Prognostic Factors of the Five-year Overall Survival from Childhood Acute Lymphoblastic Leukemia: A Medical Record-based Retrospective Study

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Tatev Arakelyan, BSc, MPH (c)

Advising Team:

Arin Balalian, MD, MPH, DrPH (c)
Vahe Khachadourian, MD, MPH, PhD

Gerald and Patricia Turpanjian School of Public Health
American University of Armenia
Yerevan, Armenia
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Abbreviations

AIC	Akaike information criteria
AL	Acute leukemia
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AUA	American University of Armenia
BFM	Berlin – Frankfurt – Münster
BM	Berlin – Moscow
BMI	Body mass index
CI	Confidence interval
CNS	Central nervous system
CR	Complete remission
CVF	Central venous fluid
DALY	Disability-adjusted life-years
Hb	Hemoglobin
HC	Hematology Center
HR	Hazard ratio
IRB	Institutional review board
KM	Kaplan-Meier
LDH	Lactate dehydrogenase
MHC	Muratsan Hospital Complex
MLL	Mixed lineage leukemia
NA	Not available

NCD	Non-communicable diseases
PC	Pediatric cancer
Ph	Philadelphia
PH	Proportional hazard
PLT	Platelets
SD	Standard deviation
SDG	Sustainable Development Goals
SDI	Sociodemographic index
US	United States
WBC	White blood cells
WHO	World Health Organization

Abstract

Introduction

Acute lymphoblastic leukemia (ALL) is the most common cancer among children worldwide. Despite being a rapidly progressive malignancy, the survival from ALL has significantly improved in the recent decades from 10% in the 1960s to >90% in 2012 in resource-rich countries. However, despite major improvements in survival from ALL, many children still may have poor outcomes or even fail to survive from the disease. The prediction of the outcome depends on various prognostic factors, identifying which would help to gain insight into strategies to optimize the available treatment modalities and to improve the understanding of disease progression and the outcome. To improve the disease outcome prediction, we aimed to assess the five-year overall survival rate in the Armenian population and to identify the demographic, cytogenetic, and clinical prognostic factors of five-year overall survival for ALL among children in Armenia.

Methods

We conducted a retrospective review of hospital inpatient and outpatient records of children aged 0-19 diagnosed with ALL from January 2010 – December 2014 in Armenia. The data was extracted from the records of two hospitals, namely Hematology Center after Prof. R.H. Yeolyan and the Muratsan Hospital Complex, covering the whole Armenian population during the study period. Kaplan-Meier analysis was utilized to assess the five-year overall survival rate in the population. Time-to-event analysis was conducted using Cox proportional hazard regression analysis to identify predictors of the overall survival. The log-rank test was utilized to assess the significance of the difference between the groups of the independent prognostic factors.

Results

Overall, 112 ALL patients were identified during the study period. The average age at diagnosis was 6.4 years (SD = 4.8), and the male:female ratio was 1.4:1. In total, 16 patients (14%) died during the study period. The five-year overall survival rate was 82%, with a median follow-up time of 5.5 years. Our study showed the delay in diagnosis for ≥30 days was an independent predictor of the overall survival (HR=3.2, 95% CI=1.02;10.13; $p<0.05$) when adjusted for gender, white blood cell count at diagnosis, and splenomegaly at diagnosis.

Conclusion

Our study confirm the delay in diagnosis is an independent predictor of survival. This finding designates the need for more research on determinants of patient- and physician-related delays in addition to introducing raising awareness campaigns among patients, primary health care providers, and community health workers in the rural areas to increasing awareness among the population to recognize the warning signs and symptoms of the disease. More methodologically rigorous research is needed to identify other principal prognostic factors of survival from ALL.

Introduction

Pediatric cancer

Pediatric Cancer (PC) is a prominent cause of death among children worldwide.[1] PC stands for malignant and benign tumors among individuals aged 0 - 19 years old.[2] PC is rare and comprises less than 1% of all cancers among all age groups.[3] The World Health Organization (WHO) reported that every year about 300,000 children get diagnosed with cancer.[4] A study conducted by the International Assessment of Cancer Registries showed that the global incidence of PC has been increasing since the 1980s.[5] The reassessment of the worldwide incidence patterns showed an incidence of 155.8 per one million person-year between 2001-2010.[6] Although, the incidence of PC is similar in countries with high and low sociodemographic index (SDI); countries with a high SDI account for only 18% of the total disability-adjusted life years (DALY) attributable to PC, while 60% of DALYs attributable to PC are from countries with low-middle SDI.[7]

Every year more than 80,000 children die because of cancer.[8] With the introduction of risk-adapted treatment and advancement in supportive care, there is a dramatic improvement in PC treatment outcomes. The overall survival rates in higher-income countries reached 84% in 2019 from 58% in the 1970s. On the contrary, the survival rate may be as low as 10% in low-resource settings.[9,10] The low survival rates from PC in the low- and middle-income countries are attributable to various factors, such as late diagnosis, financial difficulties, and low adherence to the treatment.[1,11]

The WHO introduced a global initiative to increase the overall survival rate for pediatric cancer to at least 60% by 2030.[12] Besides, one of the aims of Sustainable

Development Goals (SDG) 3.4 is to reduce premature deaths from non-communicable diseases (NCD) by 2030, where cancer is the second in the list of most common NCDs.[13] With these efforts, it is projected to prevent about one million deaths from cancer among children in the upcoming decade.[12] Thus, efforts are needed to reduce disparities in childhood cancer survival amongst high-, low- and middle-income countries.[13,14]

Childhood ALL

ALL is the most common form of malignancy among children comprising over one-fourth of all childhood cancers.[15,16,17] Between 2001 – 2010, the global age-standardized incidence of ALL was 46.4 per one million population aged 0 - 14 years.[6] The global prevalence of childhood ALL was 875,500 in 2015.[7] The peak incidence is between ages 2 - 5, and the disease is more prevalent among male population.[18,19]

Multiple risk factors contribute to the development of ALL, including genetic, environmental, sociodemographic, and parental factors.[20] ALL develops when the mutations in the lymphoblasts enable the cellular differentiation into lymphocytes and cause abnormal proliferation of the immature leukocytes, also called as blasts, which results in cytopenia.[21] The rapid accumulation of blasts in the bone marrow and other hematopoietic organs, such as liver and spleen, suppresses the normal development of the white blood cells (WBC), erythrocytes, and platelets (PLT).[21,22,23] The ALL arises in the T or B lymphocytes and is be classified into T-cell ALL or B-cell ALL as per WHO classification.[22] The clinical features of T and B immunophenotypes have major overlaps, including depression of marrow function resulting in clinical presentation with fatigue due to anemia, bruising, and petechia due to thrombocytopenia, fever due to neutropenia, and organ infiltration signs

including bone pain, lymphadenopathy, hepatosplenomegaly. Besides, the cancer cells can also spread into the CNS, testicles, eyes, gastrointestinal tract and kidneys.[18,23]

The treatment plan for ALL patients is based on the internationally accepted guidelines and is depends on the clinical subtype, risk group, and may also depend on the genetic characteristics of ALL. The treatment includes mainly chemotherapy but can also involve radiotherapy, blood product support, and targeted therapy. CNS-targeted therapy is also utilized to achieve higher survival rates.[23–25] The following are the phases of ALL treatment: induction, consolidation/intensification, and maintenance phases. The induction phase aims to rapidly kill the majority of cancer cells to achieve complete remission (CR). The consolidation phase aims to eliminate or reduce the remaining burden of the disease to the possible lowest level by providing high doses of multidrug chemotherapy. The maintenance phase aims to reduce and maintain the low or total absence of the leukemic cell in the peripheric blood and bone marrow.[26]

Despite being a rapidly progressive malignancy, the survival from ALL has significantly improved over the last decades from 10% in the 1960s to 90% and above in 2012 in resource-rich countries as reported in the study conducted by the Children Oncology Group.[27,28,29] The improvement of the survival is attributed to the provision of modified and risk-adapted therapy in addition to the central nervous system (CNS) directed therapy, and better supportive care to children.[28]

Prognostic factors of ALL

Despite major advances in the survival of children with ALL, many children fail to survive or have poor outcomes.[30] The disease outcome depends on various predictors of survival, called prognostic factors, which are defined as factors,

measures, or characteristics of the patients or the disease that can be associated with the outcome of the disease. These can be used in clinical practice to estimate the chance of survival from the disease when patients are provided with standard or no treatment.[31] Knowledge of prognostic factors of survival would help to gain insight into the strategies to optimize the available treatment modalities and to improve the understanding of disease progression and the outcome. Moreover, the identification of prognostic factors of survival will provide information to patients and families about the chances of recovery from the disease. Thus, to enhance the current understanding of disease development and improve the prediction of the outcome, it is important to investigate the independent prognostic factors and their significance in relation to overall survival.

The following prognostic factors associated with overall survival from ALL were derived through the literature review:

1. **Age at diagnosis:** Age is one of the prominent factors associated with survival from ALL.[32] Age between 1 - 9 years was shown to be associated with better prognosis, as shown in the study conducted in St. Jude Children's Cancer Research Hospital.[26] Contrary, infants and patients aged >9 years were shown to have worse outcomes.[33,34] The reason behind this difference in age groups is the biological features of lymphoblastic cells for patients within the age group of 1-9 as it was also shown to be associated with positive prognostic factors hyperdiploidy and TEL/AML1 gene rearrangement.[32]
2. **Sex:** Cancer survival rates differ among males and females for different types of cancers, including ALL. Males were shown to have more inferior survival when compared to females.[35] This gender association with survival was consistently shown in the literature.[34,36,37]

3. **Body Mass Index (BMI) at diagnosis:** Child's BMI at presentation is also considered as a prognostic factor for the survival of ALL. The results of the meta-analysis of 11 articles revealed that a higher BMI of the child was associated with a higher risk of mortality.[38] Nonetheless, the significance of this factor is not consistent in the literature.[26,39,40]
4. **Clinical subtype/Immunophenotype:** The two immunophenotypes of ALL (T-cell and B-cell) differ in their biology, cytological features, treatment response, and, finally, outcomes. Patients with T-cell ALL have an inferior prognosis compared to patients with type B-cell ALL.[41]
5. **Philadelphia (Ph) chromosome positivity:** The translocation of t(12;21)(p13;q22), named as Philadelphia chromosome, is a prominent cytogenetic abnormality among ALL patients associated with inferior survival from the disease.[41–44]
6. **Down syndrome**: ALL patients with trisomy of 21st chromosome, also known as Down syndrome, have a worse prognosis of survival from ALL.[45,46] The poor prognosis is because of the treatment-related toxicities and complications which are more common among these patients due to differing tumor biology.[47]
7. **TEL/AML1 rearrangement:** The TEL/AML1 fusion gene is a result of t(12;21)(p12;q22) translocation and its presence is an indicator of favorable prognosis, though there are divergent results on this predictor in the literature.[48,49]
8. **Mixed lineage leukemia (MLL) rearrangement:** MLL rearrangement on the chromosome band 11q23 indicates about the abnormalities in normal gene

transcription and chromatin folding, which was shown to be a significant predictor of mortality from ALL.[48,50,51]

9. **Chromosomal count:** Children with WBCs harboring hyperdiploidy, i.e., chromosomal count of >51 (normal=46) have a better prognosis in contrast to hypodiploidy (chromosome count of <46) which confers a poor outcome.[52]
10. **Hepatomegaly and splenomegaly:** Enlargement of the liver and spleen are associated with higher tumor burden and, thus, when markedly enlarged, are associated with poor prognosis for ALL patients.[20]
11. **Mediastinal mass involvement:** The presence of mediastinal mass at presentation is another factor associated with worse prognosis, though the evidence for it is controversial in the literature.[53–55]
12. **Testicular involvement at presentation:** Testicular infiltration, indicating disease dissemination, designates a poor prognosis for male patients with ALL.[56,57]
13. **CNS involvement at diagnosis:** CNS involvement at presentation, i.e., detection of blasts in the cerebrovascular fluid (CVF) is also an indicator of poor prognosis for children with ALL.[58,59,60]
14. **WBC count in the blood serum at diagnosis:** With the increasing WBC count at diagnosis the prognosis worsens.[61,62]
15. **Hemoglobin (Hb) level at diagnosis:** Children with lower Hb level at diagnosis generally have a more favorable prognosis compared to those with higher Hb level.[63]
16. **PLT count at diagnosis:** The prognosis of survival from ALL improves with the increasing PLT count at diagnosis.[64]

17. **Serum lactate dehydrogenase (LDH) level at diagnosis:** LDH plays a key role in cancer metabolism by converting pyruvate to lactate and vice versa. It is expressed in the serum during tissue damage and therefore is a biomarker for tissue damage in the body.[65,66] High expression of LDH, its association with poor prognosis for ALL, and its significance is controversial in the literature.[66–68]
18. **Delay in diagnosis:** The prolonged time-lag between the manifestation of first signs and symptoms and the diagnosis may negatively contribute to having lower rates of survival as the late referral to professional delays the initiation of the required treatment.[69] A worse prognosis was seen among patient having delay for ≥30 days.[70,71]
19. **Delay in treatment initiation:** Delaying treatment of the disease due to toxicities, infection or other may be associated with poor outcome, though this factor is poorly investigated in the literature.[72,73]
20. **Complete remission (CR) at the end of the induction phase of the treatment:** CR is defined as achieved in case of absence of blast in blood and CVF, and blast count of <5% in bone marrow analysis. Failure to reach CR at the end of the induction phase is a predictor of survival and is considered one of the most important ones.[25,26]

The situation in Armenia

According to the Armenian National Institute of Health (NIH), 77 new cases were identified among children aged 0 – 14, while 12 new cases were reported for the children aged 15-17 years in 2019. This translates into an incidence of 12.8 and 11.8 per 100,000 population among ages 0-14 and 15-17 accordingly.[74]

There were two hospitals providing care to children with hematological disorders in Armenia between 2010 and 2014, namely Hematology Center (HC) after Professor R.H. Yeolyan[75] and Muratsan Hospital Complex (MHC) of the Yerevan State Medical University.[76] After 2018, HC became the sole provider of cancer care as a result of the reunion of the two centers.[77] Within the study period, the treatment of ALL in the two Armenian institutions was based on the internationally accepted guidelines, mainly Berlin – Frankfurt - Münster (BFM), Moscow – Berlin (MB).[25,78]

To the best of knowledge, to date, there was one abstract published reporting 72% and 100% five-year overall survival rate for ALL among 3-7 year old and >7 years old respectively among children treated in HC only.[79]

The rationale of the study

An accurate estimation of the prognosis of survival requires more than a clinical experience of the physician.[80] Despite evidence from the literature on the average prognosis, each patient needs an individual approach.[81] Prognostic research helps to tackle differences between the patients and helps to make informed decisions about the future health of the patient.[80,82] Precise survival rate estimates, identification of prognostic factors are important to improve the understanding about the natural history of the disease, the characteristics of the study population to adjust treatment strategies accordingly, to assess the level of aggressiveness of cancer, for making decisions about the treatment plan, and also for clinical trial enrollment.[80]

To the best of our knowledge, prognostic factors of ALL have not been evaluated in the Armenian population. The disease was chosen as it is the predominant malignancy in children.[15,24] Considering the importance of knowing

and applying the prognostic factors and survival estimates in practice, this study aimed to assess these predictors of survival from ALL among children in Armenia.

Study objectives

The objectives of this study were:

- To assess the five-year overall survival from the childhood ALL among children in Armenia from January 2010 - December 2014
- To identify the prognostic value of demographic, clinical, and laboratory characteristics for childhood ALL survival in Armenia

Methods

Study design

For this medical record-based retrospective study, data extraction was conducted from the inpatient and outpatient records of the HC and MHC for all patients diagnosed with ALL between January 2010 and December 2014.

Study population

The target population included all patients aged 0-19 primary diagnosed with ALL between January 2010 and December 2014 in Armenia. Within the study period, all the patients received cancer care in the HC and MHC. Since the exact number of patients with ALL for the selected time frame was not known in advance, all patients were included retrospectively within the five-year period.

Study variables

Outcome Variable

The outcome variable was the five-year overall survival measured by the proportion of individuals surviving for five years after being diagnosed with ALL. The second outcome was the risk of death estimated using Cox proportional hazard (PH)

analysis. The time variable was measured from the date of diagnosis untill the last day of follow-up or death from any cause (in years). Censoring was established based on the corresponding date of the last contact with the provider, as recorded in the hospital records. The follow-up timeframe was chosen as the best and most commonly used clinical estimate in cancer survival research.[83]

Predictor Variables

Information on the following demographic, clinical, and laboratory characteristics of the patients and the disease was collected from their inpatient and outpatient records: date of birth of the patient (discrete), gender (dichotomous - male/female), region (categorical - Yerevan/marz/other), residency (dichotomous - urban/rural), hospital (dichotomous - Hematology Center/Muratsan Hospital Complex), date of admission (discrete), date of diagnosis (discrete), date of onset of first symptoms (discrete), date of treatment initiation (discrete), vital status (dichotomous - dead/censored), date of last contact (discrete), weight at diagnosis (continuous), height at diagnosis (continuous), immunophenotype (categorical - T-cell/B-cell/ALL - not otherwise described/other), Down syndrome (dichotomous - positive/negative), Philadelphia chromosome (categorical - positive/negative/not available (NA)), hyperdiploidy (categorical - positive/negative/NA), hypodiploidy (categorical - positive/negative/NA), TEL/AML1 (categorical - positive/negative/NA), MLL rearrangement (categorical - positive/negative/NA), hepatomegaly at diagnosis (categorical - positive/negative/NA), splenomegaly at diagnosis (categorical - positive/negative/NA), mediastinal mass at diagnosis (categorical - positive/negative/NA), testicular involvement at diagnosis (males only) (categorical - positive/negative/NA), CNS involvement at diagnosis (categorical - positive/negative/NA), WBC count at diagnosis (continuous), Hb count at diagnosis

(continuous), PLT count at diagnosis (continuous), LDH level at diagnosis (continuous), treatment (categorical - BFM/MB/other), response to the treatment (dichotomous - CR+/CR-).

Data collection

Three data collectors reviewed the hospital inpatient and outpatient records and hospital's registry database of the patients to collect relevant information. All the predictor variables were extracted into the developed chart abstraction form (Appendix 1). The designed form was pre-tested on two medical records before passing to the data collection stage.

Because of missing information on some patients' survival status at five years in the records (54%), additional data was requested from the hospital registry. However, there were still 30% of patients lost-to-follow-up due to the inability to reach the contact person because of the inaccurate contact details or absence of the contact person from the country.

Data management

The data entry was conducted using IBM SPSS version 23. All the electronic documents were kept in an encrypted computer, and the papers were kept in a locked drawer where only the study investigators had access. All the data collection paper forms were destroyed at the termination of the study. Data cleaning and analysis were conducted afterward using STATA/SE 13.0 statistical software. We conducted a single data entry following a range checking of 20% of the randomly selected observations. We conducted sorting, frequency testing, and graphical illustrations for detecting, deleting, editing the wrongly entered, or missing values in the database.

Statistical analysis

Descriptive analysis

We carried out descriptive analysis summarizing categorical variables by frequencies and percentages, and continuous variables by their median, mean, and standard deviations (SD). Survival function was estimated to describe the five-year overall survival via Kaplan-Meier (KM) estimated.

Time-to-event analysis

Time-to-event analysis was conducted using Cox PH regression analysis to assess the predictors of overall survival. Variables having 20% or more missing values were dropped from the analysis.

Univariable Cox PH analysis was conducted where the hazard ratios (HR), 95% confidence intervals (CI), and p values were calculated for every prognostic factor to present the significance of the association between the predictor and the outcome. Variables were selected through the stepwise forward selection technique to fit the multivariable Cox PH model after checking for PH assumptions in the univariable models. The variable inclusion and exclusion criteria were set at $p<0.25$ and $p>0.5$, respectively. The continuous variables underwent log transformation prior to the inclusion in the final model in order to reduce the effect of extreme values. The model having the least Akaike Information Criteria (AIC) and where all variables met the PH assumption was selected.

KM analysis was utilized to graphically illustrate the significant associations between independent predictors and the outcome, and the Log-rank test was utilized to assess the significance of the difference between the groups of the independent prognostic factors.

Checking for Cox PH assumptions

To check for Cox PH assumption that hazard function is time-independent, we performed the Global Schoenfeld test under the null hypothesis of "no difference" between the curves.[84] Test results with the significance level of $p>0.05$ indicated no violation of the assumption. Also, the log-log plots were illustrated for graphically checking for this assumption.

Logistical considerations

For conducting this study, the available sources were the database of medical records provided by the Pediatric Cancer and Blood Disorders Center of Armenia, which included the data of patients from HC and MHC. Other available resources are the electronic databases provided by the library of the American University of Armenia (AUA).

Ethical considerations

The study protocol was reviewed and approved by the Institutional Review Board (IRB) of the AUA. Each patient/hospital had an assigned ID in a separate form, which was kept in the encrypted computer. No individually identifiable information was gathered from the records.

Results

Baseline descriptive characteristics of the cohort

Overall, 112 patients aged 0-19 were diagnosed with ALL during the five-year interval from January 2010 to December 2014. Table 1 presents the baseline characteristics of the cohort. The average age at diagnosis of ALL was 6.4 years (SD = 4.8), and the male:female ratio was 1.4:1. More than half of the patients were from the marzes of Armenia (58%), only 4% (n = 4) were from the Artsakh Republic, and urban areas accounted for 60%. The specific clinical subtypes of the disease

were available for 44% of patients, out of which 40% (n = 45) had B-ALL, and 4% (n = 5) had T-ALL. About 4% (n = 4) of patients presented with Down syndrome. Out of all, two patients presented with the CNS involvement, and five patients had mediastinal mass involvement at diagnosis. No male patient had testicular involvement at presentation. Half of the patients (50%) had a time-lag of more than 30 days between the onset of symptoms and diagnosis. Though, only 3% (n = 3) of the patients had a delay in treatment initiation after the diagnosis of >1 day.
The mean WBC count at diagnosis was 48.44×10^9/L (SD = 100.3). The mean Hb count at diagnosis was 83.4 g/dL (SD = 27.2), and the mean PLT count at diagnosis was 81.1×10^3/μL (SD = 74.3). Mean value for LDH at presentation was 939.4 U/L (SD = 606.6). The average BMI of the child at presentation was 16.4 kg/m^2 (SD = 3.5).

Overall, the cytogenetic analysis was available for 73 patients. Among those, 18 patients were tested positive for hyperdiploidy, four had positive TEL/AML1 rearrangement, and three patients had MLL rearrangement. No patient presented with hypodiploidy.

The majority of the patients were admitted to Hematology Center after Prof. R.H. Yeolyan (83%) and 91% (n = 90) of the patients received treatment in Armenia, while 6% (n = 6) moved abroad for getting the treatment and only 4% (n = 5) refused to receive chemotherapy as prescribed by the physician. Both of the centers adapted BFM and MB guidelines, and the majority of the patients (63%) were treated according to the BFM guideline. Almost all of the patients (99%) reached CR at the end of the induction phase of the treatment.

Overall, 16 patients (14%) died during the study period. The five-year overall survival rate was 82% (Figure 1). The median follow-up time was 5.5 years. Table 2

presents the demographic, clinical, and laboratory features stratified by the disease outcome.

Univariable Cox proportional hazard regression analysis

Table 3 presents results for unadjusted predictors of five-year overall survival for the ALL from the univariable Cox PH regression analysis. We excluded 13 (52%) variables due to having more than 20% missing observations.

Delay in diagnosis for ≥30 days was a significant predictor associated with inferior survival (HR=3.23; CI: 1.02-10.20; $p<0.05$) in the univariable Cox PH regression analysis (Table 3).

We did not observe any significant association between five-year overall survival and age at diagnosis, gender, region, residency, BMI at diagnosis, WBC count at diagnosis, Hb level at diagnosis, PLT count at diagnosis, LDH count at diagnosis, blast count in bone marrow at diagnosis, having splenomegaly at diagnosis, type of treatment protocol.

Multivariable Cox proportional hazard regression analysis

Table 4 presents the results from the multivariable Cox PH regression analysis. Besides the significant factor of delay in diagnosis, the following variables were selected to be added in the multivariable models based on their clinical relevance: gender, age at diagnosis, WBC count at diagnosis, PLT count at diagnosis, and splenomegaly. The final model included gender, WBC count at diagnosis, delay in diagnosis, and splenomegaly at diagnosis (Table 4). The step-by-step results of the model development are presented in Appendix 2.

We did not observe an association between the gender and overall survival (HR=0.58; 95% CI=0.18;1.84) when adjusted for WBC count at diagnosis, delay in diagnosis, and splenomegaly.

Every 1000 µL increase in the log WBC count at diagnosis was associated with a 37% increase in the hazard of death (HR=1.37; 95% CI=0.59;3.15; p=0.46) when adjusted for gender, delay in diagnosis, splenomegaly at diagnosis.

Model evaluation

We checked the model for the proportionality assumption by using the Schoenfeld residuals (Appendix 2). All the variables in the final model met the assumption as also checked with log-log plots, except splenomegaly. Thus, the model was stratified by splenomegaly.

Discussion

This medical record-based retrospective study explored the predictors of five-year overall survival from childhood ALL among the Armenian population.

Based on the five-year data, the observed five-year overall survival was 82% without a significant difference in two hospitals. The variables delay in diagnosis for ≥30 days was predictive of lower survival when adjusted for gender, WBC count at diagnosis, and having splenomegaly at diagnosis.

The survival rate in our cohort was comparable with the research conducted by Lee et al. in Korea among 295 patients reporting 82% overall survival rate for the cohort.[85] A study conducted by Dujua and Hermandez among Filipino children followed from 2005 - 2009 demonstrated an 86% survival rate.[86] The survival rate in our cohort was comparable with a population-based Australian study conducted by Baade et al. where the estimated five-year survival rate for ALL patients was 85% between 1997 - 2006.[87]

However, our survival rate was higher compared to a study conducted by Lustosa de Sousa in Fortaleza, Brazil, among 76 ALL patients, they reported a lower

survival rate compared to ours (72% vs. 82%).[17] A study conducted by Almasi-Hashiani et al. among 173 ALL patients in Iran followed-up for five years estimated a considerably lower survival rate of 57%.[88] Also, another study conducted in Saudi Arabia among 149 patients reported lower survival rate compared to our results (73% vs. 82%).[89] The higher survival rate in our population may be associated with the improved provision of medications, increased financial support,[90] and provision of risk-adapter therapies based on international protocols.[77] Despite these results, our finding on the five-year survival rate is still comparably lower when compared to higher-income countries like the US and Germany.[29,91,92]

The survival rate assessed in our study was higher when compared with the previous study conducted in Armenia (82% vs. 72%). A possible reason for this difference could be the fact that the 72% survival rate was reported for the age group of 3-7 years only. Besides, the records were extracted from one hospital, namely Hematology Center after Prof. R.H. Yeolyan. There was also a higher possibility of selection bias due to the loss to follow-ups.

We observed that almost half of the patients presented later than 30 days after the manifestation of symptoms, hence the prognosis was significantly poor among those with delays. Our findings regarding the delay in diagnosis were similar to the findings of a study conducted in Nicaragua (50% vs. 46%).[93] An Italian study by Flores et al. reported that only 20% of patients delayed diagnosis.[94] An earlier study from Northern India identified a significantly higher value for mean days of late presentation at diagnosis among deaths when compared to cures.[95]

There is a scarcity of literature investigating the delay in diagnosis as an independent predictor of survival. Our findings confirm that the delay in diagnosis is an independent predictor of survival and designate the need to improve timely

access to specialists, which could be achieved by introducing raising awareness campaigns among primary health care providers, the parents,[96] and community health workers[97] in marzes of Armenia to be mindful of the early signs and symptoms of the disease. More research is required in the scope of patient-related and physician-related determinants of delay in diagnosis among children with ALL and its relation to the outcome. The higher delay rate may be associated with lower timely access because of the long distance to the hospital.[70,98] A retrospective study of 4,940 children conducted in Mexico showed that geographic distribution and residency could influence the delay in diagnosis.[99] Almost 60% of our cohort was from marzes of Armenia, while the only two centers providing specialized care for ALL were located in the capital of Armenia, Yerevan. Additionally, the socioeconomic characteristics, such as the educational level of the parents, place of residency should also be considered in future studies.[96]

Though age was a significant predictor in the univariable analysis, its significance attenuated in the multivariable model. The age distribution of the study participants was similar to that reported in a Brazilian study,[17] yet we did not observe significance for this variable. However, age was a historically known predictor of outcome for ALL in various studies.[17,62,100,101] A possible reason for this discrepancy in the results could be the limited power of our study to detect this predictor due to the low sample size. This suggest methodological improvements in further studies to be conducted in Armenia.

Higher WBC count at presentation is associated with an inferior outcome of the disease, as reported in the study conducted by Lustosa de Sousa et al., Al-Balwi et al., and other international studies.[17,64,89] This predictor is also one of the traditional prognostic factors, and our findings are in line with the literature showing

increasing hazard with increasing WBC count. However, the 95% CI indicated lower precision (0.59 – 3.15), perhaps due to the small sample size of the study.

Although many studies showed that the male gender is predictive of poor survival,[101–105] we did not find such association in our study. Despite the fact that we added gender to the multivariable models due to its clinical relevance, we did not observe reliable effect ranges to conclude whether it is protective or hazardous for survival. However, we detected male predominance in our study population and also predominance in the number of deaths, which is consistent with the international literature.[101,106] This gender variation in death frequencies from ALL are not yet well understood and cannot be fully explained by testicular involvement, as it is extremely rare, and we did not observe any patient with testicular involvement in our cohort. Differences may be due to varying age at presentation. However, more robust research is needed to evaluate its prognostic relevance.

There were several limitations present in our study. First of all, as the data was gathered from hospital records retrospectively, it presented with deficiencies and inconsistencies. For example, the paucity of some clinical and cytogenetic data of the patients restricted our study to a limited number of predictors to analyze. During the study period, the country had limited resources to be able to provide unlimited cytogenetic evaluations for every patient. Thus, we failed to assess cytogenetic predictors such as Ph+, Down syndrome positivity, TEL/AML1 rearrangement, MLL rearrangement, and high or low chromosomal count for prediction of the outcome. Yet these predictors were shown to have a crucial impact on survival among ALL patients.[36,42–47,49,51,52,107,108] The lack of complete follow-ups of the cohort may under or overestimate the rates of survival. Besides, we had limited information about the sociodemographic characteristics of the patients, which

may also contribute to poor prognosis. Due to limited sample, several variables were dropped from the analysis because of low or no variability within the groups of the variables. Finally, our study was underpowered (49%) to detect 0.1 point difference in survival rates as assessed with the log-rank test for two survival curves[109] given a = 0.05 and n = 112 (Appendix 3).

Conclusion

To summarize, this study showed an overall survival rate of 82% for ALL in Armenia and that delay in diagnosis was an independent predictor of survival for children with ALL when adjusted for gender, WBC count at diagnosis, and splenomegaly. The knowledge from this study can be a baseline to evaluate future progress and highlight the need for more research with a larger sample and longer follow-ups to understand differences in characteristics, gain insight into barriers to optimize survival, and improve the outcomes.

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Tables

Table 1. Baseline characteristics of the cohort

Variables Categories	Values
Hospital % (N)	
Hematology Center	83.0% (93)
Muratsan Hospital Complex	17.0% (19)
Total	100.0% (112)
Gender % (N)	
Male	58.9 % (66)
Female	41.1% (46)
Total	100.0 % (112)
Age at diagnosis	
<2 years	13.4% (15)
2-9 years	65.2% (73)
≥9 years	21.4% (24)
Total	100.0 % (112)
Marz % (N)	
Yerevan	35.7% (40)
Marz	59.8% (67)
Artsakh	3.6% (4)
Russia	0.9% (1)
Total	100.0% (112)
Residency % (N)	
Urban	75.0% (84)
Rural	25.0% (28)
Total	100.0% (112)
Year of diagnosis % (N)	
2010	21.4% (24)
2011	13.4% (15)
2012	26.8% (30)
2013	25.9% (29)
2014	12.5% (14)
Total	100.0% (112)
Clinical subtype % (N)	
T-cell	4.5% (5)
B -cell	40.2% (45)
ALL - not otherwise specified	55.4% (62)
Total	100.0% (112)
Philadelphia chromosome % (N)	
Positive	3.6% (4)
Negative	33.0% (37)
NA	63.4% (71)

Total	100.00 (112)
Down syndrome % (N)	
Positive	3.6% (4)
Negative	96.4% (108)
Total	100.0% (112)
Hyperdiploidy % (N)	
Positive	16.1% (18)
Negative	20.5% (23)
NA	63.4% (71)
Total	100.0% (112)
Hypodiploidy % (N)	
Negative	34.8% (39)
NA	65.2% (73)
Total	100.0% (112)
TEL/AML1 % (N)	
Positive	3.6% (4)
Negative	31.2% (35)
NA	65.2% (73)
Total	100.0% (113)
MLL rearrangement % (N)	
Positive	2.7% (3)
Negative	32.1% (36)
NA	65.2% (73)
Total	100.0% (112)
Hepatomegaly at diagnosis % (N)	
Positive	75.9% (85)
Negative	19.6% (22)
NA	4.5% (5)
Total	100.0% (112)
Splenomegaly at diagnosis % (N)	
Positive	69.6% (78)
Negative	27.7% (31)
NA	2.7% (3)
Total	100.0% (112)
Mediastinal mass at diagnosis % (N)	
Positive	4.5% (5)
Negative	86.6% (97)
NA	8.9% (10)
Total	100.0% (112)
Testicle involvement at diagnosis (males only) % (N)	
Negative	100.0% (62)
Total	100.0% (62)
CNS involvement at diagnosis % (N)	
Positive	1.8% (2)
Negative	94.6% (106)

NA	3.6% (4)
Total	100.0% (112)
BMI at diagnosis	
Mean (SD)	16.3 (3.5)
N	82
WBC count at diagnosis [10^9/L]	
Mean (SD)	48.4 (100.3)
N	110
Hemoglobin at diagnosis [g/dL]	
Mean (SD)	83.4 (27.2)
N	110
PLT count at diagnosis [10^9/L]	
Mean (SD)	81.1 (74.3)
N	110
LDH level at diagnosis [IU/L]	
Mean (SD)	939.4 (606.6)
N	72
Blast count in blood at diagnosis [%]	
Mean (SD)	82.7% (16.4)
N	108
Treatment modality % (N)	
BFM	63.4% (71)
MB	26.8 % (30)
Other	9.8% (11)
Total	100.0% (111)
Treatment modality (other) % (N)	
Moved abroad for the treatment	54.5% (6)
Abandoned the treatment	45.5% (5)
Total	100% (11)
Delay in diagnosis	
<30 days	50.5% (53)
≥30 days	49.5% (52)
Total	100% (105)
Delay in treatment initiation	
No (≤1 day)	97.1% (100)
Yes (>1 day)	2.9% (3)
Total	100.0% (103)
Response to the treatment % (N)	
CR +	98.9% (87)
CR -	1.1% (1)
Total	100.0% (88)
Follow-up time (in years)	
Median	5.5
N	112
Vital status % (N)	
Censored	85.7% (96)

	Dead	14.3% (16)
	Total	100.0% (112)

NA - Not available
ALL - Acute lymphoblastic leukemia
CNS - Central nervous system
BFM - Berlin-Frankfurt-Münster
MB - Moscow-Berlin
WBC - White blood cell
Hb - Hemoglobin
PLT - Platelet
LDH - Lactate dehydrogenase

Table 2. Demographic, clinical, and laboratory characteristics of the patients stratified by the outcome variable

Variable	Categories	Dead % (N)	Censored % (N)
Gender	Male	9.8% (11)	49.1% (55)
	Female	4.5% (5)	36.6% (41)
	Total	14.3% (16)	85.7% (96)
Age	<2	3.4% (4)	9.8% (11)
	2-9	7.1% (8)	58.0% (65)
	≥9	3.6% (4)	17.9% (20)
	Total	14.3% (16)	85.7% (96)
Marz	Yerevan	4.5% (5)	31.2% (35)
	Marzes	8.0% (9)	51.8% (58)
	Other	1.8% (2)	2.7% (3)
	Total	14.3% (16)	85.7% (96)
Marz (other)	Artsakh	1.8% (2)	1.8% (2)
	Russia	–	0.9% (1)
	Total	1.8% (2)	2.7% (3)
Residency	Urban	11.6% (13)	64.3% (71)
	Rural	2.9% (3)	22.3% (25)
	Total	14.3% (16)	85.7% (96)
Year of diagnosis	2010	4.5% (5)	17.0% (19)
	2011	0.9% (1)	12.5% (14)
	2012	1.8% (2)	25.0% (28)
	2013	3.6% (6)	22.3% (25)
	2014	1.8% (2)	10.7% (12)
	Total	14.3% (16)	85.7% (96)
Clinical subtype	T – ALL	2.7% (3)	1.8% (2)
	B – ALL	2.7% (3)	37.5% (42)

	ALL-not otherwise specified	8.9% (10)	46.4% (52)
	Total	14.3% (16)	85.7% (96)
Philadelphia chromosome	Positive	–	3.6% (4)
	Negative	3.6% (4)	29.5% (33)
	NA	10.7% (12)	52.7% (59)
	Total	14.3% (16)	85.7% (96)
Down syndrome	Positive	1.8% (2)	1.8% (2)
	Negative	12.5% (14)	83.9% (94)
	Total	14.3% (16)	85.7% (96)
Hyperdiploidy	Positive	–	16.1% (18)
	Negative	3.6% (4)	17.0 % (19)
	NA	8.9% (10)	54.5% (61)
	Total	14.3% (16)	85.7% (96)
Hypodiploidy	Positive	–	–
	Negative	3.6% (4)	31.2% (35)
	NA	10.7% (12)	54.5% (61)
	Total	14.3% (16)	85.7% (96)
TEL/AML1	Positive	–	3.6% (4)
	Negative	3.6% (4)	27.7% (31)
	NA	10.7% (12)	54.5% (61)
	Total	14.3% (16)	85.7% (96)
MLL rearrangement	Positive	–	2.7% (3)
	Negative	3.6% (4)	28.6% (32)
	NA	10.7% (12)	54.5% (61)
	Total	14.3% (16)	85.7% (96)
Hepatomegaly at diagnosis	Positive	13.4% (15)	62.5% (70)
	Negative	–	19.6% (22)
	NA	0.9% (1)	3.6% (4)
	Total	14.3% (16)	85.7% (96)

Splenomegaly at diagnosis	Positive	11.6% (13)	58.% (65)
	Negative	2.7 % (3)	25.0% (28)
	NA	–	2.7% (3)
	Total	14.3% (16)	85.7% (96)
Mediastinal mass at diagnosis	Positive	1.8% (2)	2.7% (3)
	Negative	11.6% (13)	75.0% (84)
	NA	0.9% (1)	8.0% (9)
	Total	14.3% (16)	85.7% (96)
Testicle involvement at diagnosis	Positive	–	–
	Negative	9.8% (11)	50.9% (57)
	NA	4.5% (5)	34.8% (39)
	Total	14.3% (16)	85.7% (96)
CNS involvement at diagnosis	Positive	–	1.8% (2)
	Negative	12.5% (16)	80.4% (90)
	NA	–	3.6% (4)
	Total	14.3% (16)	85.7% (96)
Treatment modality	BFM	7.1% (8)	56.2% (63)
	MB	3.6% (4)	23.2% (26)
	Other	3.6% (4)	6.2% (7)
	Total	14.3% (16)	85.7% (96)
Treatment (other)	Moved abroad	0.9% (1)	4.5% (5)
	Abandoned	2.7% (3)	1.8% (2)
	Total	3.6% (4)	6.2% (7)
Induction failure	No	8.0% (9)	69.6% (78)
	Yes	0.9% (1)	–
	NA	5.4% (6)	16.1% (18)
	Total	14.3% (16)	85.7% (96)
Delay in diagnosis	<30 days	9.8% (11)	36.6% (41)
	≥30 days	4.5% (5)	42.9% (48)

	NA	–	6.2% (7)
	Total	14.3% (16)	85.7% (96)
Delay in treatment initiation	<1 day	11.6% (13)	77.7% (87)
	≥1 day	–	2.7% (3)
	NA	2.7% (3)	5.4% (6)
	Total	14.3% (16)	85.7% (96)

NA - Not available
ALL - Acute lymphoblastic leukemia
CNS - Central nervous system
BFM - Berlin-Frankfurt-Münster
MB - Moscow-Berlin
WBC - White blood cell
Hb - Hemoglobin
PLT - Platelet
LDH - Lactate dehydrogenase

Table 3. Results of the Cox proportional hazard univariable analysis for overall survival among children with ALL

Predictors	Category	Overall survival		p-value
		HR	95% CI	
Gender	Female	1.00		
	Male	0.56	0.18 – 1.77	0.327
Log (Age at diagnosis, in years)	-	1.57	0.36 – 6.74	0.54
	2-9	1.00		
	≥9	1.71	0.51 – 5.68	0.383
Geographic distribution	Marz	1.00		
	Yerevan	1.10	0.36 – 3.38	0.863
Residency	Rural	1.00		
	Urban	0.79	0.22 – 2.80	0.715
Log (BMI)	-	0.54	0.03 – 21.96	0.875
Log (WBC count at diagnosis)	-	1.34	0.62 – 2.89	0.452
Log (Hb level at diagnosis)	-	3.72	0.11 – 120.85	0.460
Log (PLT count at diagnosis)	-	0.70	0.36 – 1.38	0.307
Log (LDH count at diagnosis)	-	2.63	0.21 – 32.06	0.448
Splenomegaly	Yes	1.00		
	No	0.54	0.15 – 1.91	0.340
Delay in diagnosis	<30 days	1.00		
	≥30 days	3.23	1.02 – 10.20	0.049*
Treatment	BFM	1.00		
	MB	1.31	0.38 – 4.49	0.663

Year of diagnosis	2010	1.00		
	2011	0.32	0.04 – 2.72	0.296
	2012	0.33	0.06 – 1.71	0.187
	2013	0.83	0.24 – 2.88	0.776
	2014	0.69	0.13 – 3.55	0.657

**p<0.05*
HR - Hazard ratio
CI - Confidence interval
BMI - Body mass index
WBC - White blood cells
Hb - Hemoglobin
PLT - Platelets
LDH - Lactate dehydrogenase

Table 4. Cox proportional hazard multivariable model for overall survival among children with ALL

Predictors	Overall survival		p-value
	HR	95% CI	
Gender (male)	0.58	0.18 – 1.84	0.359
Log (WBC count at diagnosis * 10^3)	1.37	0.59 – 3.15	0.461
Delay in diagnosis (≥30 days)	3.23	1.02 – 10.13	0.047*

Stratified by Splenomegaly

**p<0.05*
HR - Hazard ratio
CI - Confidence interval
WBC - White blood cell

Figures

Figure 1. Estimated survival function of the overall survival among children diagnosed with ALL within 2010-2014 using the Kaplan-Meier method

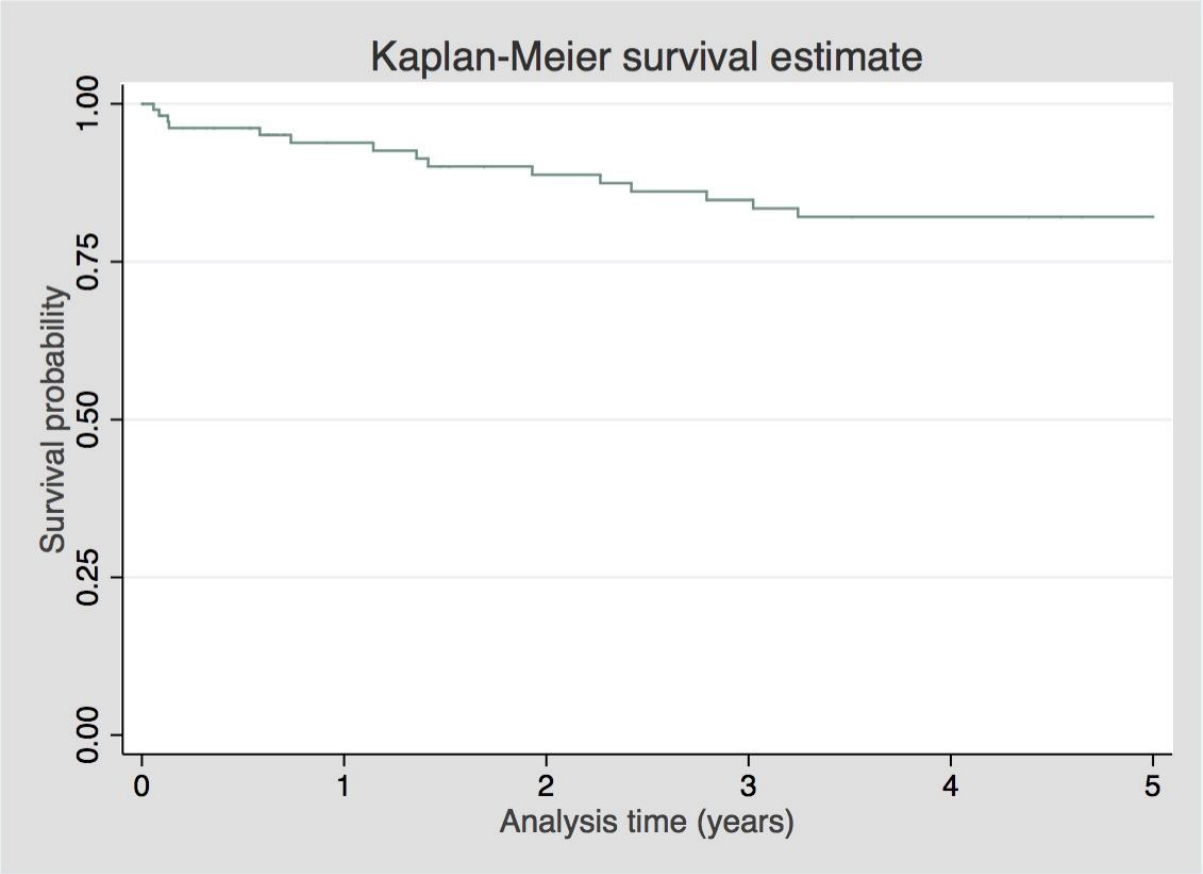
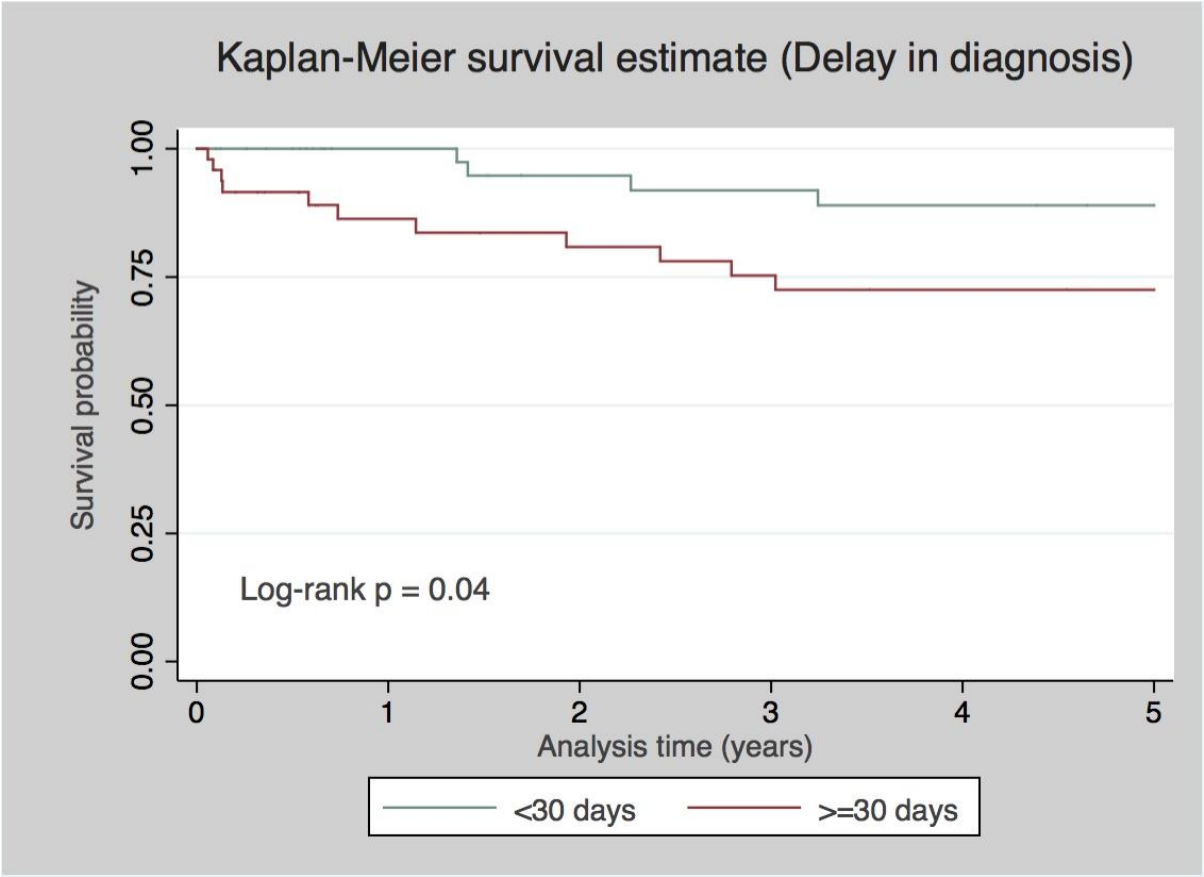


Figure 2. Estimated overall survival of delay in diagnosis for <30 days in relation to delay in diagnosis for ≥30 days using the Kaplan-Meier method



Appendices

Appendix 1. Chart abstraction form

#	ITEMS	Patient #_______	Patient #_______	Patient #_______
Q1	**Patient's ID**	___ ___ ___ ___ ___	___ ___ ___ ___ ___	___ ___ ___ ___ ___
Q2	**Reviewer's ID**	___ ___	___ ___	___ ___
Q3	**Date of the data abstraction**	___ ___ / ___ ___ / ___ ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___ ___
Q4	**Hospital ID**	___ ___	___ ___	___ ___
Q5	**Gender**	1) ……. Male 2) ……. Female	1) ……. Male 2) ……. Female	1) ……. Male 2) ……. Female
Q6	**Birth date of the patient** [day / month / year]	___ ___ / ___ ___ / ___ ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___ ___
Q7	**Geographic location**	1) ……. Yerevan 2) ……. Aragatsotn 3) ……. Ararat 4) ……. Armavir 5) ……. Gegharkunik 6) ……. Kotayk 7) ……. Lori 8) ……. Shirak 9) ……. Syunik 10) ……. Tavush	1) ……. Yerevan 2) ……. Aragatsotn 3) ……. Ararat 4) ……. Armavir 5) ……. Gegharkunik 6) ……. Kotayk 7) ……. Lori 8) ……. Shirak 9) ……. Syunik 10) ……. Tavush	1) ……. Yerevan 2) ……. Aragatsotn 3) ……. Ararat 4) ……. Armavir 5) ……. Gegharkunik 6) ……. Kotayk 7) ……. Lori 8) ……. Shirak 9) ……. Syunik 10) ……. Tavush

		11) ……. Vayots Dzor 12) ……. Other _____	11) ……. Vayots Dzor 12) ……. Other _____	11) ……. Vayots Dzor 12) ……. Other _____
Q8	**Residency**	1) ……. Urban 2) ……. Rural	1) ……. Urban 2) ……. Rural	1) ……. Urban 2) ……. Rural
Q9	**Date of the admission** [day / month / year]	___ ___ / ___ ___ / ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___
Q10	**Date of the onset of first symptoms** [day / month / year]	___ ___ / ___ ___ / ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___
Q11	**Date of diagnosis** [day / month / year]	___ ___ / ___ ___ / ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___
Q12	**Date of discharge** [day / month / year]	___ ___ / ___ ___ / ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___
Q13	**Weight of the patient at diagnosis [kg]**	___ ___	___ ___	___ ___
Q14	**Height of the patient at diagnosis [cm]**	___ ___ ___	___ ___ ___	___ ___ ___
Q15	**Clinical subtype /Immunophenotype**	1) ……. B-cell 2) ……. T-cell 3) ……. ALL not otherwise specified 4) ……. Other_____	1) ……. B-cell 2) ……. T-cell 3) ……. ALL not otherwise specified 4) ……. Other_____	1) ……. B-cell 2) ……. T-cell 3) ……. ALL not otherwise specified 4) ……. Other_____
Q16	**Philadelphia chromosome** [BCR/ABL]	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA
Q17	**Down syndrome**	1) ……. Positive 2) ……. Negative	1) ……. Positive 2) ……. Negative	1) ……. Positive 2) ……. Negative

Q18	**Hyper**diploidy [chromosomal count >51]	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA
Q19	**Hypo**diploidy [chromosomal count <44]	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA
Q20	**TEL/AML1** translocation	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA
Q21	**MLL** rearrangement	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA
Q22	**Hepatomegaly** at diagnosis	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA
Q23	**Splenomegaly** at diagnosis	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA
Q24	**Mediastinal mass** involvement	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA
Q25	**CNS** involvement at diagnosis (blasts in CVF)	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA
Q26	If male, **testicle** involvement	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA	1) ……. Positive 2) ……. Negative 3) ……. NA
Q27	**WBC** count at diagnosis [10^9 /L]	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA
Q28	**Hemoglobin** at diagnosis [g/dL]	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA
Q29	**Platelets** at diagnosis [10^9 /L]	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA

Q30	**LDH** at diagnosis [IU/L]	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA
Q31	**Blast** count in **bone marrow** at diagnosis [%]	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA
Q32	Treatment	1) ……. BFM 2) ……. MB 3) ……. Other_____	1) ……. BFM 2) ……. MB 3) ……. Other_____	1) ……. BFM 2) ……. MB 3) ……. Other_____
Q33	**Blast** count in **bone marrow** on the **33rd** day of induction [%]	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA
Q34	**Blast** count in **CSF** on the **33rd** day of induction [%]	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA	1) ……. _____ 2) ……. NA
Q35	**Date of last contact** [dd /mm/yyyy]	___ ___ / ___ ___ / ___ ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___ ___	___ ___ / ___ ___ / ___ ___ ___ ___
Q36	Patient **vital status** at the day of the last contact	1) ……. Alive 2) ……. Dead	1) ……. Alive 2) ……. Dead	1) ……. Alive 2) ……. Dead

Appendix 2. Stepwise forward variable selection for the multivariable Cox proportional hazard regression analysis

N=103
P=0.0641

Predictors	Overall survival		p-value
	HR	95% CI	
Gender (male)	0.43	0.13 – 1.40	0.163
Log (Age at diagnosis)	5.64	1.06 – 30.89	0.042*
Log (WBC count at diagnosis * 10^3)	1.26	0.86 – 1.84	0.240
Delay in diagnosis (≥30 days)	4.94	1.43 – 17.00	0.011*
Splenomegaly at diagnosis	0.72	0.18 – 2.78	0.631
PLT count at diagnosis	0.76	0.54 – 1.06	0.106

HR = Hazard ration
CI = Confidence interval
WBC = White blood cell
PLT = Platelet

Test of proportional-hazard assumption

Predictors	p-value
Gender (male)	0.047
Age at diagnosis	<0.001
Log (WBC count at diagnosis * 10^3)	0.521
Delay in diagnosis (≥30 days)	0.792
Splenomegaly at diagnosis	0.938
PLT count at diagnosis	0.018
Global test	0.006

WBC = White blood cell
PLT = Platelet

Akaike's information criterion

Obs	ll (null)	ll (model)	df	AIC

103	-64.20	-58.25	6	128.5

Obs = Observations
ll = log likelihood
df = degree of freedom
AIC = Akaike's information criterion

Variables age at diagnosis and PLT count at diagnosis did not meet the proportionality assumption and thus, were removed from the model.

N=103
P=0.1720

Predictors	Overall survival		p-value
	HR	95% CI	
Gender (male)	0.58	0.18 – 1.84	0.359
Log (WBC count at diagnosis * 10^3)	1.37	0.59 – 3.17	0.461
Delay in diagnosis (≥30 days)	3.21	1.02 – 10.13	0.047*
Splenomegaly at diagnosis	0.64	0.17 – 2.39	0.511

HR = Hazard ration
CI = Confidence interval
WBC = White blood cell

Test of proportional-hazard assumption

Predictors	p-value
Gender (male)	0.208
Log (WBC count at diagnosis * 10^3)	0.236
Delay in diagnosis (≥30 days)	0.156
Splenomegaly at diagnosis	0.625
Global test	0.310

WBC = White blood cell

Akaike's information criterion

Obs	ll (null)	ll (model)	df	AIC
103	-64.20	-61.01	4	130.0

Obs = Observations
ll = log likelihood
df = degree of freedom
AIC = Akaike's information criterion

After graphically checking for proportionality assumption, splenomegaly did not meet the assumption, thus, we stratified the model by splenomegaly.

N=103
P=0.115

Predictors	Overall survival		p-value
	HR	95% CI	
Gender (male)	0.58	0.18 – 1.83	0.354
Log (WBC count at diagnosis * 10^3)	1.37	0.59 – 3.15	0.464
Delay in diagnosis (≥30 days)	3.23	1.02 – 10.20	0.045*
Stratified by splenomegaly			

HR = Hazard ration
CI = Confidence interval
WBC = White blood cell

Test of proportional-hazard assumption

Predictors	p-value
Gender (male)	0.201
Log (WBC count at diagnosis * 10^3)	0.243
Delay in diagnosis (≥30 days)	0.161
Global test	0.198

WBC = White blood cell

Akaike's information criterion

Obs	ll (null)	ll (model)	df	AIC
103	-56.53	-53.57	3	121.05

Obs = Observations
ll = log likelihood
df = degree of freedom
AIC = Akaike's information criterion

Appendix 3. Post-hoc power analysis (STATA output)

```
. stpower logrank, n(112) hratio(0.64) nratio(0.4)

Estimated power for two-sample comparison of survivor functions
Log-rank test, Freedman method
Ho: S1(t) = S2(t)

Input parameters:

      alpha =    0.0500  (two sided)
     hratio =    0.6400
          N =       112
         p1 =    0.7143

Estimated number of events and power:

          E =       112
      power =    0.4834
```