Clinical-biological Characteristics and Poor Predictive Value of Early Treatment Response in Pediatric Acute Lymphoblastic Leukemia with CDKN2A Gene Deletion Treat with CCLG-ALL 2015 protocol

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Abstract

Background: Deletion of cyclin-dependent kinase inhibitor 2A (CDKN2A) is prevalent in pediatric acute lymphoblastic leukemia (ALL) and the prognostic importance of CDKN2A deletion is still controversial. Procedure: A total of newly diagnosed 655 pediatric ALL cases were treated with Chinese Children's Leukemia Group-acute lymphoblastic leukemia 2015 (CCLG-ALL 2015) protocol[1]. We investigated the difference among B-ALL and T-ALL patients with CDKN2A deletion for clinical characteristics at diagnosis, immunophenotype, risk stratification, cytogenetic risk group, and early treatment responses.We also analyzed the prognostic markers for event-free survival(EFS) in CDKN2A-deleted patients. Result: The incidence of CDKN2A gene deletion was presented in 14.6% (87/595) of B-ALL subgroup and 40.0% (24/60) of T-ALL subgroup. T-ALL subgroup was characterized by a higher male/female ratio, a higher proportion of older children (>10 years old) and WBC counts of greater than 50x109/L compared to B-ALL($P<0.05$). In the univariate analysis, CNS 2, cytogenetic risk groups, prednisone poor responders (PPR), poor early response (PER), and MRD[?]0.01% at day 46 ($P<0.05$) were associated with a poor event-free survival. Multivariable analysis revealed that PPR and MRD[?]0.01% at day 46 were independent inferior prognostic factors for event-free survival($P<0.05$). Conclusions: The incidence of CDKN2A deletion was more prevalent in T-ALL. CDKN2A deletion was significantly more prevalent in older (>10 years old) boys with leukocyte counts of greater than 50x109/L among T-ALL. PPR and MRD[?]0.01% at day 46 were an independent prognostic factor for EFS in pediatric CDKN2A-deleted ALL.

Introduction

Genetic alterations of acute lymphoblastic leukemia (ALL) involved in various signaling pathways are associated with the disease pathogenesis.Moreover,they may play a role as predictive biomarkers for selecting the treatment protocol and adjusting risk stratification.Inactivation of the tumor suppressor gene CDKN2Ain the 9p21.3 locus,which has been implicated in many cancer,can occurby deletion,methylation, or mutation[2].CDKN2A gene deletion is commonly present in pediatric ALL which can be detected by fluorescent in-situ hybridization(FISH)[3].It showed that CDKN2A deletion occurred in 21% BCP-ALL and 50% T-ALL patients[4].Under normal conditions,the CDKN2A gene encodes two proteins,$p16^{INK4a}$ and $p14^{ARF}$.These two proteins specifically inactivate cyclin/CDK4/6 complexes that block cell division during the G1/S phase of the cell cycle[5].A lot of research found that the deletion of CDNK2A is not only associated with T-lineage ALL but also B-lineage ALL[4,6-7].

Despite the high frequency of CDKN2A deletion in pediatric ALL,the prognosis importance in pediatric ALL is still inconclusive.Most of the results thought the deletion of CDKN2A was associated with pediatric ALL

recurrence[8-9].While Mirebeau D reported that inactivation of CDKN2A gene did not influence B-lineage acute lymphoblastic leukemia of childhood' outcome in 2006[6].

In this article we tried to describe the clinical-biological characteristics and prognostic factors in pediatric ALL patients with CDKN2A deletion.

Methods

Patients and treatment protocols

This cohort included 111 newly diagnosed ALL pediatric patients with CDKN2A deletion.All patients were diagnosed and treated according to CCLG-ALL 2015 protocol[1] at Blood Disease Hospital of CAMS & PUMC between September 2016 and December 2019.The following patients were not included in this cohort:**a** .patients who diagnosed as mature B-lineage ALL or mixed-phenotype acute leukemia (MPAL)(excluding ALL with myeloid antigen expression);**b** .patients developed from chronic myeloid leukemia blast crisis(CML-BP),another cancer or immunodeficiency disease;**c** .patients who had undergone chemotherapy or radiation in three months(excluding emergency radiotherapy to relieve the oppression symptoms);**d** .patients who used to treat glucocorticoid no less than7 days between one week to one month before enrollment.The immunophenotype was distinguished by a panel of monoclonal antibodies including markers for B-lineage cells(CD10,CD19,TdT,cyμ,sIgM,CD20,cyCD22,CD22,cyCD79a),T-lineage cells(CD1a,CD2,CD3,CD4,CD5,CD7,CD8,TCRαβ,TCRγδ,cyCD3)or myeloid cells(CD11b,CD13,CD14,CD15,CD33,CD41,CD61,CD64,CD65,CD71,GPA,cyMPO).

The protocols described in this study were approved by the Ethics Committee,Institute of Hematology & Blood Disease Hospital,CAMS & PUMC.All patients or their legal guardians signed written informed consent before treatment.

Early treatment Response and events definition

To identify the early prognostic factors, we evaluated leukemic blasts counts in bone marrow by morphology on days 46 and minimal residual disease(MRD) on days 19 and 46 during first induction.Diagnosis of complete remission(CR) was based solely on bone marrow morphology with a cutoff value of 5% of leukemic blasts on treatment day 46.Bone marrow MRD no lower than 10^{-5} is defined as positive.It is considered as good (Prednisone good responders, PGR) if the peripheral blast count on treatment day 5 is lower than 1×10^9/L, whereas it is poor(PPR) if patients show no lower than 1×10^9/L.Patients were defined as having a poor early response(PER) if MRD[?]1% on day treatment 19.

FISH andcytogenetic risk group

Pretreatment bone marrow (BM) aspirates were taken at diagnosis and at least 1-2 milliliters of bone marrow aspirates were analyzed by fluorescence in situ hybridization(FISH) for CDKN2A deletion,BCR-ABL1 fusion gene,TEL-AML1 fusion gene,E2A-PBX1 fusion gene,MLL rearrangement,MYC translocation,SIL-TAL1 fusion gene,P53-CEP17,CRLF2 rearrangement.We analyzed interphase cells according to the instructions of the probe manufacturer(America Abbott).

Patients were divided into two exclusionary cytogenetic risk groups based on the following cytogenetic abnormalities:high risk (BCR-ABL1,MLL rearrangements, chromosome <44 or t[17;19]/E2A-HIF) or intermediate risk (all extra cases with abnormal or normal cytogenetics).

Statistical analysis

Quantitative data were described by median and enumeration data was expressed as a percentage(%).We conduct Chi-square statistical significant test on categorical variables and Fisher's exact test on observations which were less than five in number.The final follow-up was on march, 2020 and the median follow-up time was 14 months (range: 1 to 39 months). Event-free survival (EFS) was calculated from the time point of diagnosis until the following events:induction treatment failure,any relapse afterCR,second malignancy (SMN),death due to any cause. Survival rates were calculated and compared using Kaplan–Meier analysis

and log-rank tests on univariable data.Cox regression analysis for multivariate data.Variables that were found statistically significant or P -value approximate to 0.1 will be included in multivariable Cox regression analysis.Statistical significance was defined as a P -value of less than 0.05.All analyses were performed using SPSS 23.0 software.

Results

Clinical characteristics,immunophenotype,risk stratification and cytogenetics abnormalities in pediatric CDKN2A-deleted ALL

Of 655 patients enrolled, CDKN2A deletion was presented in 111 cases(16.9%). The prevalence of CDKN2A gene deletion in B-ALL and T-ALL was 14.6%(87/595) and 40.0%,respectively.In the CDKN2A-deletedpatients,the median age at diagnosis was 7 years(range:0.6 to 14 years),male/female(M/F) ratio was 1.47/1.The median white blood cell(WBC) countswere35.2x10^9/L(range:0.7-565.8x10^9/L),63.1%(70/111)patients had hepatosplenomegaly at diagnosis.

In our cohort,eighty-seven (78.4%) children were presented withB-cell phenotype while 24(21.6%) children were identified asT-cell phenotype.Based onCCLG-ALL 2015 risk stratification and bone marrow MRD level during induction therapy,there were 35(31.5%),73(65.8%),3(2.7%) case for standard group, intermedium groupand high-risk group,respectively.Our research showed the high proportion of intermedium group in patients with CDKN2A deletion.

Cytogenetic and molecular analysis was available inall subjects and exhibited 41 (42.3%) cases hadco-occurrence of fusion gene:TEL-AML1 (n=13,11.7%),BCR-ABL1(n=14,12.6%),E2A-PBX1(n=4,3.6%),SIL-TAL1(n=5,4.5%),P53(n=3,2.7%),MLLrearrangement(n=1,0.9%),E2A-HLF(n=1,0.9%),other fusion gene(n=6,5.4%)(Fig.1A). n=40(46.9 %) B-ALL cases had co-occurrence of fusion gene:TEL-AML1 (n=13,14.9%),BCR-ABL1 (n=14,16.1%),E2A-PBX1 (n=4,4.6%),P53(n=3,3.4%),E2A-HLF(n=1,1.1%),other fusion gene(n=5,5.7%)(Fig. 1B).To further analyze the role of co-occurrence of fusion gene in prognosis,n=17(15.3%) children were set to high risk cytogenetic group,remaining n=94(84.7%) children were set to intermediate cytogenetic group based on cytogenetic risk group as mentioned above.Besides,12.4%(12/97) had co-occurrence ofIKZF1 deletion.Chromosomal abnormalities was observed in 41 patients(36.9%) at diagnosis, including 10 cases(9.0%) with del(9)(p21) (Table 1).

T-ALL with CDKN2A gene deletion was characterized by higher male/female ratio,more older children and higher WBC counts

We compared the difference of clinical variables and cytogenetic risk group between T-ALL and B-ALL (Table 2).The clinical features of the T-ALL subgroup(n=24) were clearly distinct from the B-ALL subgroup(n=87).The CDKN2A gene deletions were more prevalent in male than female in T-ALL subgroup(P =0.002).45.8%(11/24)patients with CDKN2A deletion were older than ten years old in the T-ALL subgroup(P =0.006).CDKN2A deletion was more prevalent in T-ALL childrenwith WBC counts of greater than 50x10^9/Lcompared to B-ALL subgroup(P =0.000).There was no significant difference among two immunophenotypic subgroupsfor age,hepatosplenomegaly,CNS2,cytogenetic risk groups, andkaryotype including del(9)(p21).

Early treatment response including PPR and MRD positive at day 46 are strong independent negative predictors for CDKN2A-deleted ALL

When monitoring for early treatment responses,results showed thatn=78 (71.3%)patients were PGR and n=110 (99.1%)patients achieved first completeremission(CR).n=10 (41.6%) cases had PPR in T-ALL subgroup while nosignificant differences between two subgroups(P =0.148).There was no significant difference among the two subgroups for other early treatment response factors.(Table 2)

By the end of follow-up,n=14 relapse(n=5 death after relapse),n=1 induction failure,n=1 death due to severe pneumonia(SP) and one patient got second malignancy(Langerhans cell histiocytosis,LCH).Unfortunately, the patients got cutaneous peripheral T-cell lymphoma soon after HLA identical hematopoietic stem cell

transplantation(HSCT) and finally died from gastrointestinal and pulmonary hemorrhage.The patient only had survived for 16 months after the first diagnosis.It should be noted that the patient had no other genetic abnormalities except CDKN2A deletion.

To test whether the co-variables described abovehad independent prognostic value in CDKN2A-deleted patients,we performed univariate analysis and multivariable analysis to assess their independent predictive power for EFS (Table 3).Univariate analysis revealed that EFS in CDKN2A-deleted ALL was associated with CNS2,cytogenetic risk groups,PPR,PER, and MRD[?]0.01% at day 46(P <0.05) while no significant difference was observed in age,sex, and WBC counts.Seven variables(CNS2,WBC counts[?]50x10^9/L,cytogenetic risk groups,PPR,PER, and MRD[?]0.01% at day 19,46) were estimated by Cox regression models,results showed both PPR(HR=3.135,95%CI:1.138-8.638) and MRD positive at day 46(HR=3.812,95%CI: 1.388-10.464)wereindependent inferior prognostic factors for event-free survival(P <0.05),suggesting the adverse impact of these factors in CDKN2A-deleted ALL.(Table 4)

Discussion

CDKN2A deletions have a high frequency in pediatric acute lymphoblastic leukemia and FISH is an accurate and reliable method for this deletion[2,4,8-10].Data from our study demonstrated the incidence of CDKN2A deletion in pediatric ALL was 16.9%,which was lower than most previous studies(22-45%)[4,6,11-15].The apparent difference in incidence is might due to the fact that previous studies had different detection methods such as MLPA or SNP and wide detection range including CDKN2Bdetection.

Most researchers acknowledged that CDKN2A-deleted patients have high WBC counts,prominent hepatosplenomegaly and expression of predominantly T-cell surface markers[2,6,16].In our cohort,CDKN2A deletion was presented in 14.6% of B-ALL and 40.0% of T-ALL.To find out whether any discrepancy between the B-ALL subgroup and the T-ALL subgroup with CDKN2A deletion,we compared clinical-biological characteristics and early treatment responsesamongthe two groups.Itrevealed that CDKN2A deletion was more prevalent among older(>10 years) males (P <0.05) and those with a WBC counts of greater than 50x10^9/L(P <0.001).However,Sulong S hasdemonstrated that CDKN2A deletion was more prevalent among older children (10>years) and high WBC counts in BCP-ALL but not among T-ALL patients[11].

According to the previous study,TEL-AML1 occurs in 20-25% of pediatric B-ALL,MLLr occurs in 2-20% pediatric ALL,BCL-ABL1 occurs in 2-5% of pediatric ALL and E2A-PBX1 occurs in 2-6% of pediatric ALL[16-17]. Sulong S had revealed that the incidence of CDKN2A deletions in pediatric ALL varying markedly by different cytogenetic subgroups and CDKN2A-deleted patients had low frequenciesof TEL-AML1,MLLr whereas had high frequenciesof BCL-ABL1,E2A-PBX1[11].Data from our research(Figure1A-B)showed a similarincidence of TEL-AML1,MLLr,BCL-ABL1compared toSulong's conclusion.

The prognostic value of theCDKN2A deletion in pediatric acute lymphoblastic leukemia is still controversial.Most results found that CDKN2A deletion was an independent prognostic indicator for poor outcomesand relapse in childhood ALLor B-ALL[4,6,9,11-15]. Because the majority of patients in this cohort are being treated on an ongoing clinical trial,we are incapable of further survival analysis.Therefore,We demonstrated the prognostic factor that might affect the event-free survival of CDKN2A-deleted patients in our cohort.It turned out that EFS ofCDKN2A-deleted patients was significantly associated with CNS2, cytogenetic risk groups, PPR,PER, and MRD[?]0.01% at day 46 in the log-rank test.It suggested that co-occurrence of BCR-ABL1,MLL rearrangements, chromosome <44, or t[17;19]/E2A-HIF in CDKN2A-deleted patients had inferior outcomes.Furthermore,both PPR and MRD[?]0.01% at day 46werepoor independent markersfor EFS.It was not surprising that PPR and MRD positive can predict the clinical outcome even in patients with CDKN2A deletion.Unexpectedly,our research showed T phenotype didn't result in apoor prognosis for event-free survival.Lack ofsignificant difference for early treatment responses among B-ALL subgroup and T-ALL subgroup was acting as a hint of theclinical outcome.

Although FISH was confirmed as a reliable technique for CDKN2A deletion,chromosomal abnormalities of CDKN2A carriers had rarely been described.In our research,we found that only afew patients(9.0%) were observed thekaryotype with del(9)(p21).According to the literature,del(9)(p13) can lead to monosomy of the

tumor suppressor gene CDKN2A in T-cell prolymphocytic leukemia[18],but it didn't been provedin childhood ALL.

One patient occurred second malignancy—Langerhans cell histiocytosis(LCH) duringmaintenance therapy who only carried CDKN2A deletion.Xerri L pointed outco-occurrence ofCDKN2A/B deletion and mutations of the MAPK pathway underlay the aggressive behavior of Langerhans cell tumors[19],it suggests that pediatric ALL with CDKN2A deletion might have potential risk in reemerging LCH.

In summary,CDKN2A deletions are significantly more prevalent in older(>10 years old) males with leukocyte counts of greater than $50x10^9$/L among T-ALL.PPR and MRD[?]0.01% at day 46 are independent prognostic factor for EFS in pediatric CDKN2A-deleted ALL.In future research,the targeted therapy such as CDK4/6 inhibitor might be an effective treatment strategy for those patients.

Conflict of Interest

The authors declare that they have no conflicts of interests with the contents of this article.

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FIGURE 1 A)co-occurrence of fusion gene in 111 cases of CDKN2A-deleted ALL;B)co-occurrence of fusion gene in 87 cases of CDKN2A-deleted B-ALL

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