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Original Article

Circulating D-dimer level is a predictor of overall survival and correlates with clinicopathologic characteristics of patients with neuroblastoma

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Abstract: Neuroblastoma (NB) is a relatively common pediatric malignancy. However, few validated blood biomarkers are available for clinical use. Here we retrospectively explored the significance of D-dimer levels of 81 patients with NB as a function of their clinicopathologic characteristics and overall survival (OS). We found that D-dimer levels were significantly associated with high-risk factors such as advanced disease stage and *MYCN* amplification. Higher D-dimer levels were observed in patients with stage 4 disease compared with those with stages 1-3 and s4 NB (P=0.004) and in those with amplified *MYCN* (P=0.041). However, no significant differences were found for sex (P=0.584) or age (≥12 months vs <12 months, P=0.201). Three-year OS was shorter for patients with higher D-dimer levels (≥0.72 mg/L; P=0.031). Multivariate analyses identified higher D-dimer levels (≥0.72 mg/L) as an independent predictor of poor OS (HR: 4.78, 95% CI: 1.98-16.1, P=0.018). Therefore, elevated D-dimer levels may serve to predict prognosis of patients with NB.

Keywords: D-dimer, neuroblastoma, predictor

Introduction

Neuroblastoma (NB) is a tumor of the developing sympathetic nervous system that typically occurs in young children and infants [1]. NBs are heterogeneous, exhibiting a broad spectrum of clinical behaviors, including spontaneous complete regression or proliferation and progression. Standard anti-tumour treatments include surgery, chemotherapy and radiotherapy. Unfortunately, many patients with NB experience poor outcome and relapse, and the disease may be disseminated when diagnosed. Therefore, it is critically important to determine the extent and stage of NB upon diagnosis.

Blood biomarkers are widely used as references for diagnosis, monitoring disease and predicting prognosis [2]. However, few clinically validated blood biomarkers are available for NB, such as lactate dehydrogenase, ferritin and neuron-specific enolase (NSE) [3-5]. Thus, suitable biomarkers are needed for evaluating NB.

Activation of coagulation and fibrinolysis is associated with malignancy, angiogenesis, tumor cell invasion, tumor progression and prognosis [6, 7]. Cross-linked fibrin in the extracellular matrix serves as a stable framework for endothelial cell migration during angiogenesis and tumor cell migration during invasion. The levels of D-dimer, a fibrin degradation product, are elevated by increased fibrin formation and fibrinolysis and are therefore widely used to assess potential thrombotic episodes such as acute venous thromboembolism [8, 9]. Elevated D-dimer levels are detected in patients with cancer, indicating venous thromboembolism. Further, D-dimers are associated with solid tumours such as lung cancer, renal cell carcinoma and colorectal cancer [10-12]. However, the role of D-dimers in NB has not been widely studied.

Here we retrospectively studied D-dimer levels and their associations with clinical features and prognosis of 81 children with NB who were

Table 1. Patient clinical characteristics

Characteristics	Number
Total number of patients	81
Age in months	
Median	24.5
Range	0.2-243
Sex	
Male	52
Female	29
Primary site	
Adrenal	37
Abdominal	25
Thoracic-abdominal	7
Others	12
INSS stage	
Stage 1	15
Stage 2	14
Stage 3	24
Stage 4	26
Stage 4S	2
MYCN status	
Amplified	26
Not amplified	47
No information	8
Risk group	
Low	20
Intermediate	26
High	35
Plasma D-dimer (mg/L)*	0.72 (0.03-3.45)

*Values represent the median (range).

treated at the Jiangxi Children's Hospital (China) between June 2010 and July 2014.

Materials and methods

Patients and disease characteristics

The medical records of all patients (n=81) who were diagnosed with NB between June 2010 and July 2014 were retrospectively reviewed after patients granted written informed consent. All patients were followed until July 2016. The extent of disease and diagnosis were determined using computerized tomography (CT), magnetic resonance imaging (MRI) or B-scan ultrasonography. The final confirmation of the diagnosis of NB diagnosis was achieved through surgery and pathological examination. Bone scans were also performed. Disease staging was evaluated according to the International Neuroblastoma System Study. *MYCN* amplification was determined using in situ fluorescence hybridization. D-dimer measurements were included in diagnostic evaluations. We define the normal level of plasma D-dimer as <0.3 mg/L.

Statistical analysis

Overall survival (OS), which was defined as the time between diagnosis and mortality, was calculated using the Kaplan-Meier method. Multivariate analyses of OS were performed using Cox regression as implemented in GraphPad Prism 5 (GraphPad Software Inc., USA). P<0.05 was considered significant.

Results

Patients' characteristics

The 81 patients included 52 boys and 29 girls, each with complete medical records (**Table 1**). The adrenal (37/81) and abdominal (25/81) regions accounted for 76.5% of primary tumor sites. *MYCN* was amplified in 35.6% (26/73) of the patients, and the median D-dimer level among the 73 patients was 0.72 mg/L.

Subgroup analysis

We performed subgroup analysis of D-dimer levels (**Figure 1**), which did not significantly differ between boys (mean ± standard deviation [SD], 1.00 ± 0.11 mg/L) and girls (0.89 ± 0.19 mg/L) (P>0.05). No significant difference was found between patients aged ≥12 months (mean ± SD, 1.06 ± 0.14 mg/L) vs <12 months (0.81 ± 0.13 mg/L) (P>0.05), although the group aged ≥12-months had a higher mean D-dimer level. Stage 4 patients had a higher median D-dimer level (mean ± SD, 1.37 ± 0.24 mg/L) compared with those with Stages 1-3 and 4s (0.78 ± 0.89 mg/L) (P=0.004). Significantly higher D-dimer levels were detected in patients with *MYCN* amplification (mean ± SD, 1.13 ± 0.13 mg/L) compared with those without (0.69 ± 0.15 mg/L) (P=0.041). These results indicate that D-dimer levels were associated with high-risk factors such as advanced stage and *MYCN* amplification.

Survival associated with D-dimer levels upon diagnosis

We further divided the cohort according to D-dimer levels above or below the median value

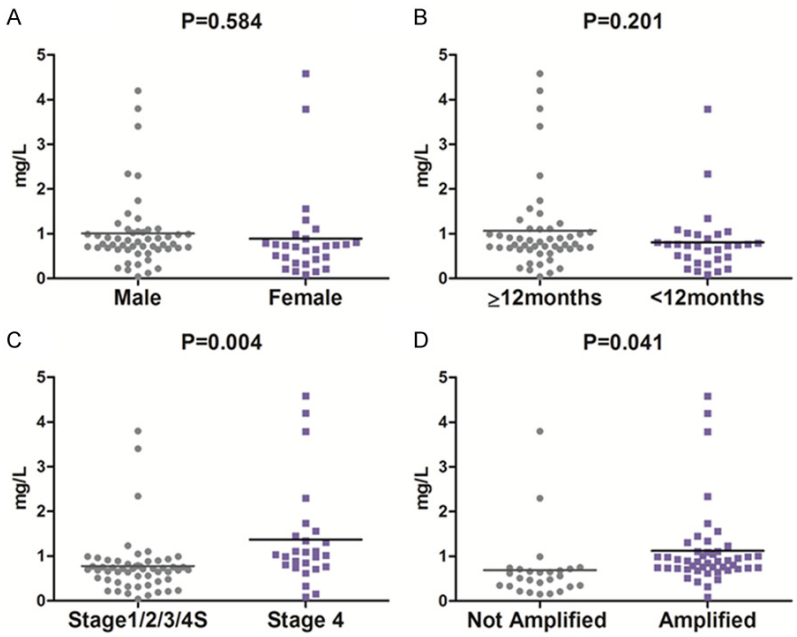

Figure 1. Association of D-dimer levels with clinicopathologic characteristics. A: Sex, boys vs girls. B: Age, ≥12 months vs <12 months. C: Disease stages, Stages 1-3 and 4s vs Stage 4. D: *MYCN* amplification, not amplified vs amplified.

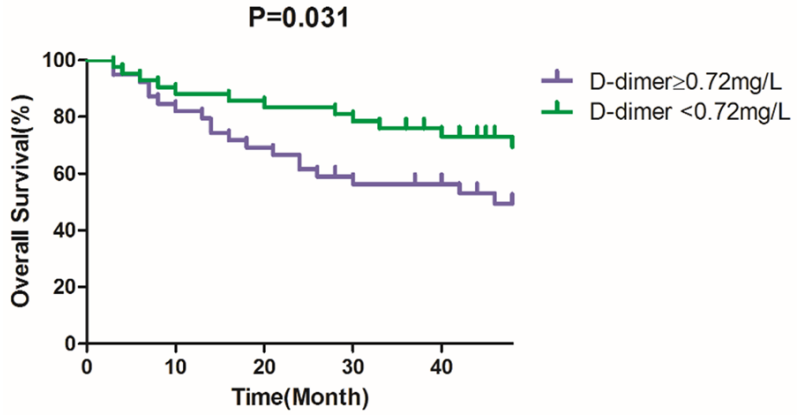

Figure 2. Kaplan-Meier analysis of overall survival (OS) as a function of D-dimer levels (above vs below the median level) in patients with neuroblastoma.

Table 2. Multivariate analysis of clinicopathologic factors affecting overall survival

Characteristics	Overall survival		
	HR	95% CI	*P*
Age in months (≥12 vs <12)	0.72	0.33-1.41	0.152
Sex (male vs female)	1.18	0.82-1.58	0.181
MYCN amplification (amplified vs not amplified)	4.15	1.92-16.17	0.012
Plasma D-dimer (≥0.72 mg/L vs <0.72 mg/L)	3.78	1.28-6.1	0.018

(0.72 mg/L). Whereas the 3-year OS of the entire cohort was 95.1% (77/81), 3-year OS was lower in the high D-dimer group compared with that of the low D-dimer group (OS: 56.8 ± 5.3% vs 77.3 ± 3.5%, *P*=0.031) (**Figure 2**).

Multivariate analysis identified D-dimer levels and *MYCN* amplification as independent predictors of OS (D-dimer, hazard ratio [HR]: 3.78, 95% confidence interval [CI] 1.28-6.1, *P*=0.018; *MYCN* amplification, HR: 4.15, 95% CI: 1.92-16.17, *P*=0.012) (**Table 2**). Sex and age were not significantly associated with OS. In general, higher D-dimer levels were associated with worse outcomes.

Discussion

To our knowledge, this is the first study to determine the clinical significance of D-dimer levels in patients with NB. We found that higher D-dimer levels in NB were significantly associated with factors raising the risk of a poor outcome (amplified *MYCN* and stage 4 disease) and may therefore serve as a predictor of unfavorable prognosis.

Higher D-dimer levels may reflect the presence of cross-linked fibrin that forms during angiogenesis and tumor invasion within a favourable host environment [13]. Fibrin remodeling is critical for the formation of new vessels and is involved in many steps of metastasis [14]. Thrombin, a central enzyme in the clotting cascade, functions as a potent tumor promoter. Thrombin-mediated platelet activation induces tumor cells to adhere to platelets, and forms clots around circulating

tumor cells [15] that protect tumor cells against immune surveillance, thereby promoting metastasis. Moreover, compared with wild-type mice, plasminogen gene-knockout mice develop larger tumors and more numerous distant metastases, which lead to shorter life spans.

D-dimer levels are associated with the clinical features of other tumors. For example, in patients with small cell lung cancer, D-dimer levels correlate with tumor stage and the number of metastases, but not with age, sex or smoking history [16]. In patients with colorectal cancer, high preoperative D-dimer levels are associated with larger tumors, deeper wall-penetration and metastasis [17]. D-dimer levels are associated with the characteristics of pancreatic cancer, ovarian cancer and breast cancer [18-20]. Together with our present findings, these results associate D-dimer levels with more aggressive disease. Further, the current study shows that D-dimer levels were associated with disease stage and OS. Thus, patients with elevated D-dimer levels had more aggressive disease and experienced shorter survival.

Current staging and risk stratification systems rely on conventional diagnostic techniques such as CT, MRI, and radioisotope scans as well as clinical characteristics such as age and sex. Patients are stratified into risk groups according to these staging systems. However, patients within each risk group experience different outcomes and differ in their responses to the same treatment.

Staging and risk stratification systems may be improved by adding new parameters. For example, tumor markers are used in the initial assessment of patients with NB. Vanillylmandelic acid, homovanillic acid, ferritin, NSE and LDH represent characteristic tumor markers for NB upon initial diagnosis and response assessment [3] and are commonly monitored in the follow-up of survivors of NB. The present study shows that the D-dimer level is a candidate staging biomarker that should be considered in efforts to develop new staging and risk stratification systems.

This study has several limitations. First, the sample size was small because of the low prevalence of NB. Although our hospital is one of the largest pediatric hospitals in Central China, we receive approximately 40 patients newly diagnosed with NB each year. After exclusion of unqualified patients such as those with incomplete records and those lost to follow-up, we only included 81 patients in the present study. Second, our study carries the inherent bias of a retrospective study. Third, because of the small study cohort, we were unable to perform subgroup analysis of progression-free-survival, which may have provided more information on the effects of D-dimer levels on patients' responses to therapy. Fourth, D-dimer is not a specific biomarker for NB or tumors, which may have affected our findings.

In summary, elevated D-dimer levels may predict more aggressive disease, and thus worse OS, of patients with NB. Considering the speed, validity and low cost of D-dimer testing, routine measurement of the D-dimer levels seems feasible for patients with NB, even in developing countries, and may provide useful information that will improve patient management.

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Disclosure of conflict of interest

None.

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