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Iron deficiency in children at the time of initial neuroblastoma diagnosis

Hongjun Fan* | Yan Su* | Chao Duan | Qian Zhao | Xisi Wang | Shuai Zhu | Wen Zhao | Mei Jin | Xiaoli Ma

Beijing Key Laboratory of Pediatric Hematology Oncology, National Discipline of Pediatrics, Ministry of Education, MOE Key Laboratory of Major Diseases in Children, Hematology Oncology Center, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China

Correspondence
Xiaoli Ma, Hematology Oncology Center, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing 100045, China.
Email: mxl1123@vip.sina.com

*These authors contributed equally to this work.

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ABSTRACT

Importance: There is a high incidence of iron deficiency in children worldwide. Notably, however, while iron deficiency is the most common cause of anemia, little is known about the prevalence and different types of iron deficiency in neuroblastoma patients.

Objective: The aim of the present study was to investigate the prevalence of iron deficiency in patients newly diagnosed with neuroblastoma.

Methods: A total of 195 newly diagnosed neuroblastoma patients from November 2015 to January 2018 were analyzed retrospectively. The survival analysis was estimated by the Kaplan-Meier method.

Results: Of the 195 neuroblastoma patients included in the study, 121 (62.1%) had iron deficiency, 55 (28.2%) had absolute iron deficiency, and 66 (33.9%) had functional iron deficiency. Being aged ≥ 18 months, tumor originating in the abdomen, International Neuroblastoma Risk Group Staging System M, high-risk neuroblastoma, lactate dehydrogenase ≥ 1500 U/L, neuron-specific enolase ≥ 100 U/L, unfavorable histologic category, *MYCN* amplification, chromosome 1p loss, and bone marrow metastasis were associated with significantly higher rates of functional iron deficiency ($P < 0.05$).

Interpretation: Functional iron deficiency at the time of initial neuroblastoma diagnosis predicted lower event-free survival. Long-term effects of iron supplementation in neuroblastoma patients with different types of iron deficiency need to be further studied.

KEYWORDS
Iron deficiency, Neuroblastoma, Event-free survival

INTRODUCTION

Neuroblastoma (NB) is a pediatric malignancy that affects 10.2 per million children under 15 years of age and is responsible for approximately 10%–12% of childhood cancer mortality.[1,2] The median age at NB diagnosis is approximately 19 months.[3] Although progress has been made in the treatment of high-risk NB, the 5-year overall survival rates is only 50%.[4]

The prevalence of iron deficiency (ID) in cancer patients ranges from 32% to 60% and most ID patients are also anemic.[5] Absolute ID (AID) and functional ID (FID) are the two most common types of ID in patients with cancer.[6] While chronic blood loss and poor nutrition deplete iron stores and cause AID, FID evidently occurs when cancer-induced inflammation elevates hepcidin, which may result in insufficient iron availability to erythrocytes despite adequate levels of total

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body iron.[6-8] Patients with advanced cancer are likely to have FID as the predominant etiology of anemia.[9] ID anemia is also associated with shorter survival times in some types of cancers, including colon cancer, lung cancer, and breast cancer.[10-12] Notably however, little is known about ID in NB patients. The aim of the present study was to identify the prevalence and causes of ID in patients newly diagnosed with NB.

METHODS

Ethical approval

The study was approved by the Ethics Committee of Beijing Children's Hospital and was conducted in accordance with the Helsinki Declaration. Written informed consent to participate in the study and for publication was obtained from all the patients' parents before their enrollment in this study. [Correction added on 17 March 2020, after first online publication. The Ethical approval section was added.]

NB patients

All patients included in this study were newly diagnosed with NB at the Hematology Oncology Center of Beijing Children's Hospital between November 2015 and January 2018. The inclusion criteria were the availability of iron status measurements including iron, serum ferritin (SF), unsaturated iron-binding capacity, total iron-binding capacity, transferrin and transferrin saturation (TSAT) all acquired at the time of initial NB diagnosis, and being aged > 6 months. Clinical data including gender, age, primary tumor site, risk stratification, bone marrow metastasis, and hemoglobin (Hb) were all collected at the time of initial NB diagnosis.

The original tumor and any metastatic tumor were evaluated via computed tomography, magnetic resonance imaging, technetium-99 m bone scanning, ^{18}F-labeled fluoro-2-deoxyglucose positron-emission computed tomography, bone marrow aspirates of the sternum and ilium, and biopsy specimens of the ilium. The patients were staged according to the International Neuroblastoma Risk Group Staging System (INRGSS).[13] In accordance with the relevant guidelines for the diagnosis, management, and prevention of ID and ID anemia in China,[14] anemia was defined as Hb < 110 g/L in children aged 6 months to 6 years, and Hb < 120 g/L in children aged between 6 years and 14 years. Anemia was classified as mild (Hb ≥ 90 g/L but < 110 g/L), moderate (Hb ≥ 60 g/L but < 90 g/L), or severe anemia (Hb < 60 g/L). ID was defined as TSAT < 20% and classified as AID or FID.[6] AID was defined as TSAT < 20% and SF < 100 ng/mL, and FID was defined as TSAT < 20% and SF ≥ 100 ng/mL.

The primary endpoint was event-free survival (EFS) based solely on recurrence or otherwise of the index cancer. Recurrence was confirmed via biopsy, bone marrow aspirates of the sternum, imaging, or a combination thereof. Follow-up was conducted for the entire study population to 31 December 2018. By the end of the follow-up period, 14 patients were lost to follow-up, resulting in a complete follow-up rate of 92.8%.

Statistical methods

Descriptive data are presented as absolute frequencies, and qualitative data are presented as percentages. For between-group comparisons, categorical variables were assessed via the Pearson's chi-square test or Fisher's exact test. Numerical variables were assessed via Student's *t*-test. Multinomial logistic regression analysis was performed to identify the risks of ID at the time of initial NB diagnosis. Survival plots were constructed using the Kaplan-Meier method.

All statistical analyses were completed using SPSS for Windows, version 19.0 (SPSS, Chicago, IL, United States), and $P < 0.05$ was deemed to indicate statistical significance.

RESULTS

A total of 195 patients newly diagnosed with NB were included in the study. Baseline characteristics of the patients including sex, age, primary tumor site, stage, risk stratification, anemia, bone marrow metastasis, and ID are shown in Table 1. The median age at the time of initial NB diagnosis was 35.0 months (range 6–148 months). The risk stratification rates were 10.3% for low risk, 28.2% for intermediate risk, and 61.5% for high risk. Anemia was observed in 128 of the 195 patients (65.6%), and 94 (48.2%) had bone marrow metastasis at the time of initial NB diagnosis. ID was identified in 121 of the 195 patients (62.1%), and among these 55 (28.2% of the total cohort) had AID and 66 (33.9% of the total cohort) had FID.

Gender distribution, age, primary tumor site, INRGSS stage, risk stratification, pathological types, histologic category, *MYCN* status, chromosome 1p status, and bone marrow metastasis in patients with AID and FID are shown in Table 2. Being aged ≥ 18 months, INGRSS M, high-risk NB, lactate dehydrogenase ≥ 1500 U/L, neuron-specific enolase ≥ 100 U/L, unfavorable histologic category, *MYCN* amplification, chromosome 1p loss and bone marrow metastasis were significantly associated with FID ($P < 0.05$). The FID rate in the high-risk group was 47.5%, which was higher than that in the low-risk group (10.0%) and the intermediate-risk group (12.8%) (Figure 1).

Figure 2 shows the EFS curves of NB patients with AID and FID. EFS of patients with FID was significantly lower than that of patients with AID ($P < 0.05$). All the statistically significant factors identified in univariate analysis were included in multivariate analysis (Table 3),

TABLE 1 Clinical characteristics of neuroblastoma patients (n = 195)

Characteristics	Number of patients, *n* (%)
Gender	
Male	100 (51.3)
Female	95 (48.7)
Age	
< 18m	31 (15.9)
≥ 18m	164 (84.1)
Primary tumor site	
Abdomen	136 (69.7)
Extra- abdomen	59 (30.3)
Stage	
L1	28 (14.4)
L2	36 (18.5)
M	119 (61.0)
MS	12 (6.2)
Risk stratification	
Low	20 (10.3)
Intermediate	55 (28.2)
High	120 (61.5)
LDH	
< 1500 U/L	164 (84.1)
≥ 1500 U/L	31 (15.9)
NSE	
< 100 U/L	77 (39.5)
≥ 100 U/L	118 (60.5)
Pathological types	
Neuroblastoma	110 (56.4)
Ganglioneuroblastoma	72 (36.9)
Unknown	13 (6.7)
Histologic category	
Favorable	62 (31.8)
Unfavorable	109 (55.9)
Unknown	24 (12.3)
MYCN status	
Amplified	22 (11.3)
Nonamplified	157 (80.5)
Unknown	16 (8.2)
Chromosome 1p loss	
No	117 (60.0)
Yes	29 (14.9)
Unknown	49 (25.1)
Anemia	
No	67 (34.4)
Yes	128 (65.6)
Mild	76 (39.0)
Moderate	49 (25.1)
Severe	3 (1.5)
BM metastasis	
No	101 (51.8)
Yes	94 (48.2)
Iron deficiency	
No	74 (37.9)
Yes	121 (62.1)
AID	55 (28.2)
FID	66 (33.9)

LDH, lactate dehydrogenase; NSE, neuron-specific enolase; BM, bone marrow; AID, absolute iron deficiency; FID, functional iron deficiency

TABLE 2 Characteristics of newly diagnosed neuroblastoma patients with absolute versus functional iron deficiency

Characteristics	AID, *n* (%)	FID, *n* (%)	*P*
Gender			
Male	28 (41.2)	40 (58.8)	0.284
Female	27 (50.9)	26 (49.1)	
Age			
< 18m	17 (73.9)	6 (26.1)	0.002
≥ 18m	38 (38.8)	60 (61.2)	
Primary tumor site			
Abdomen	38 (35.9)	47 (64.1)	0.008
Extra-abdomen	17 (62.8)	19 (37.2)	
Stage			
L1/L2/MS	39 (76.5)	12 (23.5)	< 0.001
M	16 (22.9)	54 (77.1)	
Risk stratification			
Low- Intermediate	39 (81.3)	9 (18.7)	< 0.001
High	16 (21.9)	57 (78.1)	
LDH			
< 1500 U/L	53 (49.1)	51 (50.9)	0.003
≥ 1500 U/L	2 (11.8)	15 (88.2)	
NSE			
< 100 U/L	42 (79.2)	11 (20.8)	< 0.001
≥ 100 U/L	13 (19.1)	55 (80.9)	
Pathological types			
Neuroblastoma	33 (46.5)	38 (53.5)	0.907
Ganglioneuroblastoma	20 (47.6)	22 (52.4)	
Histologic category			
Favorable	27 (73.0)	10 (27.0)	< 0.001
Unfavorable	25 (34.2)	48 (65.8)	
MYCN status			
Amplified	50 (51.5)	47 (48.5)	0.023
Nonamplified	2 (16.7)	10 (83.3)	
Chromosome 1p loss			
No	37 (50.0)	37 (50.0)	0.026
Yes	1 (9.1)	10 (90.9)	
BM metastasis			
No	48 (64.0)	27 (36.0)	< 0.001
Yes	7 (15.2)	39 (84.8)	

LDH, lactate dehydrogenase; NSE, neuron-specific enolase; AID, absolute iron deficiency; FID, functional iron deficiency; BM, bone marrow

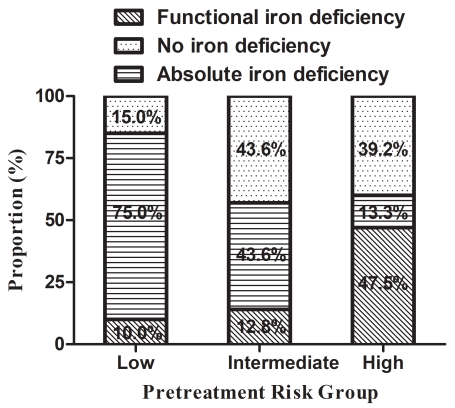

FIGURE 1 The proportion of different types of iron deficiency in newly diagnosed neuroblastoma patients with different risks.

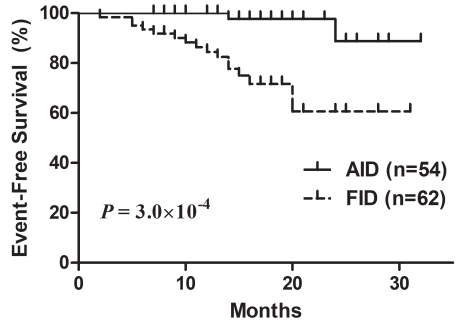

FIGURE 2 The event-free survival of neuroblastoma patients with different types of iron deficiency. AID, absolute iron deficiency; FID, functional iron deficiency.

and in that multivariate analysis the most significant independent predictor of EFS was lactate dehydrogenase ≥ 1500 U/L (hazard ratio 5.488; 95% confidence interval 2.078–14.490), followed by high-risk NB (hazard ratio 3.253; 95% confidence interval 1.120–9.448).

DISCUSSION

ID is defined by insufficient iron availability for heme synthesis for erythropoiesis.[15] AID refers to depletion of total body iron stores and insufficient iron supply, while in FID iron stores are loaded but the deposited iron is unavailable to erythroblasts and for other iron-dependent processes such as oxygen transport.[5,7,16] The main mechanism of inadequate iron supply in FID is the presence of an inflammatory process, and iron availability is reduced to approximately 44% of the normal level.[7] This is due to the release of inflammatory cytokines—particularly interleukin-6—which activate hepatic hepcidin transcription-promoting genes, increasing hepcidin concentrations and promoting the blockage of iron input into the circulation and reducing its availability.[17] An epidemiological study investigating ID reported that the prevalence rate of FID was 81.9% (335/409) in patients with different types of tumors.[18] In another study that included 105 patients with metastatic cancer in a palliative care setting, FID was diagnosed in 76.7% of anemic women and 46.8% of anemic men.[19] In the present study AID was identified in 45.5% of the 121 newly NB diagnosed patients with ID, while FID was present in 54.5%.

ID is associated with a poor prognosis in some types of cancers. Pre-existing ID anemia was reportedly independently associated with poor survival outcomes in patients with colon cancer.[20] Multiple myeloma patients with abnormal iron metabolism including iron overload and ID exhibited poorer overall survival than those with normal iron metabolism.[21] Increased DNA damage, genomic instability, and immunological dysfunction caused by ID may be responsible for the poorer prognoses reported during cancer development.[22] Ferritin has been revealed as a prognostic factor for NB in many clinical studies,[23-25] but little is known about how ferritin affects the survival of NB patients. FID was defined as TSAT < 20% and SF ≥ 100 ng/mL in the present study. These patients had high levels of ferritin but low levels of TSAT, while another group in the study had high levels of both ferritin and TSAT did not exhibit ID. FID has been shown to be associated with advanced disease and poor ECOG

TABLE 3 Univariate and multivariate analysis of prognostic factors in neuroblastoma patients

Characteristics	Univariable		Multivariable		
	OR	*P*	*OR*	95% *CI*	*P*
Stage					
M versus L1/L2/MS	14.901	< 0.001			
Risk stratification					
High versus Low-Intermediate	31.500	< 0.001	3.253	1.120–9.448	0.030
LDH					
≥1500 U/L versus < 1500 U/L	8.554	< 0.001	5.488	2.078–14.490	< 0.001
NSE					
≥ 100 U/L versus < 100 U/L	10.165	< 0.001			
BM metastasis					
Yes versus No	6.014	< 0.001			
Iron deficiency					
FID versus AID	9.822	0.003			

OR, odds ratio; *CI*, confidence interval; LDH, lactate dehydrogenase; NSE, neuronspecific enolase; BM, bone marrow; FID, functional iron deficiency; AID, absolute iron deficiency

(Eastern Cooperative Oncology Group) performance status in patients with solid tumors.[18] In the NB patients in the present study, FID reflected insufficient availability of iron despite adequate iron reserves. Hepcidin, the main regulator of iron uptake and release, plays an important role in iron homeostasis and FID in cancer patients.[6,7] Many studies have confirmed that hepcidin promotes the growth and progression of some types of solid tumors such as breast cancers, prostate cancers, and renal cell carcinomas via tumor iron retention and tumor cell survival.[26-28] Notably however, the main mechanisms involved in the causation of FID by hepcidin and how abnormal iron metabolism affects the prognosis of patients with NB require further study.

In conclusion, in the present study there was a high prevalence of ID, mainly as FID, in patients newly diagnosed with NB. FID is an important feature and independent predictor of EFS in patients with NB. The long-term effects of iron supplementation in different types of ID in NB patients need to be investigated.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Maris JM. Recent advances in neuroblastoma. N Engl J Med. 2010;362:2202-2211.
2. Duan C, Wang H, Chen Y, Chu P, Xing T, Gao C, et al. Whole exome sequencing reveals novel somatic alterations in neuroblastoma patients with chemotherapy. Cancer Cell Int. 2018;18:21.
3. London WB, Castleberry RP, Matthay KK, Look AT, Seeger RC, Shimada H, et al. Evidence for an age cutoff greater than 365 days for neuroblastoma risk group stratification in the Children's Oncology Group. J Clin Oncol. 2005;23:6459-6465.
4. Pinto NR, Applebaum MA, Volchenboum SL, Matthay KK, London WB, Ambros PF, et al. Advances in risk classification and treatment strategies for neuroblastoma. J Clin Oncol. 2015;33:3008-3017.
5. Aapro M, Osterborg A, Gascon P, Ludwig H, Beguin Y. Prevalence and management of cancer-related anaemia, iron deficiency and the specific role of i.v. iron. Ann Oncol. 2012;23:1954-1962.
6. Aapro M, Beguin Y, Bokemeyer C, Dicato M, Gascon P, Glaspy J, et al. Management of anaemia and iron deficiency in patients with cancer: ESMO Clinical Practice Guidelines. Ann Oncol. 2018; Supplement 4:iv271.
7. Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med. 2005;352:1011-1023.
8. Wilson MJ, Dekker JWT, Harlaar JJ, Jeekel J, Schipperus M, Zwaginga JJ. The role of preoperative iron deficiency in colorectal cancer patients: prevalence and treatment. Int J Colorectal Dis. 2017;32:1617-1624.
9. Neoh K, Stanworth S, Pasricha SR, Bennett MI. Estimating prevalence of functional iron deficiency anaemia in advanced cancer. Support Care Cancer. 2017;25:1209-1214.
10. Zhen L, Zhe S, Zhenning W, Zhifeng M, Zhidong L, Xiaoxia L, et al. Iron-deficiency anemia: a predictor of diminished disease-free survival of T3N0M0 stage colon cancer. J Surg Oncol. 2012;105:371-375.
11. Wang L, Shao ZH. Relationship between anemia and clinical features and prognosis of advanced lung cancer patients. Chin J Oncol. 2018;40:512-516.
12. Jian J, Yang Q, Shao Y, Axelrod D, Smith J, Singh B, et al. A link between premenopausal iron deficiency and breast cancer malignancy. BMC Cancer. 2013;13:307.
13. Brisse HJ, McCarville MB, Granata C, Krug KB, Wootton-Gorges SL, Kanegawa K, et al. Guidelines for imaging and staging of neuroblastic tumors: consensus report from the International Neuroblastoma Risk Group Project. Radiology. 2011;261:243-257.
14. Editorial Board of Chinese Journal of Pediatrics; Subspecialty Groups of Hematology and Child Health Care; Society of Pediatrics, Chinese Medical Association. Recommendations for prevention and treatment of iron deficiency and iron deficiency anemia in children. Chin J Pediatr. 2008;46:502-504. (in Chinese)
15. Evstatiev R, Gasche C. Iron sensing and signalling. Gut. 2012;61:933-952.
16. Ludwig H, Evstatiev R, Kornek G, Aapro M, Bauernhofer T, Buxhofer-Ausch V, et al. Iron metabolism and iron supplementation in cancer patients. Wien Klin Wochenschr. 2015;127:907-919.
17. Goodnough LT, Nemeth E, Ganz T. Detection, evaluation, and management of iron-restricted erythropoiesis. Blood. 2010;116:4754-4761.
18. Ludwig H, Muldur E, Endler G, Hubl W. Prevalence of iron deficiency across different tumors and its association with poor performance status, disease status and anemia. Ann Oncol. 2013;24:1886-1892.
19. Dunn A, Carter J, Carter H. Anemia at the end of life: prevalence, significance, and causes in patients receiving palliative care. J Pain Symptom Manage. 2003;26:1132-1139.
20. Teng CL, Yu JT, Chen YH, Lin CH, Hwang WL. Early colonoscopy confers survival benefits on colon cancer patients with pre-existing iron deficiency anemia: a nationwide population-based study. PLoS One. 2014;9:e86714.
21. Konig C, Kleber M, Ihorst G, Gropp A, Reinhardt H, Koch B, et al. Prevalence of iron overload vs iron deficiency in multiple myeloma: resembling or different from MDS--

and stem cell transplant (SCT)--patients? Clin Lymphoma Myeloma Leuk. 2013;13:671-680 e3.
22. Zohora F, Bidad K, Pourpak Z, Moin M. Biological and immunological aspects of iron deficiency anemia in cancer development: a narrative review. Nutr Cancer. 2018;70:546-556.
23. Morgenstern DA, Potschger U, Moreno L, Papadakis V, Owens C, Ash S, et al. Risk stratification of high-risk metastatic neuroblastoma: A report from the HR-NBL-1/SIOPEN study. Pediatr Blood Cancer. 2018;65:e27363.
24. Cohn SL, Pearson AD, London WB, Monclair T, Ambros PF, Brodeur GM, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. J Clin Oncol. 2009;27:289-297.
25. Riley RD, Heney D, Jones DR, Sutton AJ, Lambert PC, Abrams KR, et al. A systematic review of molecular and biological tumor markers in neuroblastoma. Clin Cancer Res. 2004;10:4-12.
26. Pinnix ZK, Miller LD, Wang W, D'Agostino R, Jr., Kute T, Willingham MC, et al. Ferroportin and iron regulation in breast cancer progression and prognosis. Sci Transl Med. 2010;2:43ra56.
27. Tesfay L, Clausen KA, Kim JW, Hegde P, Wang X, Miller LD, et al. Hepcidin regulation in prostate and its disruption in prostate cancer. Cancer Res. 2015;75:2254-2263.
28. Traeger L, Ellermann I, Wiethoff H, Ihbe J, Gallitz I, Eveslage M, et al. Serum Hepcidin and GDF-15 levels as prognostic markers in urothelial carcinoma of the upper urinary tract and renal cell carcinoma. BMC Cancer. 2019;19:74.

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