Prognostic value of serum lactate dehydrogenase and serum ferritin for abdominal neuroblastoma in children

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Abstract

Backgrounds: For decades, age, MYCN status, stage, and histology have been used as neuroblastoma risk factors. Serum Lactate dehydrogenase (LDH) and ferritin are reproducible, easy to obtain, and prognostic, but they are never used in risk stratification.

Purpose/Objective: The purpose of this study was to find out the prognostic value of serum LDH and ferritin in children with abdominal neuroblastoma.

Materials/Methods: Nine children with abdominal neuroblastoma from 2020-2021 in four tertiary centers of Dhaka, who treated according to Societe’ Internationale’Oncologie Pe’diatrique selected as study population and divided into low and high-risk groups. Age, sex, stage, histology, serum LDH, serum ferritin and events were analyzed using SPSS 22. Survival was calculated by Kaplan-Meier procedure.

Results: Sensitivity, specificity, positive predictive value and negative predictive value of serum LDH were 100%, 85.7%, 66.7% and 100% respectively in the prognosis of abdominal neuroblastoma in children at a cut-off point =1527.5. Sensitivity, specificity, positive predictive value, and negative predictive value of serum ferritin were 100%, 71.4%, 50% and 100% respectively in the prognosis of abdominal neuroblastoma in children at a cut-off point=581. There was no significant difference between the survival curves of both risk groups (p= 0.180) and between serum ferritin >581 and ≤581 group (p= 0.089) according to log rank test. But there was significant difference between the survival curves of LDH >1527.5 and LDH ≤1527.5 group according to log rank test (p= 0.027).

Conclusion: Analysis of this study revealed that the risk of a worse event for children with abdominal neuroblastoma was greater and outcome was also worse for patients with serum LDH >1527.5 IU/L and serum ferritin >581 ng /ml. These markers can also be used for risk stratification in low to middle income countries. But further multi-centered study with longer period of follow up on larger sample size will be required to demonstrate the independent prognostic value of serum LDH and ferritin for abdominal neuroblastoma in children.

Key Word: Abdominal neuroblastoma, Serum lactate dehydrogenase, serum ferritin.

Introduction

Neuroblastoma is the most common solid extracranial tumor in infants and children. It arises from primitive neuro blasts of the embryonic neural crest, and therefore can occur anywhere within the sympathetic nervous system 1. The most common site of the primary tumor occurs within the abdomen (65%). About half of these tumors arise from the adrenal medulla. Other common sites of neuroblastoma include the neck, chest and pelvis2. Tumors occur in approximately one in every 7,500 to 10,000 children.

This neoplasm exhibits great heterogenicity in its behaviour. The heterogenicity of neuroblastoma provides a series of clinical and biological factors with prognostic value. For decades, age, stage and histology have been used as neuroblastoma (NB) risk factors. In more recent studies amplification of the MYCN oncogene, genetic aberrations of chromosome 1p, 11q, and 17q, and specific histological features of

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the tumor are also associated with poor outcome.
Combination of prognostic variables are now routinely
used for risk-group assignment and for stratification
of treatment regimens\textsuperscript{3}. These analyses, however,
necessitate a primary tumor biopsy, which is both
costly and time-consuming\textsuperscript{4}.

Different plasma and urinary parameters have been
tested as valuable diagnostic and prognostic markers
for neuroblastoma (NB) in children. Serum level of
ferritin & lactate dehydrogenase is commonly
assessed in children suspected to have
neuroblastoma. The levels of these markers are
commonly used for diagnosis and monitoring of
neuroblastoma patients. Estimation of Serum ferritin
and lactate dehydrogenase are comparatively less
expensive and more available in Bangladesh. It can
be a good choice to facilitate screening, diagnosis,
assessment of prognosis and monitoring of
neuroblastoma patients.

LDH is widely expressed in body tissues, and because
it is released during tissue damage, it serves as a
marker of tissue injury and disease. LDH has also
been linked to tumor initiation and metabolic
processes that play a role in the survival of cancer
cells. Quantitative LDH measurements that are easily
and reproducibly obtained from a simple and
inexpensive blood assay. High levels of LDH are found
to be associated with poor overall survival (OS),
particularly in melanoma, prostate, and renal cell
carcinomas \textsuperscript{3}. The normal reference range of serum
LDH is 208 to 378 IU/L.

Ferritin is an universal intracellular protein that stores
iron and regulates its release. Serum ferritin level are
found to be elevated in cancer patients without a
Corresponding increase in tissue iron storage. Serum
ferritin is one of the tumor markers for NB and is
Elevated in most children with NB\textsuperscript{5}. The normal Thing
reference range for serum ferritin is 25 to 200 ng/ml
for newborn, 50 to 200 ng/ml for children aged 1-5
months and 7 to 140 ng/ml for children aged above 5
months.

It seems optimal to utilize biomarkers that are broadly
applicable and prognostic for risk stratification is the
best option; such biomarkers are likely to be the
downstream culmination of genomic (known and
unknown) and clinical factors. The first COG risk
stratification uses strong prognostic factors such as
histologic features, MYCN status, and ploidy, but LDH
and ferritin are overlooked, except for one German
trial\textsuperscript{3}.

Diagnosis of neuroblastoma is made through a variety
of investigations: 1) Imaging & isotopic studies 2) Histologic 3) Genetic evaluation of tumor tissue\textsuperscript{6}. Successful treatment depends upon accurate staging that includes International Neuroblastoma Risk Group Staging System (INRGSS) and International Neuroblastoma Staging System (INSS). The International Neuroblastoma Risk Group Staging System (INRGSS) is a new pretreatment risk stratification and diagnostic staging system. The International Neuroblastoma Staging System (INSS) is done post-operatively and is based on completeness of initial tumor resection and lymph node status\textsuperscript{7}.

Neuroblastoma is treated through multidisciplinary
approaches as: Operative therapy, Radiotherapy,
Chemotherapy, Myeloablative Therapy, Immunotherapy & often additional therapies. Despite
current multimodal therapies and extensive
neuroblastoma research, neuroblastoma has remained
as a challenge with unpredictable clinical course and
prognosis\textsuperscript{6}. Estimation of serum lactate
dehydrogenase and serum ferritin could be easier and
available tool to predict the prognosis of
neuroblastoma.

Patients and methods
The study was carried out at the Department of
Pediatric Surgery, Department of Pediatric
Hematology and Oncology, Department of Radiology
and Imaging, Department of Pathology of Dhaka
Medical College Hospital, Dhaka and the Department
of Biochemistry & Molecular Biology of BSMMU from
January 2020 to December 2021 for a period of two
years. Information from the department of Pediatric
Hematology and Oncology of Bangabandhu Sheikh
Mujib Medical University, National Institute of Cancer
research and Hospital, Dhaka Shishu (Children)
Hospital and Bangladesh Institute of Child Health was
collected by principal investigator with the help of
appointed doctors from respective hospitals. A total
number of 9 children with abdominal neuroblastoma
were enrolled for this study after fulfilling the selection
criteria. Eligibility for inclusion in the study included
(a) Children (0 days-14years) with abdominal
neuroblastoma staged according to INRGSS. (b)
Treated according to SIOP protocol and completed
surgical management. (c) Children with unknown
MYCN status due to unavailability. (d) Children with
parents who will provide informed consent for this
study. Prior to the commencement of this study, the research protocol was approved by The Ethical Review Committee (ERC) of DMCH. Treatment abandonment due to any cause other than progression of disease were considered as exclusion criteria for the study.

Subgroup of NB patients were created using certain parameters including age, IDRF, staging (INRG and INSS), histology (Schimada), also depending on Serum LDH and Serum ferritin level. Patients those were below 18 months, with no IDRF, in stage I, II, IV-S with favourable histology, Serum LDH level < 1400 IU/L and Serum ferritin level< 90 ng/ml were included in low risk group. Patients those were above 18 months, with presence of IDRF, in stage III, IV with unfavourable histology, Serum LDH level > 1400 IU/L and Serum ferritin level> 90 ng/ml were included in high risk group.

Event were considered as alive, death and abandonment occurring within three months of surgical management. Survival curve were generated by using methods of Kaplan-Meier.

Result:

Among nine patients four were at or below 1.5 years old and five were above five years old. Six were male and three were female. All low-risk patients had favorable histology and all high-risk patients had unfavorable histology. In the low-risk group, 2 (40%) had L1 and 3 (60%) had MS stage, in high-risk group, 2 (50%) had L2 and 2 (50%) had M stage. In low-risk group, mean serum LDH was 547.80 ± 123.24 (IU/L) at diagnosis, 468.00 ± 143.51 IU/L following chemotherapy, 259.40 ± 37.94 IU/L after one month and 219.27 ± 123.81 IU/L after 3 months. In high-risk group, mean serum LDH was 1591.75 ± 94.48 (IU/L) at diagnosis, 1645.00 ± 308.38 IU/L following chemotherapy, 1515.50 ± 535.39 IU/L after one month and 1857.00 ± 202.23 IU/L after 3 months (Figure 1.A).

The patients who died after surgery had baseline serum LDH >1527.5 U/L and among alive study subjects, 1 (14.3%) had serum LDH level >1527.5 and 6 (85.7%) had serum LDH level ≤1527.5 U/L (Table 1.A).

Table 1.A: True positive, false positive, false negative and true negative value in prognosis of abdominal neuroblastoma in children of serum LDH at a cut-off point =1527.5

<table>
<thead>
<tr>
<th>Serum LDH (IU/L)</th>
<th>Dead</th>
<th>Alive</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1527.5</td>
<td>2 (100.0)TP</td>
<td>1 (14.3)FP</td>
<td>0.083ns</td>
</tr>
<tr>
<td>≤1527.5</td>
<td>0 (0.0)FN</td>
<td>6 (85.7)TN</td>
<td></td>
</tr>
</tbody>
</table>

The patients who died after surgery had baseline serum ferritin >581 ng/ml and among alive study subjects, 2 (28.6%) had serum ferritin level >581 and 5 (71.4%) had serum ferritin level ≤581 ng/ml (Table 2.B).

Table 1.B: True positive, false positive, false negative and true negative value in prognosis of abdominal neuroblastoma in children of serum ferritin at a cut-off point > 581

<table>
<thead>
<tr>
<th>Serum ferritin (ng/ml)</th>
<th>Dead</th>
<th>Alive</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;581</td>
<td>2 (100.0)TP</td>
<td>2 (28.6)FP</td>
<td>0.167ns</td>
</tr>
<tr>
<td>≤581</td>
<td>0 (0.0)FN</td>
<td>5 (71.4)TN</td>
<td></td>
</tr>
</tbody>
</table>
Prognostic efficacy parameters for the use of serum LDH in the prognosis of abdominal neuroblastoma in children at a cut-off point =1527.5. Sensitivity, specificity, PPV and NPV of serum LDH were 100%, 85.7%, 66.7% and 100% respectively in the prognosis of abdominal neuroblastoma in children at a cut-off point =1527.5 (Table 2.A).

**Table 2.A: Prognostic efficacy parameters for the use of serum LDH in the prognosis of abdominal neuroblastoma in children at a cut-off point =1527.5**

<table>
<thead>
<tr>
<th>Prognostic efficacy parameters</th>
<th>Serum LDH (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100.0</td>
<td>22.5 – 100.0</td>
</tr>
<tr>
<td>Specificity</td>
<td>85.7</td>
<td>63.6 – 85.7</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>66.7</td>
<td>15.0 – 66.7</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>100.0</td>
<td>74.2 – 100.0</td>
</tr>
<tr>
<td>Accuracy</td>
<td>88.9</td>
<td>54.4 – 88.9</td>
</tr>
<tr>
<td>Youden index</td>
<td>0.857</td>
<td></td>
</tr>
</tbody>
</table>

CI-confidence interval; TP-true positive; TN-true negative; FP-false positive; FN-false negative.

Prognostic efficacy parameters for the use of serum ferritin in the prognosis of abdominal neuroblastoma in children at a cut-off point =581. Sensitivity, specificity, PPV and NPV of serum ferritin were 100%, 71.4%, 50% and 100% respectively in the prognosis of abdominal neuroblastoma in children at a cut-off point =581 (Table 2.B).

**Table 2.B: Prognostic efficacy parameters for the use of serum ferritin in the prognosis of abdominal neuroblastoma in children at a cut-off point =581**

<table>
<thead>
<tr>
<th>Prognostic efficacy parameters</th>
<th>Serum ferritin (%)</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100.0</td>
<td>22.2 – 100.0</td>
</tr>
<tr>
<td>Specificity</td>
<td>71.4</td>
<td>49.2 – 71.4</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>50.0</td>
<td>11.1 – 50.0</td>
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<tr>
<td>Negative predictive value</td>
<td>100.0</td>
<td>68.9 – 100.0</td>
</tr>
<tr>
<td>Accuracy</td>
<td>77.8</td>
<td>43.2 – 77.8</td>
</tr>
<tr>
<td>Youden index</td>
<td>0.857</td>
<td></td>
</tr>
</tbody>
</table>

CI-confidence interval; TP-true positive; TN-true negative; FP-false positive; FN-false negative.

According to outcome, among high-risk group 2 (50%) patients died after one month of surgery and there was no abandonment up to follow-up after 3 month of surgery (Figure 2). There was significant difference between the survival curves of LDH >1527.5 and LDH ≤1527.5 group according to log rank test (p= 0.027) (Figure 2B). There is no significant difference between the survival curves of serum ferritin >581 and ≤581 group according to log rank test (p= 0.089).

**Figure 2**: Kaplan-Meier plot of event -free survival of (A) the study patients stratified by risk group (B) patients stratified by LDH cut-off value (C) patients stratified by ferritin cut-off value.
Discussion:

The aim of this work was to investigate the prognostic value of serum LDH, and serum ferritin commonly evaluated at diagnosis in NB patients as a potential tool to stratify patients in different risk groups. The availability of a routine test may reduce the time and cost of prognostic analyses.

We should not confuse the intended purpose of normal range with intended purpose of cut-offs to define prognostic NB subgroups. For example, ferritin values within normal range portend worse outcome when applied to children with NB though not relevant to the general population.

Total nine (9) children with neuroblastoma were enrolled in the present study according to inclusion criteria. All samples were sub-divided into two subgroups (5 patient in low and 4 patients in high risk groups).

In this study patients ≤18 months aged was 44.4% and most of them were in low-risk group. Patients with age >18 months was 55.56% and most of them were in high-risk group. Which shows that patients those diagnosed at later age were usually in higher stage of NB. Uemura et al. reported in his study that 16% cases were <18 month of age and 84% cases were >18 months of age.

Overall male to female ratio of this study was 1.25:1 which indicates NB is slightly more common in boys then girls like other studies as Cangemi et al. where male to female ratio was 1.25:1 which was similar to this study. Di Cataldo et al. revealed male to female ratio 1.22:1.

All low-risk group patients (5) had favorable histology and all high risk patients (4) had unfavorable histology. In overall stage-wise distribution, 22.2% were L1, 22.2% were L2, 22.2% were M and 33.4% were MS.

Serum LDH was found significantly higher in high-risk group comparing to low risk group in our study. In the study of Moroz et al. serum LDH was evaluated and found that the risk of an event for patients with LDH >2780 IU/L was more than six times greater, while patients with LDH <265 IU/L had more than twice the risk of an event. In the study of Cangemi et al. both in patients with localized and metastatic disease high LDH values positively associated with a worse prognosis (p<0.01). They found that patients with localized disease with LDH values >1300 IU/mL had 12.9 times greater chance of relapse (95% CI: 3.34–49.78; P<0.001).

Our study also demonstrated that Serum ferritin was significantly higher in high-risk group comparing low risk group at diagnosis (p<0.001) and following chemotherapy (p=0.014). Even if serum ferritin was higher among high-risk group than low risk group after one month (p=0.061) and after 3 months (p=0.428) of surgery, there was no significant difference between the groups. In the study of Moroz et al. the risk of an event for patients with ferritin ≥425 ng/ml was approximately eight times greater.

The patients of this study who died after surgery had baseline serum LDH >1527.5 U/L and among alive study subjects, 1 (14.3%) had serum LDH level >1527.5 and 6 (85.7%) had serum LDH level ≤1527.5 U/L (Table 1.A).

We found that the LDH cut off value discriminating patients with good and bad prognosis was 1527 IU/L (Table 2.A). But in the study of Rathnakumar et al. cut off level was 190 U/L. In the study of Cangemi et al. multivariate analysis confirmed LDH predictive power only in patients without MYCN amplification (p=0.0001). In stage 4 patients, the LDH cut-off value discriminating patients with good and bad prognosis was 2500 IU/L.

In case of serum ferritin cut off value discriminating patients with good and bad prognosis was 581 ng/ml (Table 2.B). In the study of Rathnakumar et al. cut off level was 151 ng/ml. This study obtained the most discriminate cut off level when compared to other studies, which may be due to the extremely small sample size.

In this study, there was no significant difference between the survival curve of low risk and high-risk group according to log rank test (p=0.180) (Figure 2A). This is likely because such small numbers in the sample do not have the power to rule out a real difference and avoid a type two error. In the study of Di Cataldo et al., there was no survival difference between stage 4 and stage 4s patients (p=0.519, log-rank test). However, the survival curve for the stage 4s patients shows a rapid drop followed by a slow decline over the following five years, while the curve for stage 4 patients shows that early deaths were rare, with the distribution of events mostly over the first two years after diagnosis. There was significant difference between the survival curves of LDH >1527.5 and LDH...
≤1527.5 group according to log rank test (p= 0.027) (Figure 2B). There is no significant difference between the survival curves of serum ferritin >581 and ≤581 group according to log rank test (p= 0.089). This is likely because such small numbers in the sample do not have the power to rule out a real difference and avoid a type two error (Figure 2C).

There were some limitations of this study like small sample size and short period of follow up due to short study period.

**Conclusion**
Analysis of this study suggests that the risk of an event for patients with serum LDH >1527.5 IU/L and serum ferritin >581 ng/ml is greater and associated with worse outcome. It is recommended that serum lactate dehydrogenase and serum ferritin may have prognostic value for abdominal neuroblastoma in children. In future multiple centers, large sample size and long-term follow-up period will be needed to demonstrate the independent prognostic value of serum lactate dehydrogenase and serum ferritin for abdominal neuroblastoma in children.

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**Conflict of interest:** None

**References**