CHAPTER 1

General Introduction

Leukemia (or blood cancer) is a malignancy of bone marrow whereby leukemic cells undergo uncontrolled proliferation and occupy too much of the marrow, leading to bone marrow failure. Although the exact cause of leukemia remains largely unknown, several hypotheses have been proposed. In most, if not all cases, it is related to genetic alteration, either inherited or acquired after environmental exposure to, for example, ionizing radiation, or to chemicals and drugs.[1] Moreover, there is an infection-based hypothesis related to the occurrence of childhood acute lymphoblastic leukemia (ALL) in Western countries, claiming the influence of viral infections or an immunodeficiency state.[2,3] However, no direct gene-environment interactions have been established.[4]

Based on clinical presentation, childhood leukemia constitutes acute leukemia in 97% and chronic leukemia in 3% of the cases. Acute leukemia represents a clonal expansion of leukemic cells that undergo maturation arrest at a specific stage of normal myeloid or lymphoid hematopoiesis. Therefore, several subtypes can be distinguished: including acute lymphoblastic leukemia (ALL, 75%); acute myeloblastic leukemia (AML) previously called acute nonlymphocytic leukemia (ANLL, 20%); acute undifferentiated leukemia (AUL, <0.5%); and acute mixed-lineage leukemia (AMLL) nowadays called mixed-phenotype acute leukemia (MPAL).[5]

Childhood acute lymphoblastic leukemia

Currently, childhood ALL is the most common childhood cancer worldwide.[6] Also in the pediatric cancer unit (PCU) of the Dr. Sardjito Hospital (DSH), leukemia (in particular ALL) is the most common diagnosis of childhood cancer, followed by retinoblastoma and neuroblastoma (Figure 1).[7]

In the DSH the referral rate of childhood ALL continues to increase: about 65 - 70 new cases of childhood ALL per year were recorded in the last 3 years. This is a threefold increase compared with data from 10 years ago, when 25 new cases were reported in 1999 (Figure 2). In our PCU the peak incidence of childhood ALL occurs at 2 - 3 years of age (Chapter 2),[8] which is similar to the 2 - 5 years of age reported in international studies.[4]

The progress in treatment of childhood ALL from being a fatal disease to one with a 5-year cure rate of over 80% in Western countries can be used as model of childhood cancer treatment in Indonesia. The factors contributing to this success include the development of cooperative

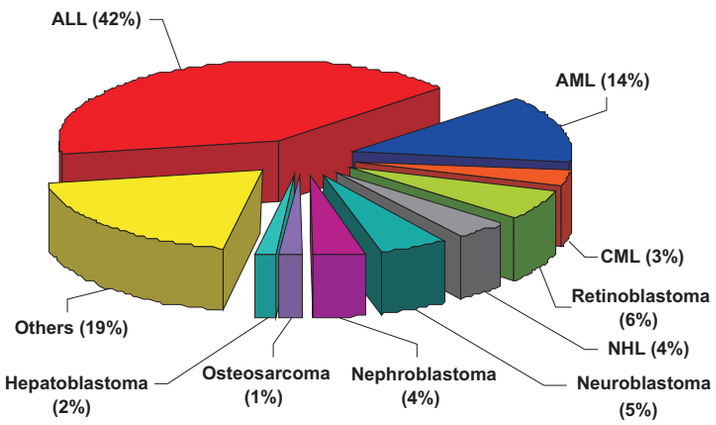

FIGURE 1. **Distribution of newly diagnosed childhood cancer in the Pediatric Cancer Unit of Dr. Sardjito Hospital (Yogyakarta, Indonesia) during 1999 - 2010 (n=1,351).** The incidence of childhood cancer has increased in recent years.

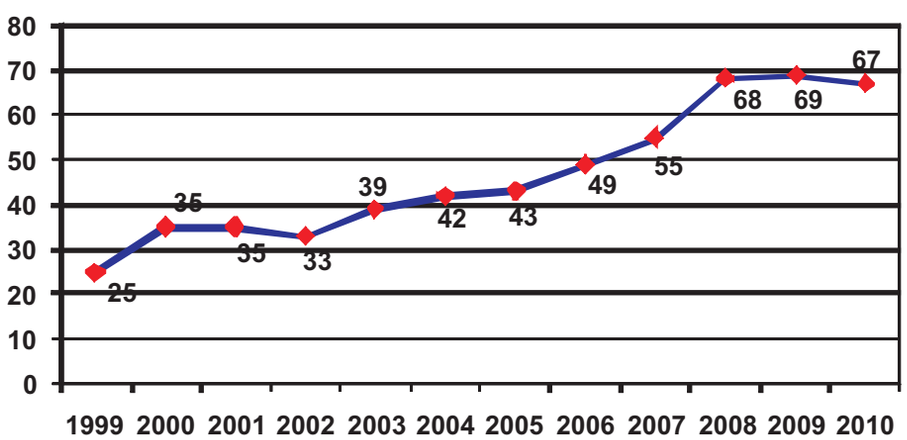

FIGURE 2. **Number of newly diagnosed patients with childhood ALL in the Pediatric Cancer Unit of Dr. Sardjito Hospital (Yogyakarta, Indonesia) during the period January 1999 to September 2010.**

medical groups, well-organized clinical trials, good access to supportive care, and progress in the characterization of patients at high risk for treatment failure. Moreover, the development of tailored risk-group-adapted therapy has also played an important role in achieving the high cure rate.

Although significant progress in the treatment of childhood cancer has been made, cancer continues to burden families and is associated with significant costs for treatment.[9] In our PCU setting, treatment costs often result in financial problems for the families as their income may be decreased due to time lost from work, or to total loss of employment. Such problems often result in poor adherence to treatment.[10]

Diagnosis

The signs and symptoms of childhood ALL are due to bone marrow failure caused by the infiltration of lymphoblasts, and extramedullary infiltration of organs such as liver, spleen, lymph nodes and practically every other organ. Bone marrow failure may also manifest as anemia, susceptibility to infections, and a tendency to bleeding. In Western countries, at diagnosis about two-thirds of children with ALL show signs and symptoms of leukemia that have been present for less than 4 weeks.[11] In contrast, in low-income countries the delay between the first signs and symptoms, and diagnosis and treatment may be longer due to less effective health systems and limited resources.

A definite diagnosis of childhood ALL is established based on examination of bone marrow. In PCUs with limited resources, the diagnosis is based on identification of lymphoblasts in bone marrow by light microscopy for morphology and (sometimes) by cytochemical assessment (e.g. PAS, Sudan Black B, or esterase staining). Morphologically, lymphoblasts are classified as L1, L2 or L3 according to the French-American-British classification. However, except for the rare case of L3, this classification has no correlation with the prognosis of patients. Advanced examination using immunophenotype, karyotype, genotype, and pharmacogenetic studies provide more significant biological characteristics of lymphoblasts related to the prognosis.[4] Based on the surface and intracellular expression of antigens of the leukemic cells, the immunophenotype examination can help to differentiate the leukemic cells as myeloid or lymphoid lineage, and the origin of lymphoid cells as B-cell or T-cell lineage. This differentiation is

important to select the most appropriate treatment. Within ALL, mature B-cell ALL (often morphologically recognizable as L3) also requires a specific treatment protocol, similar to Burkitt's lymphoma. Precursor B-cell ALL is the most frequent subtype and has the best prognostic characteristics at diagnosis, e.g. lower white blood cell counts, and favorable age group (1 - 10 years). Patients with T-cell ALL are usually associated with the presence of hyperleukocytosis at diagnosis, bulky disease (hepato-splenomegaly with peripheral lymphadenopathy), mediastinal mass or testicular infiltration. Based on these clinical and immunophenotypic characteristics, ALL is divided into various risk categories and the treatment is then adapted to the risk category. Besides clinical and specific cell surface characteristics, leukemic cells can also be classified based on specific genetic alterations, which have both prognostic and therapeutic relevance. These alterations can be identified using karyotyping or specific polymerase chain reaction (PCR)-based techniques, as well as by FISH. In addition, small nucleotide polymorphisms can be present in genes relevant for drug metabolism. Therefore, PCR-based techniques are used to explore the genes that influence the pharmacological effects of chemotherapy and thereby determine the response to treatment. This has, for instance, been shown in our patient cohort for the enzyme thymidylate synthase, which is important for the effectiveness of methotrexate.[12] The result raises the question as to whether the characteristics of ALL in Indonesia and other Asian countries are similar to those in Western countries. If there are indeed differences, these may be important for the prognosis and optimal treatment. Therefore, a treatment that is 'best practice' in Western countries may not necessarily be the best option for the situation in Indonesia. This work presented in thesis mainly evaluates aspects related to the therapeutic options for childhood ALL.

In almost all of the 14 PCUs in Indonesia, the diagnosis of childhood ALL was based on morphology and cytochemistry examination, since the immunophenotype examination was not yet available (with the exception of Jakarta). In 2006, at the start of our Dutch Cancer Society project, this method was introduced in DSH and was used for risk stratification in patients treated on the Indonesia-ALL-2006 protocol.[13] Other advanced biological diagnostic tools are not yet available in Indonesia, apart from some used on a research basis in a limited number of PCUs.

Treatment

Treatment of childhood ALL is aimed at eliminating the leukemic cells and restoring bone marrow to obtain normal hematopoiesis. Since treatment with chemotherapy is highly toxic to body organs, the treatment regimen is developed on a risk-adapted principle; this means that patients are treated according to their individual risk of treatment failure. This strategy aims to prevent excessive treatment or toxicity in patients with less aggressive and/or progressive disease and, conversely, to avoid under-treatment in patients with aggressive and/or progressive disease at high risk for treatment failure. Therefore patients are classified as being standard (SR), intermediate (IR) and high risk (HR) patients; some protocols may also sub-classify high-risk patients into a very high-risk (VHR) category. Basically, the Indonesian treatment protocols utilize the simple risk stratification developed by the Rome consensus[14] that was refined in the National Cancer Institute (NCI) workshop.[15] According to this, patients are classified as SR when diagnosed at age 1 - 9 years and with a white blood cell count less than 50,000/µl. The remaining patients are classified as HR ALL patients. The additional criteria for HR ALL in the Indonesian protocol are the presence of mediastinal mass, testicular or central nervous system (CNS) involvement, and an absolute number of lymphoblasts in circulation of 1,000/µl or more at the end of the one-week prephase treatment with a glucocorticoid plus one dose of intrathecal methotrexate (MTX). After developing the immunophenotype examination in DSH, the T-cell phenotype was used as an additional HR criterion; thus all patients with T-cell ALL are treated on the HR protocol. Recent international risk classification has become more complicated due to combining clinical presentation at diagnosis with many biological or molecular features and minimal residual disease as risk parameters.

The basic treatment of childhood ALL consists of remission induction, consolidation, prevention of CNS involvement, and maintenance or continuation treatment.[11,16] Type of drugs, dose intensity and time of administration vary among the childhood ALL protocols. The induction phase is aimed at inducing complete remission status with the disappearance of leukemic cells from circulation and less than 5% leukemic blasts in bone marrow showing normal hematopoiesis, and no clinical signs of leukemia elsewhere. This phase comprises 4 - 6 weeks and consists of three or four drugs including an oral steroid (prednisone or dexamethasone), intravenous vincristine, and intravenous L-asparaginase

as the third drug.[16] In some protocols intravenous anthracycline (usually daunorubicin or doxorubicin) has been used as the fourth induction drug. The Indonesian protocols used a 3-drug induction in the WK-ALL-2000 protocol and a 4-drug induction in its successor the Indonesia-ALL-2006 protocol. The consolidation phase is conducted after achievement of complete remission, and aims to maximize eradication of leukemic cells. In comparison with induction, the consolidation phase is less standardized. Some studies investigated the effect of treatment intensification with additional doses of L-asparaginase.[17,18] Since L-asparaginase is the most expensive drug in the protocol, we performed a study to examine whether additional doses would improve prognosis or would lead to more toxicity (Chapter 7). Randomized trials in the USA showed a better outcome in HR patients who received treatment intensification with L-asparaginase, and in HR patients with poor response to induction treatment who received a post-induction intensification.[19,20] Other randomized studies confirmed that additional blocks of treatment intensification during the first few months of treatment improve survival outcome.[21,22] These results confirmed the idea that treatment modulation or intensification may abrogate the prognostic factors at diagnosis and the response during treatment.

The CNS-directed therapy aims to prevent CNS infiltration or relapse. This strategy is based on the assumption that CNS is a sanctuary site for leukemic cells due to the fact that they are protected by the blood-brain barrier from therapeutic concentrations of systemic chemotherapy. In earlier protocols, treatment consisted of cranial irradiation in combination with a few intrathecal methotrexate injections.[23] Considering that cranial irradiation may generate neurocognitive late effects, influence the growth and development of the children, and cause secondary brain tumors, recent protocols omit the use of cranial irradiation or have restricted its use to HR patients or to patients with initial CNS involvement.[24,25] To decrease the incidence of CNS relapse, most protocols now replace cranial irradiation with the more frequent administration of intrathecal methotrexate, or triple intrathecal therapy consisting of hydrocortisone, cytarabine and methotrexate, and utilize high-dose intravenous methotrexate with citrovorum factor rescue.[26-28] Intrathecal methotrexate as pre-symptomatic CNS-directed therapy should be instituted early during induction and consolidation treatment, and be continued through at least part of the maintenance therapy to prevent the development of CNS leukemia. The occurrence of CNS relapse is also

associated with the type of steroid used in the protocol; dexamethasone seems to be superior to prednisone or prednisolone in preventing CNS relapse.[29-31] This benefit is based on the finding that dexamethasone has better penetration into the cerebrospinal fluid and a longer half-life than prednisone.[32] Specifically for the Indonesian situation, we performed a randomized study to investigate whether dexamethasone or prednisone is the more effective and less toxic steroid in childhood ALL treatment (Chapter 8).

The aim of maintenance or continuation therapy is to control the disease and/or prevent relapses, since the leukemic cells may resurge and occupy many organs after remission has been achieved. The re-appearance of leukemic cells or relapse may occur in bone marrow, CNS, testicles and other sites, either as isolated or combined relapses in those sites. A combination of daily oral 6-mercaptopurine and weekly oral methotrexate with periodic pulses of oral steroid and intravenous vincristine is generally used in the maintenance treatment phase, as is also the case in the Indonesian protocol. It is recommended to take a 6-mercaptopurine tablet in the evening rather than in the morning since it seems to be more effective and causes less nausea at that later time.[33] The main toxicity during maintenance treatment is myelosuppression, the incidence and severity of which is related to the dosage of 6-mercaptopurine and methotrexate. Polymorphisms of thiopurine methyltransferase influence the effect of 6-mercaptopurine.[34] Therefore, because not all patients are equally susceptible to the effect of this drug, dose adjustment is needed during maintenance treatment. From a practical viewpoint, the white blood cell count is used as an indication for the effectiveness of methotrexate and 6-mercaptopurine. During maintenance treatment the white blood cell count should be in the range 2,000/µl to 4,000/µl with a platelet count of more than 50,000/µl in the Indonesian protocol, as it is in many other protocols worldwide. A weekly or fortnightly white blood cell count, absolute neutrophil count, and platelet count should be performed as the basis of the dosing schedule. Treatment duration varies between different protocols. The Italian AIEOP study showed that a total treatment of 2 years was adequate in low or average-risk childhood ALL.[35,36] The MRC UKALL-VIII trial, using less intensive induction treatment, indicated that completing maintenance treatment in two years provided the same overall outcomes as compared with three years.[37] The Berlin-Frankfurt-Munster trials confirmed that two years of treatment was superior to 18 months.[38]

Based on these data, it was decided to adopt a total treatment duration of two years for the Indonesian protocols.

Other important components for the success of childhood ALL treatment are early diagnosis of complications, good access to supportive care (including availability of blood products), a good nutritional program, and continuous psychosocial support for optimal treatment adherence.[11] Late detection of the first symptoms of a complication and poor access to supportive care may have resulted in the high rate of toxic death and the low event-free survival as observed in most low-income countries, including Indonesia. This correlation is easy to understand, since leukemia or chemotherapy (and the combination of both) will induce myelosuppression that predisposes to severe infections and bleeding. Thus in the PCU where supportive care is poorly developed, it will be dangerous to implement more intensive treatment regimens in an attempt to improve the outcome.[39]

Adverse events

The adverse events in childhood ALL treatment consist of refusal or abandonment of treatment, early death, resistant disease (failure to achieve complete remission after the induction treatment), and death in remission or relapse. All these events will adversely influence the success of treatment, represented by low event-free survival. By definition, treatment refusal occurs before entering treatment and abandonment occurs whilst following treatment. Both are the most common events in low-income countries, but are almost nonexistent in Western countries. Similar to treatment refusal or abandonment (35%), in Indonesia death during treatment (23%) is also a prominent adverse event (Figure 3).[40]

The high death rate in low-income countries might be related to the fact that when patients come for diagnosis the disease is already advanced, or because they suffer from co-morbidities such as infection and malnutrition, or because these factors are combined with limited access to supportive care in most hospitals. This situation is very different from the much lower death rate achieved in Western countries. In the Dutch ALL-VI protocol, which was the basis for the development of the Indonesian childhood ALL protocol, the death rate was very low during induction (3/190 or 1.6%) and in remission during maintenance treatment (4/184 or 2.2%).[26] In the UKALL-VIII to XI study the death rate decreased from 3% to 1% during induction, and from 6% to 1% during remission.

This achievement was attributed to the success in avoiding fatal infections, while other causes of death (mainly hemorrhage) had not declined.[41]

This considerable difference in death rates between Western and low-income countries is mainly due to the poor general health of our patients, the late diagnosis of complications, and inadequate access to supportive care, medicines and other life-support facilities. Adequate access to supportive care, medicines and other life-support facilities are essential when patients face the toxic or life-threatening conditions of their disease. In addition, in our PCU setting as a university hospital, it is also related to inadequate staffing, the too frequent rotation of residents, lack of expertise, lack of reliable and fast laboratory analyses, late delivery of blood products, and lack of protocol adherence by healthcare providers - to name but a few. In addition there is a certain lack of urgency that might be rooted in the cultural background.

In Western countries, relapse or the re-appearance or infiltration of leukemic blasts in body organs after achieving remission, during therapy or after finishing treatment, is the most common cause of treatment failure.[42] Relapse is a devastating event for patients and families due to the low chance of survival, the intensive treatment required to achieve survival, the additional costs, and the problems of being confronted with toxicity again. Problems in the management of patients with relapse are associated with the resistance of leukemic cells to a second round of treatment after having received intensive treatment at initial diagnosis.[43] Since the outcome of patients with relapse is poor,[42,43] the treatment plan for these patients requires extra care and should be individually adjusted to the needs of each patient. The relapse rate in the Indonesian protocol is not as high as that in Western countries, this may be due to the timing of patient monitoring (which is relatively short to show relapses) and/or because of other competing events (e.g. many patients abandon treatment or have died).

Many factors are associated with relapse, including the biological characteristics of leukemic blasts, the intensity level of the protocol, and adherence to the protocol. *In vitro* studies have shown that leukemic blasts at relapse lost their sensitivity to glucocorticosteroid[44] as reflected by decreased apoptosis induction. This may explain the development of relapse and the difficulty to cure relapsed patients.[45] Patients with isolated extramedullary relapse showed better prognosis than those with isolated bone marrow relapse.[43] Patients with T-cell ALL tend to experience early relapse, and the outcome after marrow relapse is poor.[46]

Poor early response to treatment is also related to the higher relapse rate in Western studies,[47] and this was the reason to study early response to treatment in our setting (Chapter 5).[48] Treatment of relapse in childhood ALL varies between protocols, and can include chemotherapy, bone marrow transplantation or hematopoietic stem cell transplantation. Since a protocol specifically directed for patients with relapse is not yet available in DSH, these patients are usually re-treated with the HR protocol (or with some modification of the protocol), or a change is made to palliative treatment.

Treatment results of childhood ALL

Although an increasing cure rate has been achieved in childhood ALL, there is still considerable disparity between Western countries and low-income countries (LICs). The high cure rate of more than 80% is only reported in Western countries, where 20% of all children with ALL in the world reside. The remaining 80% of patients with ALL live in LICs with cure rates of less than 40%, as is the case in Indonesia.[40] In an attempt to close this gap, twinning programs between PCUs in Western countries and the developing PCUs in LICs have been initiated. This 'North-South' program has enabled improvements in human resources and clinical outcomes in PCUs in LICs; examples of these are the twinning programs between Italy and Nicaragua,[49] between North America (St. Jude Children's Research Hospital) and El Salvador and Guatemala,[50,51] and between the Netherlands and Indonesia.[52]

The Indonesian experience

The setting of Pediatric Cancer Unit in Indonesia and Yogyakarta

Indonesia is the fourth most populous country in the world, with a population of around 230 million. Indonesia can be classified as a LIC or developing country. Being an archipelago state with almost 17,000 islands, the island Java is inhabited by about 60% of the country's population; this has led to an uneven spread of medical specialist services, which in Indonesia are mainly concentrated in Java. Of the 14 PCUs in Indonesia, the majority has very limited resources with only 1 - 3 pediatricians/PCU, limited supportive care, and insufficient availability of medications.

Unfortunately, because up to now the governmental health program has focused on infection, malnutrition and the high infant mortality rate, cancer has no priority at this moment.

Yogyakarta is located in central Java. It is the second smallest province (after Bali) in Indonesia with a population of about 3.5 million. The life expectancy in Yogyakarta is the highest among the 33 provinces in Indonesia. The city of Yogyakarta is known as the 'student city' of Indonesia with 3 state universities and about 60 private institutes/universities. The largest and oldest university (also at national level) is the Universitas Gadjah Mada (UGM), which was established in 1949. The DSH was built in 1982 and served as the university teaching hospital of the UGM. Currently the DSH consists of 24 departments, including the Department of Pediatrics, with 2,579 medical and non-medical staff. In 1992 the Yogyakarta Pediatric Oncology and Hematology Centre (YPOHC) of DSH established international collaboration with the VU University Medical Center (VUmc; Amsterdam, the Netherlands), pioneered by Prof. AJP Veerman,[52] which has generated a series of PhD students who graduated either in the VUmc or in UGM. The national Indonesian treatment protocols of childhood ALL and AML were also developed thanks to this collaboration. In 2001 the YPOHC also established collaboration with Saskatchewan University (Saskatoon, Canada) in the field of computerized childhood cancer registry. These twinning programs have resulted in many international publications. In 2010 the Estella Foundation (the Netherlands) sponsored a ward in DSH with 37 beds dedicated to children with cancer. Also, the Dutch Koningin Wilhelmina Fonds (KWF *kankerbestrijding*) supported our PCU in DSH to develop our research activities and improve our human resources. Since 1992 the Estella Foundation has provided a considerable proportion of our medicines and/or chemotherapies. Currently, the YPOHC of DSH consists of two professors (one emeritus) and three pediatric consultants of hematology-oncology to serve the 40 - 45 pediatric hematology-oncology patients in the ward and the 10 - 15 outpatients for daily works. Two professors (Prof. AJP Veerman from VUmc, Amsterdam and Prof. Kaiser Ali from Saskatchewan University, Saskatoon, Canada) visit our PCU each year. During the last 3 years a teleconference via internet connection has been conducted weekly between the VUmc Amsterdam and the PCU of DSH to discuss our complicated cases.

Most patients in the PCU of DSH have a low socio-economic status.[40] At the start of implementation of the first Indonesian childhood

ALL treatment protocol, the costs were mainly paid by the parents or families themselves. Since 2004 treatment costs are partly covered by the government *Jamkesmas* (medical insurance) program that is intended for poor people.[53] This insurance covers some of the costs of some medicines, chemotherapy and hospitalization (in the 'economy class' section only). Unfortunately, because this insurance coverage is far too low to cover the treatment of a catastrophic disease such as cancer, parents still have to pay for some of the laboratory tests and some of the medicines (as well as the other costs for daily living and transportation) from their own income, savings or loans.

Achievements and obstacles in Dr. Sardjito Hospital

In the 1970s, before developing an Indonesian childhood ALL treatment protocol, the protocol used in the UGM teaching hospital was adopted from the Dutch childhood ALL protocol, but the data on patient outcome were never published. In 1990, on the initiative of one of Prof. AJP Veerman's Dutch parents, a new protocol was developed for Yogyakarta: the COM-ALL 92 protocol. This was based on the Dutch Childhood Leukemia Study Group protocol ALL-VI, with elements of the Berlin-Frankfurt-Munster protocols for high-risk patients. The first national Indonesian childhood ALL, the WK-ALL-2000, was inspired by the success of the Dutch ALL-VI which used a 3-drug induction and dexamethasone instead of prednisone or prednisolone as steroid.[26] This protocol was generated during a workshop held in Yogyakarta and was attended by representatives from PCUs in Indonesia as well as by experts in childhood ALL from Europe (Prof. AJP Veerman and Prof. G Henze) and from the USA (Prof. J Nachman).

At the beginning of implementation of the Indonesian childhood ALL protocol in DSH (1997 - 2002), of the 164 newly-diagnosed patients 35% refused or abandoned treatment, 23% experienced toxic death, 22% relapsed, and 20% were alive at a median of 3 years post diagnosis (Figure 3).[40] Treatment refusal and abandonment were more common in poor patients than in prosperous patients (47% vs. 2%). In DSH, treatment refusal or abandonment were mostly related to financial problems in the family, as well as to other factors including their perceptions regarding the curability of leukemia, experienced side-effects of treatment, transportation to and from hospital, and trust in the healthcare providers in DSH.[40,54-56]

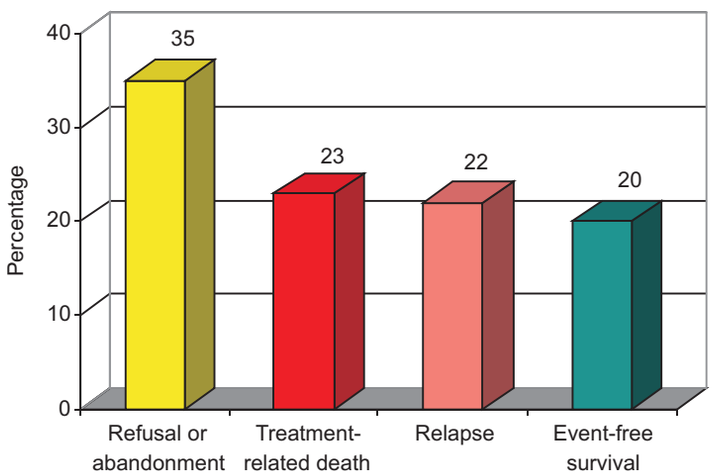

FIGURE 3. **Treatment outcome in childhood ALL in the Pediatric Cancer Unit of Dr. Sardjito Hospital, Yogyakarta (1997 - 2002).**

To improve treatment outcome in DSH several measures were implemented, including the provision of a medication diary for parents, a structured parental education program, and improved access to donated chemotherapy. The medication diary was used to help parents/families with the complicated schedule for oral chemotherapy and to attend hospital in time for scheduled appointments or admissions. In children whose mother had an educational level of senior high school or higher, we found that the 3-year event-free survival was significantly higher in patients whose parents received the medication diary than in those who did not receive it (62% vs. 29%, P=0.04) (Sitaresmi et al., manuscript submitted). After introduction of the parental education program, especially among poor families treatment refusal decreased from 14% of 120 patients to 2% of 96 patients (P=0.001), while event-free survival showed a significant increase (P=0.004).[57]

Aim of the studies

To investigate the low survival rate, as well as the high rate of treatment abandonment and toxic death associated with the Indonesian childhood ALL protocols, we conducted studies aimed to improve diagnosis and treatment outcome.

The following objectives were addressed:

1. To establish the clinical and laboratory characteristics of patients in the PCU of Dr. Sardjito Hospital.
2. To evaluate the efficacy of the Indonesian childhood ALL protocols, i.e. the WK-ALL-2000 protocol and the Indonesia-ALL-2006 protocol.
3. To study randomized interventions for improving outcome suitable for the setting of Indonesia: these interventions concerned the use of antibiotic prophylaxis, the dosage of L-asparaginase (in the WK-ALL-2000 protocol), and the efficacy of dexamethasone versus prednisone (in the Indonesia-ALL- 2006 protocol).

Outline of the thesis

Chapter 1 **General introduction.** This chapter presents a brief overview of childhood ALL with regard to diagnosis, treatment and outcome. The PCU of the DSH is also briefly introduced.

Chapter 2 **Incidence of childhood leukemia in Yogyakarta, Indonesia, 1998 - 2009.** This study was conducted to provide epidemiological data on the incidence rate of childhood acute leukemia in the DSH catchment area, that was previously was not available. The trend in incidence rates by year over the past ten years, and the proportion of ALL to AML, are discussed. These data are also compared with international data.

Chapter 3 **Immunophenotypic patterns of childhood acute leukemias in Indonesia.** This study evaluates the diagnosis of childhood acute leukemia after introduction of the immunophenotype examination in 2006. The level of agreement in the diagnosis of acute leukemia between this method and that of the simpler morphological examination is also compared.

Chapter 4 **Apoptotic cell identification: an *in vivo* study during induction treatment of childhood acute lymphoblastic leukemia.** The study was performed to evaluate the profile of apoptosis as representing response to treatment during the induction phase.

Chapter 5 **Early response to dexamethasone as prognostic factor: results from the Indonesian Childhood WK-ALL Protocol in Yogyakarta.** This study was designed to classify patients based on their early response to treatment. The prognostic value between the group classifications is analyzed.

Chapter 6 **Randomized double-blind trial of ciprofloxacin as prophylaxis for clinical sepsis and mortality during the induction treatment in childhood acute lymphoblastic leukemia.** This study was performed with the aim to improve treatment outcome in the Indonesian protocol. The role of oral ciprofloxacin as prophylaxis against sepsis and toxic death during the induction phase is compared with the placebo arm.

Chapter 7 **L-asparaginase: Long-term results of a randomized trial on the effects of an additional three doses during consolidation in the Indonesian WK-ALL-2000 Protocol.** This study was designed to evaluate whether treatment intensification using additional doses of L-asparaginase improves treatment outcome in the Indonesian protocol.

Chapter 8 **Dexamethasone versus prednisone in childhood acute lymphoblastic leukemia treatment, results of the Indonesia-ALL-2006 Protocol: a randomized trial of standard-risk patients.** This trial was performed to compare the outcome of dexamethasone versus prednisone in a dexamethasone-based Indonesian protocol.

Chapter 9 **General discussion.** The results of these studies are discussed in order to evaluate the efficacy and/or adverse outcomes during implementation of the Indonesian protocols.

Chapter 10 **Summary, conclusions, recommendations and perspectives.** Based on the studies described in this thesis, we discuss the most important implications and present an overview of the rationale for the next Indonesian protocol.

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