Iron deficiency in children at the time of initial neuroblastoma diagnosis

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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.5 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...
bodies. Patients with advanced cancer are likely to have FID as the predominant etiology of anemia. ID anemia is also associated with shorter survival times in some types of cancers, including colon cancer, lung cancer, and breast cancer. 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, 18F-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). In accordance with the relevant guidelines for the diagnosis, management, and prevention of ID and ID anemia in China, 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. 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
and in that multivariate analysis the most significant independent predictor of EFS was lactate dehydrogenase \( \geq 1500 \text{ U/L} \) (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.\(^5\) 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.\(^5\) 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 \(\geq 100 \text{ ng/mL}\) 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          | 95% CI        |
| Univariable                      | P           |   | P          |
| Stage                            |             |               |
| M versus L1/L2/MS                | 14.901      | < 0.001       | 3.253 | 1.120–9.448 | 0.030 |
| Risk stratification              |             |               |
| High versus Low-Intermediate     | 31.500      | < 0.001       | 5.488 | 2.078–14.490 | < 0.001 |
| LDH \(\geq 1500 \text{ U/L} \) versus \(< 1500 \text{ U/L}\) | 8.554       | < 0.001       |
| NSE \(\geq 100 \text{ U/L} \) versus \(< 100 \text{ U/L}\) | 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**, neuron-specific enolase; **BM**, bone marrow; **FID**, functional iron deficiency; **AID**, absolute iron deficiency
Survival.

Carcinomas via tumor iron retention and tumor cell such as breast cancers, prostate cancers, and renal cell carcinomas via tumor iron retention and tumor cell growth and progression of some types of solid tumors. Many studies have confirmed that hepcidin promotes the investigated.

NB. The long-term effects of iron supplementation and independent predictor of EFS in patients with NB. FID is an important feature 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.

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.

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