


Characteristics of Central Nervous System (CNS) Involvement in Children With Non-Hodgkin's Lymphoma (NHL) and the Diagnostic Value of CSF Flow Cytometry in CNS Positive Disease

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Abstract

Objective: To investigate the characteristics of central nervous system (CNS) involvement in children with non-Hodgkin's lymphoma (NHL) and the value of flow cytometry (FC) in the diagnosis of CNS disease in pediatric NHL. **Methods:** The data of 56 newly diagnosed pediatric NHL patients with CNS involvement (CNS+/mass, CNS+/palsy, CNS+/CSF) were analyzed. The proportions and formats of CNS disease in different pathological types were compared. In addition, FC and conventional cytology (CC) of cerebrospinal fluid (CSF) were carried out in 383 newly diagnosed NHL cases. **Results:** A total of 383 children with NHL were enrolled. Among these patients, 56 (14.6%) were diagnosed with positive CNS involvement (CNS+), 33 had bulky disease (tumor diameter >10 cm), 32 had bone marrow invasion, 32 had lactate dehydrogenase levels >1000 U/L, and 25 had invasion of more than 4 organs at the time of diagnosis. There were 14 patients with T lymphoblastic lymphoma (T-LBL), 9 with B lymphoblastic lymphoma (B-LBL), 26 with Burkitt's lymphoma (BL), and 2 with Epstein-Barr virus-positive diffuse large B cell lymphoma (EBV + DLBCL). Among the 56 CNS+ patients, 35 were CSF-positive (CSF+); there were 2 patients who were CSF+ via CC detection and 35 who were CSF+ via FC detection. The difference between CC and FC was statistically significant ($P < 0.01$). In the T-LBL group, 14 patients were CNS+/CSF, and in the B-LBL group, 8 were CNS+/mass. In the BL group, 22 patients were CNS+/mass and 15 were CNS+/CSF. In the anaplastic large-cell lymphoma group, 5 patients were CNS+/mass. Nine of the 56 CNS+ patients had events. The 2-year overall survival rate was $87\% \pm 0.046\%$, and the 2-year event-free survival rate was $76.2\% \pm 0.07\%$. **Conclusion:** CNS+ diagnoses were more common in pediatric NHL patients with bulky disease and/or bone marrow involvement and/or involvement of more than 4 organs at the time of diagnosis, and they were also common in the EBV + DLBCL and BL groups. FC of CSF showed important clinical significance in the diagnosis of CNS disease in pediatric NHL patients, and it can be used to significantly improve the CNS+ detection rate.

Keywords

flow cytometry, cerebrospinal fluid, central nervous system involvement, children, non-Hodgkin's lymphoma

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Introduction

Central nervous system (CNS) involvement is a poor prognostic factor of non-Hodgkin's lymphoma (NHL) in children. Therefore, early diagnosis and treatment of CNS disease is important to improve long-term disease-free survival rates in children.¹⁻³ With the continuous progress of science and technology, the methods and technologies used for the diagnosis of CNS disease are constantly improving, from imaging and cerebrospinal fluid

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(CSF) morphology studies to cerebrospinal fluid CSF flow pattern and genetics studies, and so on. With the continuous progress of diagnosis technology, the clinical staging of patients, which has a vital role in follow-up treatments, has also become more accurate. In 2015, the international organization for pediatric NHL redefined the diagnostic criteria for CNS involvement⁴: cranial nerve palsy symptoms, intracranial and intraspinal space-occupying lesions on imaging, and lymphoma tumor cells detected by CSF smear. Most patients with early CNS invasion have no obvious clinical manifestations. Therefore, in the diagnosis of CNS invasion, the results of CSF analysis are particularly important. Some studies have showed that it is a common occurrence in CNS cancers that the tumor DNA shed into CSF. A recent study also investigated that when resect primary brain or spinal cord tumors the CSF was collected, 26 out of 35 CSF samples (74%) contained tumor DNA, and another study confirmed that oncogenic mutations in tumor derived DNA was found from CSF in a small number of patients.⁵⁻⁷ While these studies suggest that the shedding of tumor DNA into CSF may be a frequent occurrence in CNS cancers, it is unclear whether comprehensive sequencing of CSF can routinely and reliably identify clinically relevant genomic alterations without prior knowledge of mutations in the primary tumor and whether this can be done successfully without the need for surgery in a large series of patients.⁷ Improving the diagnosis of positive CNS involvement (CNS+) and strengthening the treatment of CNS disease are key factors to improve the prognosis of the disease. Therefore, to improve the diagnostic rate of CNS invasion, FC and conventional cytology (CC) of CSF were performed in newly diagnosed NHL patients for the first time of intrathecal since April 2017 in our center. This article summarizes the clinical characteristics, treatment protocols, and prognoses of NHL patients with CNS disease who were newly diagnosed in our center from April 2017 to December 2019. Moreover, the value of FC in the diagnosis of CNS disease is explored.

Materials and Methods

Patients

A total of 383 newly diagnosed NHL patients in Beijing Children's Hospital (BCH) from April 2017 to December 2019 were collected, and the patients with CNS disease were selected. This study was discussed and approved by the ethics committee of our hospital (iec-c-006-a03-v.05). All subjects gave signed informed consent via their guardians.

Diagnosis of Lymphoma

Tumor biopsy tissues and/or bone marrow tissues were taken for pathological sections, and immunohistochemical and fluorescence in situ hybridization (FISH) examinations were performed at the same time. The pathological data were reviewed by pathology experts from more than 2 hospitals, and treatment was started only after the diagnosis was confirmed. According

to the histological and/or bone marrow pathology findings, BM were classified by morphology, immunology, cytogenetics, and molecular biology.

Diagnostic Criteria and Staging of Central Nervous System Disease

The revised international pediatric NHL staging system (IPNHLSS⁷) was used.

CNS involvement was considered to be when there was CNS tumor mass (identified by imaging techniques); when there was cranial nerve palsy that could not be explained by extradural lesions; and when blasts were morphologically identified in CSF. CNS positivity conditions were thus defined as: CNS+/mass, CNS+/palsy, and CNS+/blasts. CSF+ determinations were based on the morphological evidence of lymphoma cells. The types of CSF involvement were determined as: CSFm, CSF positivity by morphology; CSFi, CSF positivity by immunophenotyping; CSFc, CSF positivity by cytogenetic or FISH analysis; and CSFmol, CSF positivity by molecular techniques.

Treatment Protocols

Burkitt's lymphoma (BL): BCH 2010 mature B-cell lymphoma C-CNS+ group (modified from LMB 96 C1 protocols).

For Epstein-Barr positive-diffuse large B cell lymphoma (EBV + DLBCL), the treatment was the same as that of the BL group.

Lymphoblastic lymphoma (LBL): BCH 2010 lymphoblastic lymphoma (modified from BFM-LBL95 protocols).

Anaplastic large-cell lymphoma (ALCL): BCH 2010 anaplastic large-cell lymphoma (modified from BFM-ALCL99 protocols, methotrexate 5 g/m² ± alecetinib).

Evaluation Criteria

Complete remission was considered to be complete disappearance of the tumor; partial remission was considered to be tumor shrinkage more than 50%, but not complete remission; stable disease was considered to be tumor shrinkage less than 50% or tumor increase not more than 25%; disease progression was considered to be when tumor enlargement was more than 25%; and recurrence was considered to be when new lesions reappeared after the disease reached complete remission.⁸

Cerebrospinal Fluid Detection Methods

For CC detection of CSF, 0.1 mL of CSF was blown evenly, dropped into a centrifuge tube, fixed with filter paper, and paddled together, and a CSF smear was obtained by using a centrifugal smear machine. After drying, Wright-Giemsa staining was performed, and cell morphology was observed with a microscope. The results were negative in immature leukemia.

For FC detection of CSF, the monoclonal antibody was selected according to the immunophenotypic expression of

Table 1. Baseline Information of the 56 CNS+ Patients With NHL.

Pathology	NHL (n)	(n) %	M	F	CNS+ with NHL Bulky disease	BM+	≥4 organs involved	LDH ≥1000 U/L	ATLS
T-LBL	103	14 (13.6%)	14	0	14	14	12	11	14
B-LBL	54	9 (16.7%)	3	6	2	8	1	2	2
BL	135	26 (19.2%)	20	6	17	9	12	19	18
ALCL	47	5 (10%)	3	2	0	0	0	0	0
EBV + DLBCL	4	2 (50%)	2	0	0	1	0	0	0
Total	383	56 (14.6%)	42	14	33	32	25	32	34

Abbreviations: CNS, central nervous system; NHL, non-Hodgkin's lymphoma; LBL, lymphoblastic lymphoma; BL, Burkitt's lymphoma; ALCL, anaplastic large-cell lymphoma; EBV, Epstein-Barr virus; +, positive; M, male; F, female; BM, bone marrow; LDH, lactate dehydrogenase; ATLS, acute tumor lysis syndrome.

tumor pathological tissue at the time of initial diagnosis. The expression of monoclonal antibodies labeled with fluorescein and FC antigen, the size of the cells, and the number of particles in the cells were used to identify normal and abnormal cells. The monoclonal antibody and matching reagent were prepared by BD company (United States), as was the instrument. CellQuest Pro and FACSDiva software were used for analysis. After centrifugation, the supernatant was discarded, and red cell lysate was added for 3 minutes. Phosphate-buffered saline was used to wash the cells until the solution ran clear. Then, 20 μ L of monoclonal antibody was selected and reacted in the dark for 15 minutes at room temperature. Phosphate-buffered saline was used to fix the volume at 1 mL. All CSF samples were sent to the Lu Daopei Hospital of Yan Da, Hebei Province, for detection studies. The antibodies used for different lymphoma subtypes were: (1) BL: CD38, CD10, CD34, CD19, CD45, CD20, kappa, lambda, CD19, CD10, and bcl-2; (2) T/NK and ALCL: CD7, CD2, CD3, CD5, CD4, CD56, CD8, CD45, CD57, CD30, and ALK; (3) B-LBL: CD38, CD10, CD34, CD19, CD13+33, CD45, CD20, CD34, and CD22; and (4) T-LBL: CD34, CD2, CD3, CD4, CD5, CD45, CD8, CD7, CD99, CD56, and CD3.

Regarding FC positivity, if the lumbar puncture was successful without injury and if CSF was not mixed, then the immunophenotype of leukemia cells could be detected. If CSF was mixed or bloody, then the immunophenotype of leukemia cells was higher than that of peripheral blood immature cells. Regarding FC negativity, if the lumbar puncture was successful without injury and if no abnormal immunophenotype was found on FC of CSF, then the abnormal immunophenotype of CSF was lower than that of peripheral blood immature cells.

Follow-Up

The patients were followed up regularly in the lymphoma clinic, and follow-up was continued until March 31, 2020. Event-free survival (EFS) time was defined as the period from the date of diagnosis to the date of primary disease progression/recurrence, chemotherapy-related death, or follow-up endpoint. The overall survival (OS) time was defined as the period from the date of diagnosis to the date of death due to any cause or the end of follow-up.

Statistical Analysis

SPSS 20.0 statistical software was used for data processing. If the quantitative data obeyed a normal distribution, then the mean \pm standard deviation was used to represent the data, and the t-test was used to compare 2 groups. If normal distribution was not obeyed, then the median (minimum to maximum) was used to represent the data, and the Wilcoxon rank sum test was used for intergroup comparison. Classification data were described by frequency (percentage), and comparisons between groups were performed by using the chi-squared test. The Kaplan-Meier method was used for survival analysis. Cox regression was used to screen risk factors, and when the *P*-value was <0.05 , the difference was considered statistically significant.

Results

Clinical Data

A total of 383 NHL patients were included in this study, including 103 T-LBL patients, 54 B-LBL patients, 35 BL patients, 13 DLBCL patients, 11 high-grade B-cell lymphoma patients, 2 follicular lymphoma patients, 4 EBV + DLBCL patients, 47 ALCL patients, and 14 peripheral T-cell lymphoma patients. A total of 56 patients (14.6%) were diagnosed as CNS+, including 41 males and 15 females, with an average age of 7.3 years (range, 2.5-13.7 years). In the T-LBL group, 14 patients (13.6%) were CNS+; in the B-LBL group, 9 patients (16.6%) were CNS+; in the BL group, 26 patients (19.2%) were CNS+; in the EBV + DLBCL group, 2 patients (50%) were CNS+; and in the ALCL group, 5 patients (11%) were CNS+. Among the 56 CNS+ patients, 33 had bulky disease (tumor diameter > 10 cm), 32 had bone marrow invasion, 32 had lactate dehydrogenase (LDH) levels >1000 U/L, 25 had invasion of more than 4 organs, and 34 had tumor lysis syndrome in the early stage of chemotherapy. Further, 35 of the 56 patients had CSF positivity. Specifically, 14 patients with CSF positivity were in the T-LBL group; 5 were in the B-LBL group, 1 was in the EBV + DLBCL group, and 15 were in the BL group. See Table 1 for details.

Table 2. Detection of CNS Invasion in the 56 CNS+ Patients With NHL.

CNS invasion detection method	EBV + DLBCL (n = 2)	T-LBL (n = 14)	B-LBL (n = 9)	BL (n = 26)	ALCL (n = 5)	Total (n = 56)
Mass	1	0	2	2	3	8
Paralysis	0	0	0	1	0	1
CSF+	1	13	1	3	0	18
Mass & paralysis	0	0	2	8	2	12
Mass & CSF+	0	1	4	3	0	8
Paralysis & CSF+	0	0	0	0	0	0
Mass & paralysis & CSF+	0	0	0	9	0	9

Abbreviations: CNS, central nervous system; NHL, non-Hodgkin's lymphoma; EBV, Epstein-Barr virus; +, positive; DLBCL, diffuse large B cell lymphoma; CSF, cerebrospinal fluid; LBL, lymphoblastic lymphoma; BL, Burkitt's lymphoma; ALCL, anaplastic large-cell lymphoma.

Table 3. Comparison of CC and FC.

Pathology	% lymphoma		CC+	X2	P-value	
	CSF+	FC+				
T-LBL	14	14	1-14.5 (3.5)	2	90.012	0.023
B-LBL	5	5	1-4.6 (2.3)	0	-	-
BL	15	15	0.3-4.5 (2.5)	0	-	-
EBV + DLBCL	1	1	2.6	0	-	-
Total	35	35		2	-	-

Abbreviations: CC, conventional cytology; FC, flow cytometry; CSF, cerebrospinal fluid; LBL, lymphoblastic lymphoma; BL, Burkitt's lymphoma; EBV, Epstein-Barr virus; DLBCL, diffuse large B cell lymphoma; +, positive.

Diagnosis of Central Nervous System Invasion

In the T-LBL group, 14 patients were CSF+, and 1 was CNS+/mass. In the B-LBL group, 8 patients were CNS+/mass, and 5 were CNS+/CSF. In the BL group, 26 patients were CNS+/mass, and 15 were CNS+/CSF. In the ALCL group 5 patients were CNS+/mass. In the EBV + DLBCL group, 1 patient was CNS+/mass, and 1 was CNS+/CSFi. See Table 2 for details.

Flow Cytometry and Conventional Cytology for Cerebrospinal Fluid Detection

FC and CC of CSF were performed for all 383 newly diagnosed NHL patients. A total of 35 patients were CSF+. All 35 of these patients were also identified as CSF+ by the FC method, while only 2 were identified as CSF+ by the CC method. The difference between the FC and CC methods was statistically significant. In the T-LBL CNS+ group, 14 patients (100%) were identified as CSF+ by the FC method, and 2 were detected as CSF+ by the CC method; the difference was statistically significant ($P < 0.01$). In the B-LBL CNS+ group, 5 patients were identified as CSF+ by the FC method, while the CC detection rate was negative. In the BL CNS+ group, 15 patients were identified as CSF+ by the FC method, while the CC detection rate was negative. In the EBV + DLBCL CNS+ group, only 1 case was identified as CSF+ by the FC method, while the CC test was negative. See Table 3 for details.

Follow-Up and Prognosis

During the follow-up until March 31, 2020, the average follow-up time was 15.96 months (3-36 months). In the BL group, 5 patients had events: 2 had CNS recurrence, 2 died of infection, and 1 developed a second tumor (acute myeloid leukemia). In the T-LBL group, 3 patients had events: 1 patient had CNS and bone marrow (BM) recurrence, and 2 had BM recurrence. In the EBV + DLBCL group, 1 patient had CNS recurrence. Of the 56 patients, 47 had complete remission. The 2-year OS rate was $87\% \pm 0.046\%$, and the 2-year EFS rate was $76.2\% \pm 0.07\%$. A total of 327 patients identified as CNS-negative in the same period were also analyzed. The 2-year OS rate was $90\% \pm 0.035\%$, and the 2-year EFS rate was $83.2\% \pm 0.05\%$, which were significantly higher than the rates of those identified as CNS+.

Discussion

The prognosis of NHL complicated by CNS invasion is poor, and the incidence rate is about 5% to 30%.⁹⁻¹¹ High-risk factors include: clinical stage III-IV bone marrow invasion or leukemia; pathological types that are mostly highly malignant and aggressive subtypes, especially BL; and pharyngeal lymph node involvement and ocular and maxillofacial invasion.¹ The clinical manifestations of CNS invasion are diverse and related to the affected site, including cerebral palsy, limb movement disorder, intracranial hypertension, and other symptoms. Those with spinal cord involvement may exhibit limb movement disorder and paraplegia.^{2,3} CNS invasion can be seen in various CNS regions, including the meninges, cranial nerves, brain parenchyma, spinal cord, or a combination of these, and meningeal invasion is the most common.⁹⁻¹¹ The incidence of CNS invasion is related to the pathological subtype and disease progression of lymphoma, and BL and LBL are the most common types in pediatric lymphoma to have CNS invasion.¹²⁻¹⁴ The median time of CNS invasion is 4 months (range, 1-13 months).^{9,14} The route of CNS invasion varies with the location of the tumor.^{9,10,15} The results of this study showed that the majority of school-age boys were boys, and the ratio of male to female patients was about 3:1. More than half of the patients exhibited giant tumor and bone marrow invasion, with more than 4 organs

involved at the time of initial diagnosis, and most of them were middle- and late-stage patients, which was consistent with the current literature. According to the pathological types, the results showed that the central invasion rates of the EBV + DLBCL and BL groups were significantly higher than those of other groups, which was also consistent with the literature.

In 2015, at the fifth international conference on NHL in children, adolescents, and young adults, the international pediatric NHL staging system was revised,⁴ and the diagnostic criteria for CNS involvement were updated: CNS+/mass, CNS+/palsy, and CNS+/blasts. In addition, CSF positivity was defined as CSFm, CSF+ by morphology; CSFi, CSF+ by immunophenotyping; CSFc, CSF+ by cytogenetic or FISH analysis; and CSFmol, CSF+ by molecular techniques (based on polymerase chain reaction).⁴ Therefore, since April 2017, all newly diagnosed NHL patients in our hospital have been evaluated per the above revisions, and increased the method of CSF immunophenotype method at the time of first intrathecal, and the above methods have significantly improved the diagnostic rate of CNS invasion and recurrence. After the diagnosis of CNS involvement, the results of this study showed that 100% of CNS+ children in the T-LBL group were CNS+/CSF, 88.9% of CNS+ children in the B-LBL group were CNS+/mass, 84.6% of CNS+ children in the BL group were CNS+/mass, 57.7% of CNS+ children in the BL group were CNS+/CSF, and all CNS+ children in the ALCL group were CNS+/mass. To sum up, the children with CNS invasion in the T-LBL group were mostly CNS+/CSFi, while in the B-LBL and ALCL groups, CNS invasion mostly showed as CNS+/mass. Therefore, for NHL children, cranial magnetic resonance imaging, spinal magnetic resonance imaging, positron emission tomography-computed tomography, and CSF detection are very necessary in the judgment of CNS involvement.

The results of this study showed that the proportion of CSF+ in the T-LBL group central invasion group was the highest (100%), followed by the BL and B-LBL groups. All these cases were diagnosed by FC. The difference between FC and CC was statistically significant. In conclusion, FC analysis of CSF is helpful in the diagnosis of CNS invasion, and its positive detection rate and accuracy are significantly higher than those of the traditional CC method, which is consistent with the current literature. FC has great application value in the diagnosis of CNS invasion, and it can be used to significantly improve the detection rate of severe CNS invasion, which is a poor prognostic factor of NHL. Therefore, early diagnosis and intensive treatment of CNS invasion are of great significance to improve the long-term disease-free survival rate of children.

Conclusion: Central invasion was found to be more common in pediatric NHL patients with bulky disease and/or BM involvement and/or involvement of more than 4 organs. It was also common in the EBV + DLBCL and BL groups. CSF detection by FC has important clinical significance in the diagnosis of

CNS invasion in children with NHL, as it can significantly improve the detection rate of CNS involvement.

Authors' Note

All analyses were based on previous published studies; thus, no ethical approval and patient consent are required. Guarantor of integrity of entire study: Huang Shuang; Study concepts: Huang Shuang and Jin Ling; Study design: Huang Shuang, Zhang yonghong; Literature research: Huang Shuang, Yang Jing, Duan yanlong; Statistical analysis: Huang Shuang, Zhang Meng; Manuscript editing: Huang Shuang, Zhou chunju; Manuscript revision/review Huang Shuang, Zhou chunju, Zhang Yonghong; Manuscript final version approval: Zhang Yonghong.


Declaration of Conflicting Interests

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