, patients will undergo bone marrow assessment either on day 7 or day 14. Arika M (Japan) from 1988-1999 on 116 patients, which assess on day 14 shows that: 69 children are M1 (59.5%), 25 patients are M2 (21.6%), 22 patients are M3 (18.9%). This result is similar to ours. Before the end of induction phase, bone marrow aspirate will be carried out, we have 90 patients whose bone marrow assessment on day 28 confirms complete remission (M1) reach 100%. Schrappe M shows that failure therapy in this phase is 2.4%. Table 3.5 shows that complete remission after induction phase has 88.24%, death rate in this phase is 11.76%. Meanwhile, the American CCG 1961 research group has 21/2057 (1.02%), other groups in the world also have death rates in this stage between 1-2%. CV Ha (Hue) reported the ALL treatment death rate is 44% in the first 28 days of treatment. The reason for the high death rate is severe infection due to neutropenia and uncontrolled bleeding, brain hemorrhage. Comparing with other research groups in the world shows that death rate during induction phase is a serious problem that requires attention, supportive care such as proper & effective antibiotic usage and adequate supplement of blood products to prevent possible strokes has large impact on treatment results.

**4.3.2 Side effect and toxic during induction phase:**

The induction phase uses 4 drugs, thus the patient has to endure a large amount of chemotherapy, leading to more side effect as myelosuppression due to leukopenia, anemia, thrombocytopenia. There are some, but not significant, differences between the side effect ratio researched and that of researches by LT Phuong and BN Lan. These side effects are usually most serious from day 7-14 of the first stage, corresponding to mycositis, severe infection and fever neutropenia. When the bone marrow recovers, infection and mycositis in children also decrease and return to normal on week 4 of induction phase.

**4.3.3 Post induction of CCG 1961:**

Among the 102 patients treated and follow up according to CCG 1961 protocol by the end of the research, 31/5/2015, the longest monitoring period from diagnosis to the end of the research is 84 months, the shortest period is 1 week when the patient died. There are 47 patients alive and among them 5 are expected to stop treatment in August (2 patients), December (2 patients) and 1 patient will stop in October 2015. By the end of the research, among the 38 deaths: 12 were during induction phase with the earliest being 1 day after starting treatment and latest being 26 days before the completion of induction phase (28 days).

Relapse percentage is 16.67% (17 patients), among them 15 patients relapse while the treatment (2 patients relapse very early in less than 6 months), this may be explained by unfavorable factors such as high level WBC ≥ 50G/L at diagnosis, hepatosplenomegaly, in 1 child we also find translocation Philadelphia t(9;22), monosomy chromosme 7 and hypodiploid, 1 child with 11q deletion. 2 patients relapse late. Other groups with the same relapse results: Ma-Spore 17.9%; CCG 1961 16.92%; UKALL 97-99: 16%. All 17 cases are bone marrow relapse with 1 case of testicular relapse and marrow relapse, no CNS relapse found. The number of patient’s dies of post induction phase is 26 (25.5%). Our number is rather higher than Ma-Spore group, high-risk ALL has 78 patients and they have 7 patients died during treatment (9%). Australia uses the ANZCHOG (Study VIII) protocol for 66 high-risk ALL, death rate during treatment is 4.5% (3 patients). All these patients’ deaths in our research are at home or local hospitals due to uncontrolled bleeding or infection before being transferred to NHP.

**4.3.4 Outcome based on Kaplan-Meier:**

Graph 3.1 & 3.2 shows OS and EFS rates 5 years. Based on this estimation, our OS result is 48,6 ± 5,0% and EFS is 46 ± 5,0%. This is a humble result when compared to the result of CCG 1961 protocol published by Nita LS in 2007 with 80,4 ± 1,4% for OS and 71,3 ± 1,6% for EFS. Allen Yeoh (Singapore 2012) applied Ma-Spore protocol in 2003 give the results of 71.8% for OS rate 5 years and 50.6% for EFS rate 5 years. Arika M (Japan) has OS rate for high-risk group at 68,7 ± 8,3%. Veeman A (Holland) published the high-risk ALL treament based on Dutch ALL-9 (1997-2004) results of 71% for OS rate and 78% for EFS rate (5 years). This shows that not only using the correct medicine based on the protocol but also the doctor must have enough experience in supportive care and side effect treatment well, isolation therapy and healthy nutrition also increase patients’ survival rates. Patients’ deaths in our research are mostly due to infection as a result of severe neutropenia decrease and uncontrolled bleeding. Comparing OS and EFS 5 years ratio between boys and girls in our research shows significant difference: boys have better ratio than girls, this difference is statistically significant when p < 0.05. Allen Yeoh published the treatment results based on Ma-Spore protocol in 2003 when comparing between 2 genders show no difference, EFS rates after 8 years are 80% in boys and 81.1% in girls. Chritensen MS shows that boys have worse prognosis factors than girls but did not die of infection, girls have higher death rates due to infection of 4.4% compared to 2.1% in boys. Comparison results of OS and EFS between 2 age group in our research are different from that of other authors in the world. Allen Y research based on Ma-Spore protocol shows that there is a statistically significant difference between the EFS rate of 2 groups above and below 9 years old (p=0.000), survival rate for age group >9 is 73.4% while age group <9 is 83.8% after 8 years. Bauruchel A shows that survival rate for age group <10 is higher than that of age group >10 in research by Dana Farber Cancer Institute (1991-2000), children aged below 10 (n=685) and children aged above 10 (n=108) shows EFS rates after 6.5 years is 85%±1% & 77%±4%. However, these results do not have statistical significance (p=0.09). Among 90 patients continued with CCG 1961 protocol there are 77 show RER and 13 show SER on day 7 of induction phase. Comparison in terms of OS and EFS rates between these 2 groups show that RER patients have higher survival rate than SER patients. However, this difference is not statistically significant (p>0.05), maybe because the number of SER patients is small when among 16 SER patients only 6 complete treatment.

**CONCLUSION**

Based on research on 129 high-risk ALL patients and treatment based on CCG 1961 protocol for 102 patients at the Department of Oncology at National Hospital of Pediatrics, we reach these conclusions:

**1. Clinical features and investigations of high-risk ALL patients:**

- Children afflicted with high-risk ALL at NHP are usually between age 1-10 (69.2%), male patients are more common than female patients (boys: girls ratio is 2.07). The noticeable clinical characteristics of children admitted to hospital and suspected of leukemia is fever, pain in the bone, petechie, hepatosplenomegaly and lymphadenopathy. There is no difference in terms of clinical characteristics between B cell and T cell ALL.

- Hematologic characteristics: Anemia with Hb <90g/L takes up 76%, WBC count ≥ 50G/L is 57.4%, platelet ≤ 20G/L takes up 1/3 of the cases (29.5%). Lymphoblast rate of bone marrow is high (average 82.6%).

- Based on FAB classification, L1 high-risk ALL is more usual than L2 (55% versus 40.3%), a small percentage (4.7%) are AML but immunophenotype assessment shows ALL. Pre B high-risk childhood ALL takes up 81.4%, T is less common (13.18%), pre B or T ALL can be found but with other markers or biphenotype.

- Karyotype culture from bone marrow detect 40.2% with chromosomal abnormalities, among them hypodiploid is 23.7% and structural chromosomal abnormalities is 12.4%. Chromosomal abnormalities ratio with unfavorable prognosis [hypodiploid, translocation t(9;22) & 11q deletion] takes up 63.4% (26/41) of cases with abnormalities.

**2. Treatment based on CCG 1961 protocol results:**

- Complete remission of induction phase is 88.2%.

- OS rate and EFS rate 5 years based on Kaplan-Meier estimation are 48.6% and 46% respectively; boys have higher survival rates than girls (54.8% and 52.9% compare to 30.5% and 29.6%) with statistical significance with p<0.05; the ratio of RER is higher than that of SER (49.6% & 47.8% compare to 31.5% & 30.4%) (p> 0.05).

- Common death rate is 37.25%, most are in induction phase and intensification phase. The common cause of death is serious infection and bleeding.

- Relapse percentage is 16.7%. Unfavorable factors such as gender, lymphoblast on day 7 of induction treatment, tumor lysis syndrome, hypodiploid and translocation t(9;22) have effect on outcome.

**CONTRIBUTION VALUE OF RESEARCH**

- This is the first research in which systematic assessment on high-risk ALL treatment based on an international protocol for Vietnamese children is made. The research indicates that half of the children afflicted with high-risk ALL can be cured in conditions provided at NHP.

- Although the survival rates for high-risk ALL children in NHP is still low compared to CCG 1961 protocol treatment results in other places in the world, the relapse results is similar to that of other international authors, deaths usually occur at induction phase and intensification phase thus it is possible to improve patients’ survival rate by paying better attention to collaborative treatment.

**REQUEST**

1. When CCG 1961 protocol is applied, it is necessary to improve the quality of chromosomal abnormalities detection. To improve treatment results, adequate medicine must be provided, especially for patients who seek treatment at home.
2. Training and imparting treatment protocol for doctors and nurses in local hospitals to improve the overload situation in NHP.
3. According to international researches, the treatment result of twice intensifications is not better than one, the death rate in intensification phase is pretty high, thus application of arm A of CCG 1961 protocol should be considered.

**LIST OF SCIENTIFIC RESEARCHES RELATING**

**TO THE THESIS**

1. Evaluate the result of high risk acute lymphoblastic leukemia at induction phase, CCG 1961 protocol in national hospital of pediatrics. *Journal of pediatrics*, Volume 6, N0 2, April, 2013.
2. Reseach the risk factors of patient with high risk acute lymphoblastic leukemia in national hospital of pediatrics. *Journal of pediatrics*, volume 6, No 5, October, 2013.