Prognostic Importance of Serum Ferritin in Patients with Stages III and IV Neuroblastoma: The Children's Cancer Study Group Experience

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ABSTRACT

Ferritin was measured in sera obtained at diagnosis from 241 patients with neuroblastoma to determine (a) the incidence of elevated ferritin and (b) the relationship between ferritin level and outcome. Ferritin was infrequently elevated in sera from patients with Stages I and II disease but was abnormally elevated in 37 and 54% of those with Stages III and IV neuroblastoma, respectively. The mean and median levels for each stage were compared and were highest for Stages III and IV disease. Analysis of progression-free survival for children with Stages III and IV disease indicated that elevated ferritin was associated with a significantly poorer prognosis than was normal ferritin and that this correlation was independent of stage and age at diagnosis. Progression-free survival at 24 months of follow-up for patients with Stage III disease with normal ferritin was 76% and with elevated ferritin was 23%. For those with Stage IV disease, progression-free survival was 27 and 3% with normal and elevated ferritin, respectively. We conclude that determination of the level of ferritin in serum at diagnosis is useful for selecting appropriate therapy for patients with Stage III neuroblastoma. Those with normal ferritin (63% of patients) have a good outcome with current therapy, but those with elevated ferritin (37%) do poorly and require more effective therapy. Although ferritin defines subgroups with Stage IV disease, the outcome of all groups must be improved.

INTRODUCTION

The prognosis for patients with neuroblastoma varies with the stage of disease and the age at diagnosis. Stage, as described by Evans et al. (1), correlates with survival; localized disease (Stage I) has the best and widespread disease (Stage IV) has the worst outcome. One special form of widespread neuroblastoma (Stage IV-S) is an exception in that spontaneous regression is frequent, and the prognosis is excellent (1, 2). Younger children, stage for stage, have a better outlook (4, 5). In general, it has not been obvious at diagnosis what distinguishes those patients with metastatic disease who survive from those who die. Recently, however, Zeltzer et al. (6, 7) reported that quantitation of neuron-specific enolase in serum can identify infants with Stage IV disease who have a good or poor prognosis.

In another study, we found that serum ferritin levels were different in patients with Stages IV and IV-S neuroblastoma. Ferritin levels were elevated in those with Stage IV disease, while they were usually low or normal in patients with Stage IV-S tumors (8). In addition, for 55 children with various stages of neuroblastoma treated at the Children's Hospital of Philadelphia, it appeared that an increased serum ferritin level at diagnosis was associated with a poorer outcome than a normal level.3

The current study was designed to determine (a) the incidence of elevated ferritin at diagnosis in relationship to stage of disease and (b) whether the level could identify subsets of patients with Stages III and IV disease with different prognoses.

MATERIALS AND METHODS

Patients. Serum ferritin was measured in 7 children with Stage I and 21 with Stage II neuroblastoma. There was no CCG therapeutic study open for Stages I and II patients, thus accounting for the small number of specimens. Ninety-four children with Stage III neuroblastoma were enrolled in CCG Protocols CCG-373P and CCG-373 (April 1978 to June 1983), while 331 patients with Stage IV disease were entered on CCG-371P and CCG-371 (April 1978 to November 1982). Serum was received for testing from 38 of 94 patients with Stage III and 175 of 331 with Stage IV neuroblastoma; these sera were from 24 boys and 14 girls with Stage III and 105 boys and 70 girls with Stage IV disease. All patients were under 16 years of age.

Stage III patients who were entered into CCG-373P received CPM, VCR, dacarbazine, doxorubicin, and teniposide, whereas those in CCG-373 received CPM, VCR, dacarbazine, doxorubicin, and teniposide. Stage IV neuroblastoma patients enrolled in CCG-371P received CPM, VCR, dacarbazine, doxorubicin, and teniposide. Those on CCG-371 were treated with CPM, VCR, and dacarbazine for 10 weeks after diagnosis and then with one of 3 regimens: (a) CPM, VCR, dacarbazine; (b) CPM, VCR, dacarbazine, doxorubicin, and teniposide; or (c) CPM, VCR, dacarbazine, doxorubicin, teniposide, and cisplatin.

Ferritin Measurements. Serum samples were obtained at diagnosis before therapy by CCGS investigators, frozen, sent to the CCGS Neu-
Serum ferritin levels were measured by CEP and RIA. CEP used rabbit polyclonal antibodies to human placental ferritin (Behringwerke AG, Marburg, West Germany), which detect a wide spectrum of isoferritins (9, 10); a positive precipitin line between the sample and the antibodies indicates >400 ng of total ferritin per ml (11, 12). Normal children have negative serum ferritin by CEP (12). Results of testing by CEP were expressed as positive or negative. RIA used rabbit polyclonal antibodies to human liver ferritin (RIANEN kits; New England Nuclear, Boston, MA), which preferentially detect basic isoferritins and underestimate acidic isoferritins (13, 14). By RIA, the normal range of serum ferritin levels in children over 6 months of age is 7 to 142 ng/ml with a median of 30 ng/ml (15). Infants under 6 months of age have higher levels of serum ferritin (15, 16).

Statistical Analyses. Results of ferritin measurements were sent to the CCSG Statistical Center for analysis. Differences in mean and median ferritin levels between stages of disease were analyzed for significance with the Student t test and the Wilcoxon nonparametric rank test, respectively.

Survival and PFS, which is defined as survival terminated by progression of disease or death, were both used as measures of outcome. The difference in outcome for patients with elevated and normal levels of ferritin was tested with the Mantel-Peto-Cox statistic (17). Although the relationship between ferritin level and outcome generally was similar whether CEP or RIA data were analyzed, CEP gave slightly more significant differences between prognostic groups. Therefore, outcome analysis was based upon ferritin level as measured by CEP unless otherwise indicated.

Results of protocols for Stage III disease were combined as were those for Stage IV disease, because there were no interstudy differences (CCG-373P versus CCG-373; CCG-371P versus CCG-371) with respect to patient characteristics, PFS, or survival. The relationship between ferritin and outcome was the same when this was measured by either PFS or survival. However, PFS was chosen to present results because patients are removed from the primary study protocol after tumor progression occurs and subsequently receive a variety of treatments which theoretically could affect survival in different ways. When PFS results are stated, the life table-estimated 24-month rate is often given, since the follow-up period for these patients generally makes that a stable estimate, and relatively few patients have had the initial progression of disease after that length of PFS.

Serum samples were available from 40 and 53% of patients with Stages III and IV disease, respectively, who were enrolled in the CCSG protocols. The distributions of various important clinical characteristics were compared for those patients who had serum samples versus those who did not. These groups had no significant differences in the distributions for any of the characteristics examined (age, sex, presence and sites of disease and metastases, and treatment regimens). In addition, PFS was compared for those patients with and without serum samples. For Stage III neuroblastoma, PFS was the same for 38 children with and 56 children without serum ferritin measurement (P = 0.30). There also was no difference in PFS among 175 patients tested and 158 not tested who had Stage IV neuroblastoma (P = 0.83).

RESULTS

Ferritin Level and Clinical Stage of Disease. Serum ferritin levels for different clinical stages are shown in Table 1. Levels ranged from 0 to 1600 ng/ml by RIA, and testing the same samples by CEP (positive or negative) and RIA (≥ or ≤ 142 ng/ml) demonstrated a concordance rate of 84%. The proportion of patients with an abnormal level was highest among those with Stages III and IV disease. While there was no difference between Stages I and II and between Stages III and IV, the mean and median levels (by RIA) for Stages II versus III were significantly different (P = 0.012, t test; P = 0.001, Wilcoxon test). Correction for these 3 multiple comparisons by the Bonferroni method still shows significant differences for Stages II versus III (P = 0.036, t test; P = 0.004, Wilcoxon test). If one were to compare any stages separated by more than one level (e.g., I versus III, I versus IV, II versus IV), the differences are statistically significant.

Ferritin Level and PFS for Patients with Stage III Neuroblastoma. Age at diagnosis predicts outcome independently of stage (18, 19). To determine if ferritin is a third independent prognostic factor, age categories with the greatest impact on outcome were defined; then, the relationship between ferritin and PFS was determined within these categories. Chart 1 shows the estimated PFS for patients with Stage III disease according to age at diagnosis. The probability of PFS for all patients with Stage III disease at 24 months was 48%. Children less than 2 years of age at diagnosis had 62% probability of PFS at 24 months, whereas those 2 years or older had a 32% chance of PFS (P = 0.03).

Results of protocols for Stage III disease in relation to age at diagnosis. The probability of PFS for all patients enrolled in CCG-373P and -373 studies was determined for the following age groups: all ages; <2 years; and ≥2 years. The difference between groups diagnosed before and after 2 years is significant (P = 0.03). The median follow-up was 16 months, and the range was 1 to 56 months.

Table 1. Level of serum ferritin in relation to clinical stage at diagnosis

<table>
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<tr>
<th>Clinical stage</th>
<th>CEP assay</th>
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* +, abnormally elevated ferritin; -, normal level. See "Materials and Methods" for details.
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(A)

Serum ferritin (+) n=14

Serum ferritin (-) n=24

(B)

Serum ferritin (+) n=3

Serum ferritin (-) n=14

(C)

Serum ferritin (+) n=11

Serum ferritin (-) n=10

Time in months from diagnosis

Estimated Percent Surviving without Progression of Disease

0 10 20 30 40 50 60

0 25 50 75 100

0 25 50 75 100

0 25 50 75 100

Chart 2. PFS for patients with Stage III neuroblastoma in relation to normal (-) or elevated (+) serum ferritin. Patients were grouped as follows according to age at diagnosis: A, all ages (P = 0.002); B, <2 years (P = 0.03); and C, ≥2 years (P = 0.02). The median follow-up was 30 months, and the range was 3 to 52 months.

When patients of all ages were grouped according to positive or negative serum ferritin, the probability of PFS 24 months after diagnosis was 23% for 14 with positive ferritin and 76% for 24 with negative ferritin (P = 0.002; Chart 2A). For 3 patients with positive ferritin who were diagnosed before 2 years, the PFS was 0% at 13 months; in contrast, 14 patients with negative ferritin had 68% PFS up to 48 months after diagnosis (P = 0.03; Chart 2B). In those over 2 years at diagnosis, the probability of PFS at 24 months was 30% for 11 patients with positive ferritin and 88% for 10 with negative ferritin (P = 0.02; Chart 2C). Thus, the ferritin level predicted outcome independently of stage and age.

Ferritin Level and PFS for Patients with Stage IV Disease. Age also is an independent prognostic factor for those with Stage IV disease; however, for these patients, the greatest difference in outcome is predicted by diagnosis before or after 1 year of age (20). Patients between 12 and 23 months of age have an outcome similar to older children. Chart 3 demonstrates that the probability of PFS at 24 months for all Stage IV patients was 17%. However, patients under 1 year at diagnosis had a 42% chance of PFS at 24 months, whereas those older than 1 year had only a 13% probability of PFS.

The relationship of serum ferritin to outcome for patients of all ages and for those under and over 1 year was determined. When patients of all ages were analyzed, 94 with positive ferritin had only a 3% probability of PFS at 24 months, whereas 81 with negative ferritin had 27% PFS (P = 0.007; Chart 4A). Analysis of outcome of 21 patients diagnosed before 1 year of age with Stage IV disease revealed 29 and 54% PFS rates at 12 months for those with positive and negative ferritin, respectively (Chart 4B). Although these differences were not statistically significant (P = 0.26), 6 of the 14 infants with negative ferritin have PFS for 18 to 55 months. Only 2 of 7 infants with positive ferritin survive progression free, and they are doing so at 12 and 14 months. Longer follow-up and more patients will clarify the prognostic value of ferritin for infants. For children 1 year or older with Stage IV neuroblastoma, the probability of PFS at 24 months was 87 with positive ferritin and 67 for negative ferritin, 21% (P = 0.03; Chart 4C).

Quantity of Ferritin and PFS. To determine if ferritin levels within and above the normal range related to outcome, patients were divided into 3 groups with the most distinct outcomes according to quantity of ferritin determined by RIA: <75 ng/ml; 75 to 142 ng/ml; and >142 ng/ml (Chart 5). For Stage III patients, the estimated 24-month PFS rates were 79, 69, and 31% in the low-, intermediate-, and high-ferritin groups, respectively (P = 0.02, test for trend with Mantel-Peto-Cox statistic). For Stage IV patients, the estimated 24-month PFS were 38, 27, and 4% in the 3 respective groups (P = 0.005). Thus, the quantity of ferritin as measured by RIA separates patients with Stages III and IV disease into groups with different outcomes.

DISCUSSION

This study demonstrated that subgroups of patients with Stages III and IV neuroblastoma with different outcomes can be
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Chart 4. PFS for patients with Stage IV neuroblastoma in relation to normal (−) or elevated (+) serum ferritin. Patients were grouped as follows according to age at diagnosis: A, all ages (P = 0.007); B, <1 year (P = 0.26); C, ≥1 year (P = 0.03). The median follow-up was 21 months, and the range was 1 to 56 months.

Defined at diagnosis by measuring serum ferritin. Patients with either stage of disease who have normal levels of ferritin before treatment have a better prognosis than those with high levels. Furthermore, a low normal level (<75 ng/ml) predicts a better outcome than an intermediate level (75 to 142 ng/ml), which in turn predicts a better result than an abnormal level (>142 ng/ml). Subgroups were identified within different age categories indicating that prediction of outcome is independent of age.

Tumor cells are likely to be the source of increased ferritin in serum. Ferritin is produced by neuroblastoma cells in vitro (12), and nude mice bearing human neuroblastomas have detectable levels of human ferritin in their sera (21, 22). Ferritin is present in tumors tested directly from patients (9, 12), and levels in serum usually become normal with clinical remission (12). Anemia or damage to liver cells from tumor invasion are not likely explanations for the elevation, since there is no correlation between ferritin and hemoglobin (12), transaminase (12), or iron5 levels in serum of patients with neuroblastoma.

The incidence of abnormal levels and mean levels in patients with Stages I and II disease (although the number of patients was small) was lower than in those with Stages III and IV disease. This indicates a general correlation with clinically defined tumor burden. However, there was a wide intrastage range for those with stages III and IV disease, suggesting that additional undefined variables influence the quantity in serum. Release of tumor ferritin into serum may vary for different neuroblastomas. This is supported by the observation that primary tumors from patients with widespread metastases, Stages IV and IV-S, contain comparable amounts of ferritin, whereas serum levels are increased only in those with Stage IV disease (8). Another explanation is that tumor burden is not accurately quantitated by clinical staging; if so, the highest serum level for a given clinical stage would indicate the largest tumor burden and vice versa.

The reason(s) for a correlation between the level of ferritin in serum and outcome is unknown. The quantity of serum ferritin may be related to tumor cell characteristics, such as proliferation and/or differentiation, which are likely to influence tumorigenicity. If an antitumor immune response occurs in vivo, it could be adversely affected by high ferritin in the tumor environment and in serum. In vitro, ferritins from HeLa and neuroblastoma cells or ferritins from serum of patients with Hodgkin’s disease or breast cancer can bind to the surface of T-lymphocytes, inhibiting formation of rosettes with sheep erythrocytes (23, 24); and ferritins can suppress the response of lymphocytes to mitogens (25, 26).

Other tumor variables such as serum neuron-specific enolase

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6 H-W. L. Hann, M. W. Stahlhut, and A. E. Evans, unpublished data.

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(6, 7, 27), tumor cell DNA content (28), and histopathology of primary tumor (29) have recently been shown to have prognostic value. As we identify more predictive factors, it should be possible to manage individual patients more efficiently.

We conclude that measurement of ferritin in serum at diagnosis will be useful for managing patients with Stage III neuroblastoma. Those with normal levels account for 63% of patients, and they will be useful for managing patients with Stage III neuroblastoma. Those with normal levels account for 63% of patients, and they have a 76% probability of PFS 24 months after diagnosis when given conventional therapy. Thus, they should not be subjected to new therapies with potentially high toxicities. In contrast, 37% of Stage III patients who have high ferritin do extremely poorly, and they need more effective treatment immediately after diagnosis, and prognosis is established. Although ferritin levels also divide patients with Stage IV disease into subgroups with different outcomes, even the best group has only a 38 and 27% PFS as defined by RIA and CEP, respectively. Identification of patients within this subgroup who will have PFS may be possible with additional prognostic markers such as neuron-specific enolase; if not, all patients in this and poorer risk groups should receive new and potentially more effective therapy. With new therapy protocols, measurement of ferritin will be an important aspect of defining the study population for reliable interpretation of results.

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APPENDIX

Principal Investigators of CCGS

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