Case Report
B-acute lymphoblastic leukemia occurring in two pediatric patients with a history of prior Wilms tumors

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Abstract: Therapy-related acute leukemia (t-AL) is an observed carcinogenesis due to the toxic side effects of ionizing radiation and/or conventional chemotherapy for primary tumors. To our knowledge, therapy-related acute myeloid leukemia (t-AML) accounts for a large proportion in t-AL. While B-acute lymphoblastic leukemia (B-All) has never been reported following the treatment of pediatric Wilms' tumor (WT). This condition is also not well understood. Here we report B-All occurring in two pediatric patients with a historyof prior WTs. The pre-operative chemotherapy, surgery, and postoperative chemotherapy were all timely implemented in these two children. Unfortunately, B-ALL both occurred in them after treatment. Thus, they were then treated for leukemia. Thankfully, six months after the treatment of leukemia, case 1 underwent haematopoietic stem cell transplantation (HSCT). She is currently in remission. Nevertheless, thrombosis on precava and right atrial occurred in case 2. It was a pity that this child died from pulmonary embolism and disseminated intravascular coagulation six months after the diagnosis of leukemia. *Conclusion:* It is noted that some t-AL secondary to prior pediatric WTs, which attention should be paid by the clinician, especially for the surgeon. It can not only obviously reduce the mortality rate but also notedly prolong the disease-free survival attribute to the early diagnosis of secondary leukemia and timely HSCT. What is Known: It is noted that some t-AL secondary to prior pediatric WTs. HSCT can notedly prolong the disease-free survival of secondary leukemia. What is New: No data on B-All secondary to pediatric WT.

Keywords: Therapy-related acute leukemia, Wilms' tumor, secondary malignant neoplasms, haematopoietic stem cell transplantation

Introduction

Therapy-related acute leukemia (t-AL) is defined as a heterogeneous group of neoplasms, which occurs in ionizing radiation and/or conventional chemotherapy including alkylating agents, topoisomerase-II-inhibitors and antimetabolites for primary neoplastic. Most secondary leukemia are myeloid, which represent 5% to 10% of all acute myeloid leukemia (AML) [1, 2]; while therapy-related acute lymphoblastic leukemia (t-ALL) is relatively rare, t-ALL represents 1.5% to 2.5% of all adult acute lymphoblastic leukemia (ALL). t-ALL is much less frequent than therapy related acute myeloid leukemia (t-AML), which accounts for approximately 12% of all t-AL and 1.2-4% of adult ALL [3, 4].

Wilms' tumor (WT), one of the most common abdominal solid tumors in children, represents the most common pediatric renal embryonic malignancy. With the improvement of chemotherapy and medical support of patients treated with chemotherapy and radiation, the number of cancer survivors has been significantly growing. However, the long-term cancerization and teratogenesis are also highlighted on account of chemotherapy and radio-therapy. Adult t-AL has been researched relative systematically whether in domestic or foreign [5-8]. Nevertheless, few data is available on pediatric t-AL developing after chemotherapy and/or radiotherapy. Only several scattered cases are reported, such as primary AML transformation to therapy-related myelodysplastic syndrome (t-MDS) [9], T lymphoblastic lymphoma (T-LBL) transformation to t-AML, B-ALL transformation to t-AML, B-ALL transformation to t-MDS [10], osteogenic sarcoma transformation to t-AML [11], cerebrum transformation to t-AML [12].
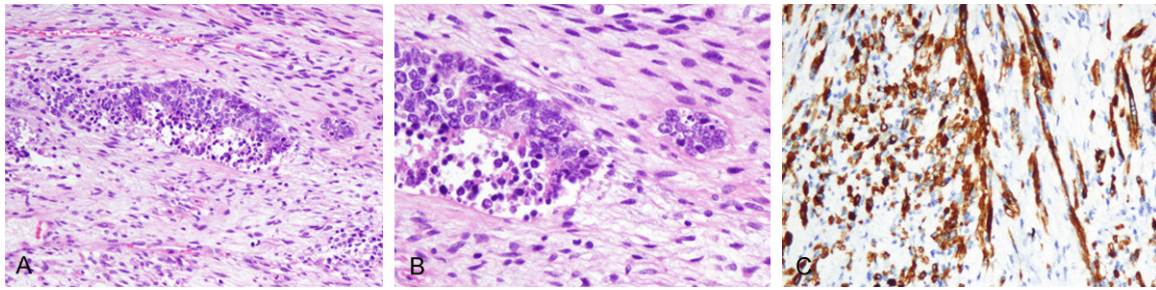
Figure 1. Histopathological images of case 1. A: The left renal and tumor by resection, hematoxylin-eosinstain; original magnification ×200. B: Hematoxylin-eosin stain; original magnification ×400. A broad range of spindle cells were in a woven arrangement. Part of the cell cytoplasm was with rich red dye. Cell atypia and deeply stained nuclear were significantly observed. C: Desmin (one of immunohistochemistry) of spindle cells was remarkably positive.
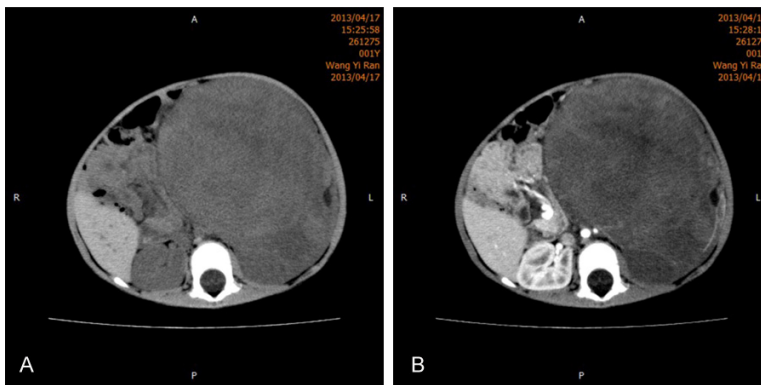

Figure 2. Initial abdominal CT of case 1. A: CT plain scan, B: CT contrast enhanced scan. Huge mass in the left kidney (118 mm×110 mm×101 mm). The density of the lesions was heterogeneous. The lower density cystic shadow could be seen. Normal renal tissue was obviously compressed. The left renal artery was wrapped by the mass and several abdominal aortic lymph nodes were enlarged.

hospital. Giant left renal tumor resection plus retroperitoneal lymph node dissection were carried out on April 24, 2013 at our hospital. Wilms' tumor was finally made a definite diagnosis by pathology and immunohistochemistry and abdominal CT scan on October 18, 2013 (**Figures 1** and **2**). The patient had adjuvant postoperative chemotherapy on the same day. In the subsequent visits, the patient had recovered, with noadditionalcomplications.Nevertheless, on April 14, 2013, the baby suddenly developed a high fever (Tmax 38.7°C) accompanied with dry cough.

More importantly, no data is available on secondary B-ALL in children with WT whether in domestic or foreign. In order to improve the recognition of ALL in pediatric WT, two unusual relevant cases are reported as follows.

Case presentation

Case 1, a female infant with one year and two months old, was hospitalized with hematuresis and an abdominal enclosed mass for five weeks on April 24, 2013. The malignant tumor, possibly a left side Wilms' tumor, was initially prompted by the renal pathology biopsy at the local hospital on March 11, 2013. The baby then underwent 2 cycles of chemotherapy with Pirarubicin (THP) (50 mg/m^2 on day 1), vincristine (VCR) (1.5 mg/m^2 on day 1) and Actinomycin D (Act-D) (15 ug/kg on day 1 to day 5) since March 13, 2013 in the same

Complete blood count analysis with differential indicated an obvious increase in white blood cells (WBC 340×10^9/L). Peripheral and bone marrow smear analysis revealed an abnormal increase in the amount of lymphoblast and Immature lymphocytes (60% and 88.5%, respectively) (**Figure 3A**), which indicated ALL (L2). Flow cytometry showed abnormal B cells (68.6%) (**Figure 3B-F**). Fluorescence in situ hybridization (FISH) indicated mixed lineage leukemia (MLL) fusion gene was positive. Additionally, Ig and TCR Gene rearrangement detection were negative. Chromosome-based analysis showed structural rearrangements were also negative. According to World Health Organization (WHO)-based classification, these results verified the diagnosis as B-ALL (L2, BII, MLL-ENL positive, high-risk). Regular chemotherapy was began with chemotherapy regimen of high-risk B-ALL according to the modified
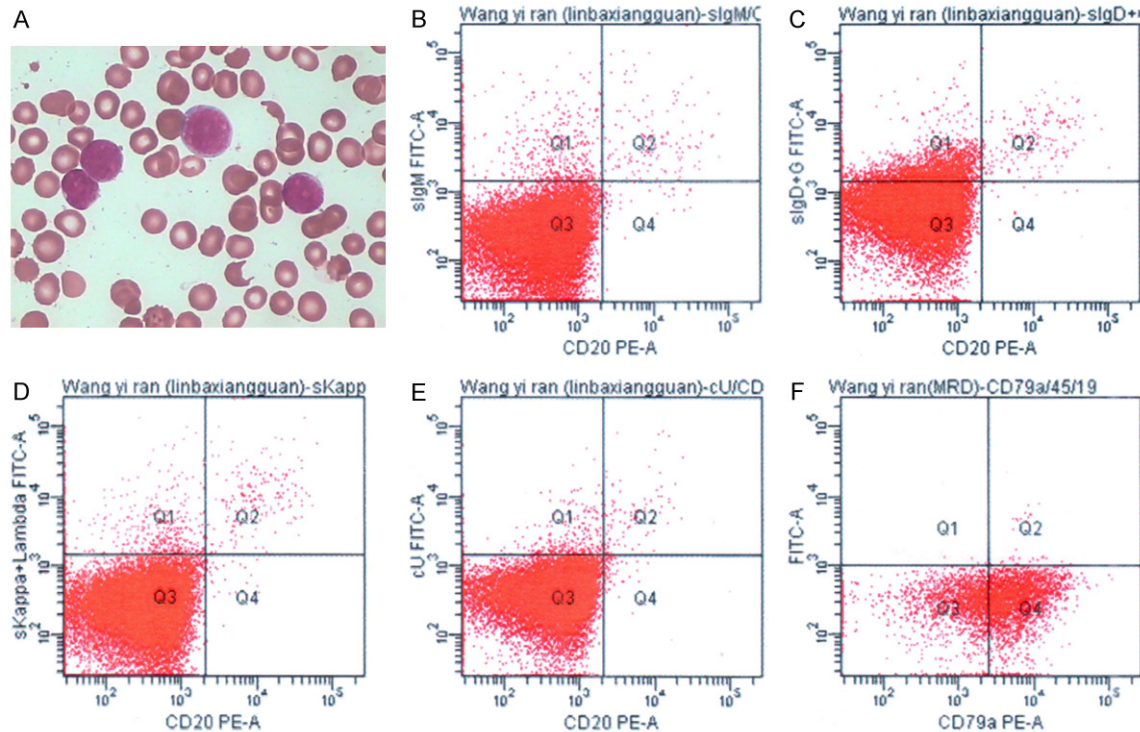
Figure 3. Bone marrow hemocytology (A) and flow cytometry (B) of Case 1. (A) The result revealed an abnormal increase in the amount of lymphoblast and Immature lymphocytes (60% and 88.5%, respectively). (B-F) The result showed abnormal B cells.
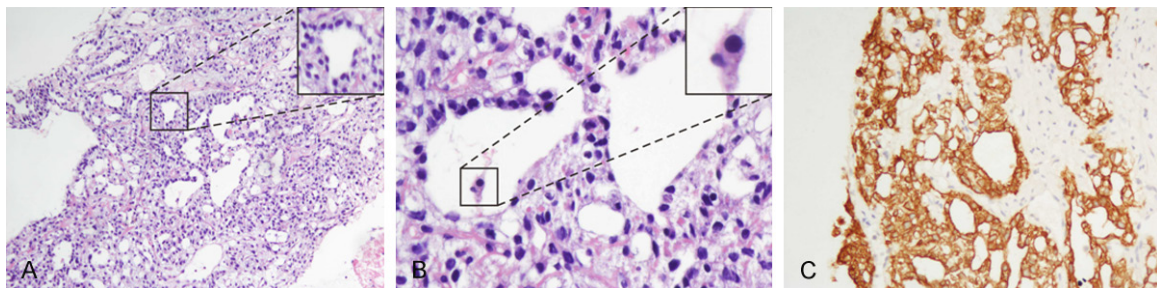

Figure 4. Histopathological images of case 2. A: The left kidney tumor by preoperative puncture. hematoxylin-eosin stain; original magnification ×200. B: Hematoxylin-eosin stain; original magnification ×400. The tumor cells were arranged in the adenoid structure. The system and size of gland were irregular. Cell atypia and deeply stained nuclear were significantly observed. C: Ck (one of immunohistochemistry) of tumor cells was remarkably positive.

ALLIC BFM2008 protocol on April 24, 2014. Fortunately, timely allogeneic haematopoietic stem cell transplantation (HSCT) was carried out in October 2014. The infant is currently in remission.

Case 2, a male infant, one year and nine months old, was admitted with a left abdominal mass for more than one month on September 19, 2011. Left Wilms' tumor was indicated by the renal pathology biopsy of other hospital

(Figure 4). Then the baby experienced 2 cycles chemotherapy, firstly with THP (50 mg/m^2 on day 1), VCR (1.5 mg/m^2 on day 1) and Act-D (15 ug/kg on day 1 to day 5); secondly with Etoposide (VP16) (100 mg/m^2 on day 1 to day 5), Cisplatin (DDP) (20 mg/m^2 on day 1 to day 5) since March 2013 at the local hospital. Giant left renal tumor resection plus retroperitoneal lymph node dissection were carried out on September 22, 2011 at our hospital. Because the pathological tissues of resection were all
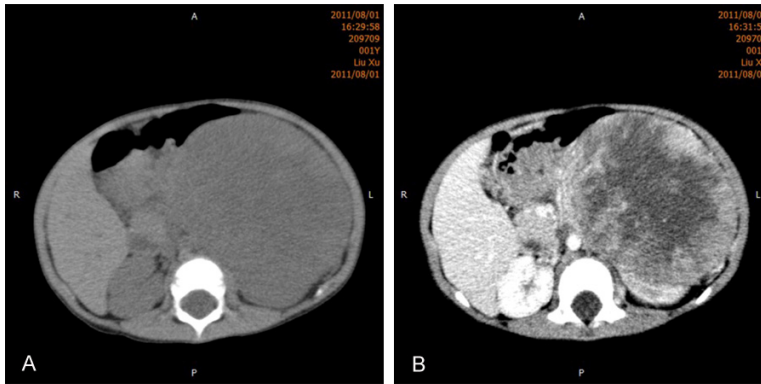
Figure 5. Initial abdominal CT of case 2. A: CT plain scan, B: CT contrast enhanced scan. Huge mass in the left kidney (121 mm×113 mm×105 mm). The density of the lesions was heterogeneous. The lower density cystic shadow could be seen. Normal renal tissue was obviously compressed. The left renal artery was wrapped by the mass and several abdominal aortic lymph nodes were enlarged.

necrotic, Wilms' tumor was definitely confirmed by previous the renal pathology biopsy and abdominal CT scan (**Figure 5**), on top of adjuvant postoperative chemotherapy until March 14, 2012. After discharge from our hospital, the baby suddenly developed recurrent high fever (Tmax 38.9°C) accompanied with retro auricular lymph nodes swelling on April 18, 2014. A mass in the right upper abdomen was also observed by his parents. Blood examination manifested moderate anemia (Hb 79 g/L). Multiple swelling lymph nodes between the postcava and aortaventralis were obviously hinted by abdominal CT in our hospital, suggesting probable tumor metastasis. Peripheral and bone marrow smear analysis revealed anabnormal increase in the amount of lymphoblast and Immature lymphocytes (36% and 95%, respectively) (**Figure 6A**), which indicated ALL (L2). Flow cytometry showed abnormal B cells (27%) (**Figure 6B-F**). Ig and TCR Gene rearrangement detection indicated IgVH, TCRγ and TCRδ genes had rearranged. Additionally, thirty leukemia fusion genes were all negative. FISH indicated MLL and BCR/ABL fusion genes were both negative. Based on WHO classification, t-ALL (L2, BIII, high-risk) were confirmed. Regular chemotherapy was put into effect according to the modified ALLIC BFM 2008 protocol on May 14, 2014. Thrombosis on the precava and right atrium were prompted by cardiac ultrasound and chest CT on October 20, 2014. Immediately, heparin was used for anticoagulation. Then after one day, urokinase was applied to thrombolysis. Unfortunately, the patient died from pulmonary embolism and disseminated intravascular coagulation (DIC).

Additionally, due to myelo suppression, we injected these two patients with recombinant human granulocyte-colony stimulating factor (rHu-G-CSF).

Discussion

WT, or nephroblastoma, is an embryonal tumor of the kidney remarkable for its replication in early renal development. WT is the most common pediatric renal cancer and is the second most widely observed in all pediatric abdominal tumors. WT, accounting for more than 95% of all pediatric renal cancers, occurs mostly before the child is five years old. The peak incidence is between 2 to 3 years old [13]. The estimated annual incidence is one child in every 10,000 before the age of 15 years in Europe and North America in the United States [14], while approximately 100 children occur annually in Germany [15]. With the continuous improvement of current treatment strategies, patients with WT can be cured with a high event free survival of over 90% [16]. However, a long-term therapy is required for fear of relapse and/or SMN [17].

We herein report two pediatric patients who were both hospitalized for an observed abdominal mass. Poorly differentiated WTs were confirmed by the affected renal fine needle aspiration biopsy. The diseased renal resection and retroperitoneal lymph node dissection were promptly carried out after two cycles of stage-specific preoperative chemotherapy by our expert surgeon. Postoperative pathology confirmed that case 1 was an uncomplicated nephroblastoma, while the examined pathological tissue of case 2 was accompanied with necrosis. Adjuvant postoperative chemotherapy was immediately implemented on the same day of the pathological report. But, it is so unfortunate that therapy-related B-ALL happened in these two pediatric cases after treatment. The intervals from primary WT to secondary B-ALL were 12 months and 30 months, respectively. SMNs were verified by pathological ex-
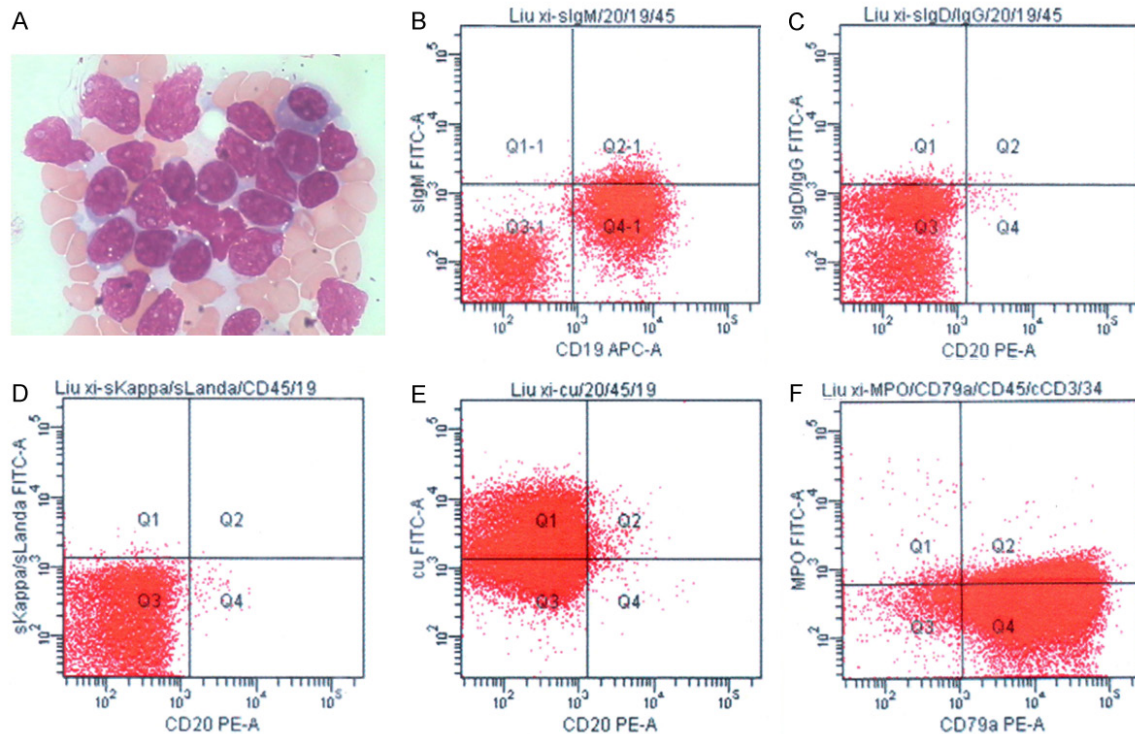
Figure 6. Bone marrow hemocytology (A) and flow cytometry (B) of Case 2. (A) The result revealed an abnormal increase in the amount of lymphoblast and Immature lymphocytes (36% and 95%, respectively). (B-F) The result showed abnormal B cells.

amination, which eliminated the emerging tumor was metastatic carcinoma.

According to an international collaborative study, the patients with WTs developed secondary solid tumors, such as lymphoma, breast cancer, thyroid cancer, and so on. Some patients developed 3 or even 4 secondary solid tumors. Also, t-AL, accompanied with malignant solid tumors, happened in a very few patients. There was a marked difference between secondary solid tumors and secondary leukemias in the age-time incidence patterns. For solid tumors, the risk increased sharply with age and time since WT had been confirmed, while decreased with calendar period of WT diagnosis; For leukemias, the risk was highest during the few years immediately following the WT diagnosis [18], above all in the first 5 years [19], which is in accordance with these two cases reported here. t-ALs account for 15%-20% of all patients with SMN following WT, which have been reported only in t-AML [20].

Based on WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues, t-MNs are serious long-term consequences of cytotoxic treatments for primary tumors and autoimmune diseases. t-MNs comprise t-MDS, t-AML and myelodysplastic/myeloproliferative neoplasm [21]. t-MNs happen in ionizing radiation and/or conventional chemotherapy including alkylating agents, topoisomerase-II-inhibitors and antimetabolites. It is commonly observed in chemotherapeutic and/or radiotherapeutic treatment for primary malignant hematologic diseases (mainly hodgkin lymphoma and non-hodgkin lymphoma), and solid tumors (mainly breast cancer, ovarian cancer, prostate cancer). Moreover, t-MNs have also been observed in patients receiving immunosuppressive treatment for rheumatologic/autoimmune diseases or solid organ transplantation [22, 23]. t-MDS/AMLaccounts for 10%-2% of all AML. 70% of t-AML transforms from t-MDS, while 20% of t-AML is primary AML [24]. Compared with primary AML, the prognosis of t-AML is much worse. The median survival time of t-AML is 6-8 months and the five-year survival rate is only 10% [25]. Most secondary leukemia are myeloid, however, recently, t-ALL make up a small but growing body, account-

ing for approximately 12% of all t-AL with or without the MLL gene rearrangement [26, 27]. No data, so far, is available on secondary B-ALL in children whether in mainland China or in other countries. In addition, nearly all cases are reportedly associated with translocations involving chromosome 11q23, the site of the MLL gene. This translocation appeared to occur in patients treated with or without DNA topoisomerase II inhibitors [28]; however, as far as we know, amp (MLL) has not been previously reported in t-ALL. Therefore, it should be noted this case 1 was MLL-ENL positive, which is extremely uncommon.

So far, t-AL secondary to WT, of which the definite pathogenesis is still unknown, might be related to joint multiple chemotherapy and hypoimmunity. At present, alkylating agents were considered as the first chemotherapeutic compounds to be associated with leukaemia development after successful treatment of solid and haematological cancers [29]. They induce DNA damage by transferring alkyl groups to oxygen or nitrogen atoms of DNA bases, resulting in highly mutagenic DNA base lesions [30]. In addition to DNA base lesions, they also can form intra- and interstrand crosslinks by attacking two bases within the same or on opposing DNA strands respectively. During replication, interstrand crosslinks stall replication forks, which can result in the formation of DNA DSBs. If misrepaired or left unrepaired, DNA DSBs can give rise to translocations, inversions, insertions and loss of heterozygosity [31]. A large sample of ovarian cancer study, among the women who received platinum-based combination chemotherapy, the relative risk of secondary leukemia was 4.0. Evidence of a dose-response relation was observed, with relative risks reaching 1.9, 2.1, 4.1, 7.6, respectively at doses of less than 500 mg, 500 to 749 mg, 750 to 999 mg, 1000 mg or more of platinum [32]. t-AML induced by alkylating agents mostly transfers from t-MDS, which is slow onset and usually occurs in 5 to 7 years after the initial treatment [33]. A total or partial deletion were discovered in chromosome 7 or 5 [34]. Case 2 used Cisplatin at the second cycle of chemotherapy.

Besides alkylating agents, DNA topoisomerase inhibitors were affirmed as inducing a distinct form of secondary leukemia [35]. DNA topoisomerases are critical enzymes responsible

for unknotting and relaxing supercoiled DNA, thus allowing DNA replication to occur. To relax supercoiled DNA, topoisomerases bind covalently to the DNA strand and create transient single (type I topoisomerases) and DSBs (type II topoisomerases). These DNA strand breaks are readily religated after topoisomerases are released from the DNA [36]. However, persistent DNA DSBs are also highly mutagenic and can result in chromosomal deletions, insertions, inversions and translocations, all of which are characteristic of the leukaemic cell clone [37]. According to a previous report, among the patients with breast cancer who received DNA topoisomerase inhibitor-based combination chemotherapy, the incidence rate of t-AL was 0.4%, which rose with the increase of accumulated dose. The incidence rate of t-AL was as high as 9.94% at doses of 900 mg/m^2 [38]. Thus, these two cases both applied pirarubicin and case 2 used etoposide in the second cycle. Pirarubicin and etoposide are both anthracyclines, belonging to DNA topoisomerase II inhibitor. Incubation period of t-AL induced by DNA topoisomerase II inhibitor was about two years, usually without first expression of MDS [39]. Compared with t-ALs induced by alkylating agents and radiation, balanced translocation of chromosome was apt to occur in t-AL induced by DNA topoisomerase II inhibitor [40]. The karyotype of t(8;21) and t(15;17) were common [41]. Moreover, t(9;11) of the MLL gene in chromosome 11q23 was the most popular [42].

These two children both utilized rHu-G-CSF because of myelo-suppression. Nevertheless, unexpectedly, several latest researches have expressed concerns about an increased risk of developing secondary leukaemia in patients receiving G-CSF during chemotherapy [43-45]. As is known to all, G-CSF stimulates the proliferation of granulocytic progenitors and promotes their differentiation into mature neutrophils [46]. It also causes premature release of neutrophils from the bone marrow and enhances their capacity for phagocytosis. The patients treated with chemotherapy usually present with severe myelo suppression, which are apt to sepsis and even sepsis shock. G-CSF has been widely used to reduce the severity and the duration of neutropenia, infection-related mortality and increase the success rate of chemotherapy. But, G-CSF might promote secondary tumors by two mechanisms below:

Firstly, G-CSF-induced production and release of bone marrow neutrophils may result in increased DNA damage and mutation rates in HSPC [47]. Secondly, repeated application of G-CSF may cause a continuous discharge of neutrophile granulocyte from their protective bone marrow niche, which may render them more susceptible to genotoxicity [48]. However, according to the international chemotherapy scheme, the intensity of treatment in the children with neuroblastoma is stronger than that of the patients with WT. Thus, theoretically, the children with neuroblastoma after chemotherapy are liable to myelosuppression, which need more G-CSF to resist agranulocytosis. Nonetheless, why is few data available on t-AL in the children with neuroblastoma? After treatment with similar chemotherapy, why does secondary tumor occur in only a small portion rather than all of the patients? We speculate the answer might be linked with a genetic susceptibility to cancer. Genetic predisposition to t-AL is regarded as a complex trait determined by multiple pathogenetic variants and their interaction with specific exogenous toxicities [49].

t-AL has also been reported in the adult population. Studies have reported breast cancer patients, and ovarian cancer patients who accepted DNA topoisomerase inhibitor-based joint chemotherapy, and platinum-based combination chemotherapy respectively observed an increased incidence rate of secondary leukemia with the growing dose of chemotherapeutics was reported [32, 38]. Nonetheless, until now, there is no report on secondary tumor due to the excessive chemotherapy. Even so, case 2 in this article underwent excessive preoperative chemotherapy, which could not be ruled out a possible certain relationship with secondary leukemia. It needs further in-depth study. Furthermore, chemotherapeutics can not only inhibit the body immunity surveillance but can also damage the body immune function, which provides the conditions for the proliferation of tumor cells.

t-AL, characterized by high early mortality, progresses rapidly and tolerates the conventional chemotherapy [50, 51]. According to random group study, the survival median age is 8-10 months and the five-year survival rate is less than 10% [52, 53]. The causes of insensitivity to treatment are the primary or secondary drug resistance. Recently, Lenalidomide, an immu-nomodulatory agent, has been approved by the US Food and Drug Administration for patients with low-risk therapy-related leukemia, which is associated with interstitial deletion of the long arm of chromosome 5. Azacytidine, a DNA methyltransferase inhibitor, has significantly improved overall survival in patients with high-risk t-AML with low bone marrow blast counts [54, 55]. But cases for clinical trials are still insufficient, the further study is needed.

With the significant improvement of allogeneic stem cell transplantation, in order to achieve long-term disease-free survival, HSCT is the only way for the majority of patients with leukemia [56]. It is gratifying! Recent studies by The European group for blood and marrow transplantation and Center for international blood and marrow transplant research have shown that the total five-year survival for patients treated with HSCT were respectively 63% and 50% [57, 58]. Attributing to early diagnosis and treatment of secondary leukemia, the mortality declines and the disease-free survival prolongs. Therefore, timely HCST may be the best way for this kind of patients.

All in all, the combined application of alkylating agents, topoisomerase II inhibitor, G-CSF, as well as single drug cumulative dose may be the important reasons for these two cases who occurred to be t-All. Aiming to prolong the survival time of tumor patients, under the premise of no influence of therapeutic effect of the primary tumor, we aim to research on how to prevent and reduce the occurrence of t-AL, which is a new subject in tumor discipline.

Disclosure of conflict of interest

None.

Abbreviations

t-AL, Therapy-related acute leukemia; ALL, Acute lymphoblastic leukemia; t-ALL, Therapy-related acute lymphoblastic leukemia; AML, Acute myeloidleukemia; t-AML, Therapy-related acute myeloidleukemia; B-All, B-acute lymphoblastic leukemia; WT, Wilms' tumor; WHO, World Health Organization; HSCT, Haematopoietic stem cell transplantation; SMN, Secondary malignant neoplasms; t-MDS, Therapy-related myelodysplastic syndrome; T-LBL, T lymphoblastic lymphoma; THP, Pirarubicin; VCR, Vincristine; Act-

D, Actinomycin D; VP16, Etoposide; DDP, Cisplatin; FISH, Fluorescence in situ hybridization; MLL, Mixed lineage leukemia; DIC, Disseminated intravascular coagulation; rHu-G-CSF, Recombinant human granulocyte-colony stimulating factor.

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