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Research Paper

Osteopontin as a predictive factor of requirement to primary surgical intervention and a prognostic factor in small intestinal non-Hodgkin lymphoma

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ABSTRACT

The treatments for intestinal non-Hodgkin lymphoma (NHL) have not been established. Primary surgical resection seemed a rational choice because it can establish the diagnosis and reduce the tumor burden. The authors found that NHL with jejunum involvement may more require for primary surgical intervention following chemotherapy. However, the surgical rate of jejunum involvement was 9.3 to 23.8%. Osteopontin (OPN) plays a role in tumorigenesis, invasion, and metastasis of human cancers. This study is aimed to explore the influence of OPN in small intestinal NHL. A retrospective analysis between 1995 and 2010 was collected from clinical data. Fourteen cases of primary small intestinal NHL were identified. Anti-OPN mouse monoclonal antibody was applied to these lymphoma tissues. Seven patients (50%) experienced abdominal pain and/or distention. Jejunum involvement was presented in 8 of 14 patients (57.1%). Surgical treatment was performed in 5 of 8 patients with jejunum involvement. We found those with OPN expression ≥ 50 had significant higher operation risk (OR = 18, p = 0.044). Linear regression confirmed that there was a significant association of score of OPN in intestinal NHL and survival time (P = 0.034, r = -0.641, respectively), indicating a correlation of the increased OPN score and decreased survival time. In conclusion, the results explain that OPN and the involved site may be as predictive factors of requirement to primary surgical intervention. We found a trend that OPN can be considered as a prognostic factor in small intestinal NHL, although the subgroups were too small for individual analysis.

Keywords: Osteopontin, intestinal, non-Hodgkin lymphoma.

INTRODUCTION

The gastrointestinal tract is the most common extra-nodal site involved by non-Hodgkin lymphoma (NHL). Primary intestinal NHL is prominently different from primary gastric NHL regarding to clinical features, pathological subtype, treatment and prognosis. The small intestine involved by lymphoma is less often than large intestine. The treatment strategies for intestinal NHL have not been established yet. According to the results of small series of intestinal NHL surgical resection has been commonly accepted as a primary treatment modality. Primary surgical resection seemed a rational treatment of choice since it can simultaneously establish the diagnosis as well as reduce the tumor burden (Lee et al., 2009).

Besides, the authors found a trend that NHL with jejunum involvement may require for primary surgical intervention following chemotherapy (Chen et al., 2014). However, the surgical rate of intestinal NHL with jejunum involvement was around 9.3 to 23.8% (Daum et al., 2003; Gregory and Turowski, 1995; Koch et al., 2001; Ibrahim et al., 2001; Kako et al., 2009), and the authors want to find someone's biomarker as a predictive factor for increasing the sensitivity of the primary surgical rate.

OPN is a member of the small integrin-binding ligand N-linked glycoproteins (SIBLINGs) family, a major protein of

extracellular bone matrix, and gene expression is upregulated in specific phases of osteoblastic lineage differentiation (Bellahcene et al., 2008). In recent decade studies, OPN have been found overexpressed and associated with tumor progression and metastasis in various cancers, such as breast cancer (Khan et al., 2005; Furger et al. 2003), prostate cancer (Khodavirdi, 2006), gastric cancer (Ue et al., 1998), melanoma (Kiss et al., 2015), colorectal cancer (Ng et al., 2015), hepatocellular carcinoma (Deng et al., 2013; Zhu et al., 2014), nasopharyngeal carcinoma (Hou et al., 2015) and renal cell carcinoma (Ramankulov et al., 2007). The mechanisms revealed that the binding of OPN with several integrins and/or CD44 family members through Arg–Gly–Asp (RGD)-dependent and RGD-independent interactions initiates the pathways involved in cancer cell adhesion, proliferation, invasion, extracellular matrix degradation, migration as well as angiogenesis, leading to cancer progression and metastases (Bellahcene et al., 2008; Khan et al., 2005; Furger et al. 2003).

Interestingly, OPN is also a secreted extracellular matrix protein which also performs a potent inhibitor of soft tissue mineralization and, consequently, it is able to block ectopic calcification of the vasculature *in vivo* (Giachelli et al., 2005; Scatena et al., 2007). Besides, OPN is also a soluble cytokine involved in inflammation and tissue remodeling (Scatena et al., 2007). In acute and chronic inflammatory responses, OPN is highly expressed by both macrophages and CD4 lymphocytes and plays a functional role in early Th1 response (Giachelli et al., 1998). Because of all these properties, OPN is thought to exacerbate inflammation in several chronic diseases, including atherosclerosis (Scatena et al., 2007).

However, the role of OPN in intestinal NHL is still unknown. The aim of this study is to explore the OPN as a predictive factor of requirement to primary surgical intervention and a prognostic factor in small intestinal non-Hodgkin lymphoma.

METHODS

Patients

A retrospective analysis between 1995 and 2010 was collected from clinical data in Tri-Service General Hospital. Of these, 14 cases of primary small intestinal NHL were identified. A Cox model was used for multivariate analysis. The Kaplan-Meier method was used for survival analysis.

The collected clinical data including: age at diagnosis, Ann Arbor stage, Eastern Cooperative Oncology Group (ECOG) performance status, lactate dehydrogenase (LDH) level, extra-nodal sites, and International Prognostic Index (IPI). Initial staging included past history and physical examination, serum blood tests (including LDH and other biochemical markers), chest X-ray, computed tomography of the neck, chest, abdomen, and pelvis, as well as bone marrow aspiration and biopsy.

CHOP chemotherapy consisted of cyclophosphamide, 750 mg/m^2 as intravenous (i.v.) infusion on day 1, doxorubicin, 50 mg/m^2 i.v. on day 1, vincristine, 1.4 mg/m^2 (maximum dose; 2 mg/body) i.v. on day 1, and prednisone 60 mg/m^2 per orally (p.o.) on days 1 to 5 (CHOP) or cyclophosphamide, 750 mg/m^2 i.v. on day 1, epirubicin, 80 mg/m^2 i.v. on day 1, vincristine, 1.4 mg/m^2 (maximum dose; 2 mg/body) i.v. on day 1, and prednisone 60 mg/m^2 p.o. on days 1 to 5 (CEOP). The R-CHOP or CHOP was prescribed for 3 or 4 courses following radiotherapy for localized disease, and for 6 to 8 courses for advanced disease. CEOP or COP regimen was given for patients who have cardiac dysfunction or were older than 70 years. The dosage and schedule of rituximab included in R-CHOP regimen was 375 mg/m^2 every 3 weeks combined chemotherapy.

The secondary line chemotherapy contains ICE (ifosfamide, carboplatin and etoposide) and ESHAP (Etoposide, Methylprednisolone, Cytarabine, and Cisplatin).

The complete remission (CR) and partial remission (PR) were evaluated by Japanese 327123 International Working Group criteria (Cheson et al., 1999). Stable disease (SD) is defined as less than a PR but not progressive disease (PD). PD was defined as the occurrence of new lesions or an increase of 25% in the sum of the products of the cross-sectional diameters of all previously detected lesions.

The primary endpoint was overall survival (OS). The final date for OS was defined as the day of death from any cause or the day last known alive. OS was assessed using the Kaplan–Meier method and compared between groups using the log-rank test (Kaplana and Meier, 1958; Mantel, 1966). All the survival analyses were conducted with the STATA with $P<0.05$ being defined as statistically significant.

Immunohistochemical staining of OPN

Anti-OPN mouse monoclonal antibody (mAb53, prepared against recombinant glutathione S-transferase human OPN fusion protein, dilution at 1:1000) was applied to intestinal NHL tissue, using the avidin-biotin-peroxidase complex method, following the manufacturer's instructions. This method has been reported in our previous publication (25).

In brief, the immunostaining was performed manually at room temperature. Endogenous peroxidase and nonspecific background staining were blocked by incubating slides with 3% aqueous hydrogen peroxide for 10 min. After washing with PBS for 5 min, slides were blocked with normal serum for 20 min, followed by incubation with the anti-OPN primary antibody, at the given dilution, for 60 min. After rinsing with PBS for 5 min, sections were incubated with a biotinylated secondary antibody for 20 min. After washing with PBS for 5 min, slides were incubated with avidin-biotin complex for 30 min and washed again. Chromogen was developed with 10 mg of 3,3'-diaminobenzidine

Table 1. Patient population: Primary with jejunum vs. without involvement in primary small intestinal non-Hodgkin lymphoma.

Parameter	No. of patients (%)	
	Jejunum (n = 8)	Without (n = 6)
Age (yr)		
Mean	63.75	47.1
Range	47-87	17-75
Sex		
Male	5	5
Female	3	1
Cell type		
B	6	4
T	2	2
ECOG		
0-1	4	5
≥2	4	1
IPI		
1-2	2	3
3-5	6	3
Surgery		
Yes	5	2
No	3	4
Surgical rate (%)	62.5	33.3
Outcome		
Overall survival time (m)	28.68	53.33
3-year overall survival, n (%)	4/8 (50)	4/6 (66.6)

tetrahydrochloride diluted in 12 ml of Tris buffer for 2 min. All samples were lightly counterstained with Mayer's hematoxylin for 30 s before dehydration and mounting. A section of colon cancer that was previously proven to be OPN positive by western blot was used as a positive control. Mouse IgG was used as the negative control serum. Membranous and cytoplasmic immunostaining intensity was assessed for OPN on an intensity scale of 0 (negative), 1+ (weakly staining), 2+ (moderately staining), to 3+ (strongly staining). Also, the percentage of immunostaining density in tumor cells was calculated. The OPN IHC score, obtained by multiplying the percentage of cells by its corresponding immunostaining intensity, ranged from 0 to 300.

Statistical analysis

Student t test and Chi-square test was checked to compare baseline demographic difference between jejunum cohort and non-jejunum cohort. Kaplan–Meier survival curves were plotted to ascertain subsequent mortality in the patients with intestinal NHL and were stratified according to the jejunum involvement and without jejunum involvement.

Correlation between immunostaining score of OPN in intestinal NHL and survival time was performed by linear regression analysis. A $P<0.05$ value was considered to be statistically significant.

RESULTS

Patient characteristics

In 14 patients with primary small intestinal NHL, 10 patients were men (71.4%) and 4 patients were women (28.5%); 10 patients (71.4%) were diagnosed as having B-cell lymphoma, in whom 6 (60%) were diffuse large B-cell lymphoma (DLBCL). Seven patients (50%) presented with abdominal pain and/or distention. Jejunum involvement was present in 8 of 14 patients (57.1%). The clinical characteristics of the jejunum involvement group and without jejunum group are summarized in Table 1.

Treatment

Surgical treatment was performed in 5 of 8 patients with jejunum involvement due to acute abdomen or perforation related peritonitis. The surgical rate of NHL with jejunum involvement was higher than without jejunum, although this difference was not significant (62.5%; 33.3%, P = 0.28).

Outcomes and survival time

The median overall survival time was 39.24 months and the 3-year survival rate was 57.1%.

1. The median overall survival time for patients with jejunum involvement was shorter than those without jejunum involvement (39.5 months; 81 months), although the difference was not significant (P = 0.067).
2. T cell lymphomas (n = 4) seemed to have a poorer overall survival time than B cell lymphomas, but still without significance (29.9 months vs. 43 months; P = 0.967).

Clinicopathological characteristics and analysis of OPN expression

The pathological diagnosis comprises 11 tumors located in intestinal area. The different immunostaining expressions of OPN in cytoplasmic components of tumor cells are demonstrated in Figure 1a and b. Moreover, Table 2 lists results from quantitative analysis of OPN IHC scores.

Linear regression analysis confirmed that there was a significant association of score of OPN in intestinal NHL and survival time (P = 0.034, r = -0.641, respectively, Figure 2), which indicating an exponential correlation of the increased OPN score and decreased survival time.

Chi-Square test confirmed that there was a significant association of OPN expression more than 50 and the surgical rate of intestinal NHL (OR = 18, p = 0.044), which indicating an exponential correlation of the score of OPN >50 and increased the surgical rate.

DISCUSSION

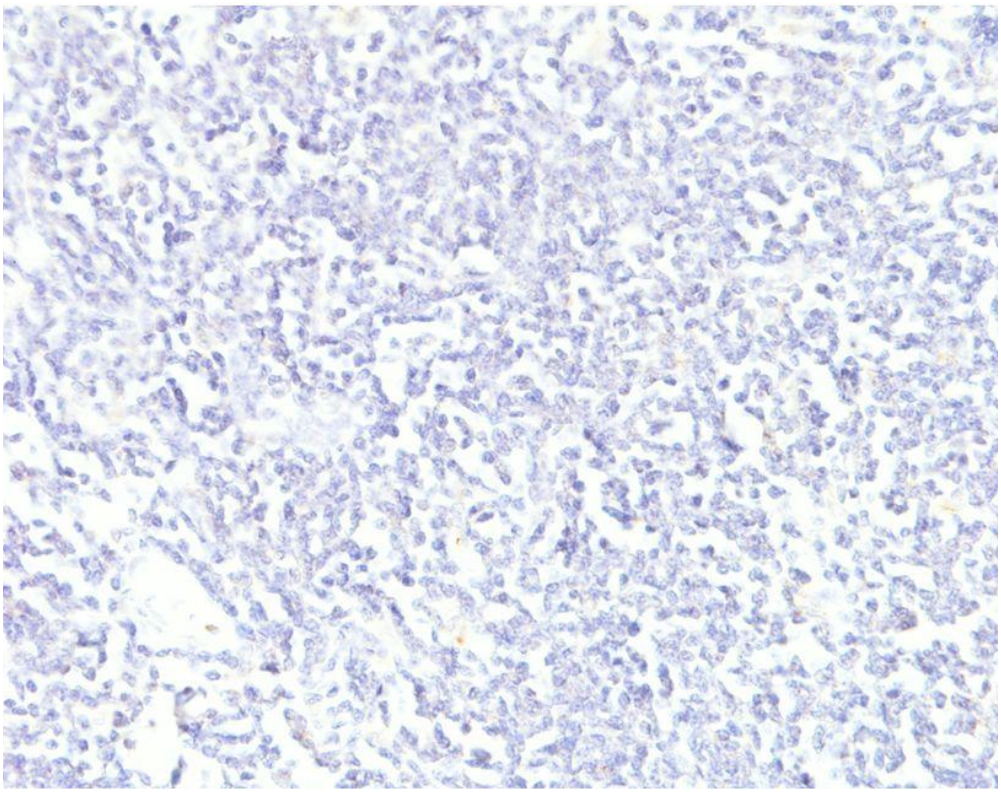
Previous studies have shown that ileocecal lymphomas frequently present with complications requiring surgical intervention on an emergency basis due to its anatomical site. Even if 54% of ileocecal lymphomas required immediate surgery, 30% of non-ileocecal lymphoma presented with complications (Daum et al., 2003; Koch et al., 2001; Gurney et al., 1999; Liang et al., 1995). Consequently some anatomical location of lymphoma also presented with complications requiring emergency surgical intervention. Earlier studies are summarized in Table 3 (Daum et al., 2003; Gregory and Turowski, 1995; Koch et al., 2001; Ibrahim et al., 2001; Kako et al., 2009; Cheson et al., 1999; Kim et al., 2011). The emergent surgical rate was around 9.3 to 23.8%, but 50% was noted in our study, especially up to 62.5% in NHL patients with jejunum involvement. In our study, two cases had emergency surgery for intestinal perforations during chemotherapy. Thus, small intestinal NHL with jejunum involvement was suggested for primary surgical intervention and then chemotherapy. In our results, the membrane and cytoplasm of the tumor cells showed positive for OPN with different staining intensities in the lymphoma specimens, OPN expression > 50 and the surgical rate of intestinal NHL (OR = 18, p = 0.044), which indicating an exponential correlation of the score of OPN >50 and increased the surgical rate. Thus, OPN scores was found as a predictive factor for increasing the sensitivity of the primary surgical rate.

B-cell lymphoma is the most common type in the small intestinal NHL and DLBCL accounts for most common type. Some studies have shown that T cell lymphomas and NHL with disseminated disease had been a poorer overall survival time, although this difference was not significant in our study (P>0.05) (Daum et al., 2003; Gregory and Turowski, 1995; Koch et al., 2001; Ibrahim et al., 2001; Kako et al., 2009). However, the authors found a trend that the overall survival time of jejunum involvement was shorter than without jejunum involvement, it may be caused by anatomy of small intestine.

Osteopontin is categorized as both a matrix protein and a cytokine. Increased protein levels of osteopontin have been detected in the circulating plasma or serum-derived from patients with a number of solid neoplasms (Jae-Hoon et al., 2002; Le et al., 2003; Standal et al., 2004). In the circulating blood, osteopontin molecules may contribute to an interaction among cancer cells and endothelial cells, indicating that the expression of osteopontin may regulate cancer invasion, intra- and extravasation, and colonization at distant sites. The osteopontin levels in the plasma of the SCLC were consistent with our findings of immunohistochemistry staining of osteopontin in the tumor tissues, suggesting that osteopontin is critically involved in the aggressive progression of MM, which indicating an correlation of the increased OPN score and decreased survival time.

Limitations

Most studies of primary GI NHL (1 case per 100,000 persons per year) were small, retrospective studies reporting only small patient numbers or to studies that have been conducted over periods of up to one decade and more. These studies are often heterogeneous, combining different types of GI NHL and using varying histological classifications, different staging systems, and different forms of treatment.



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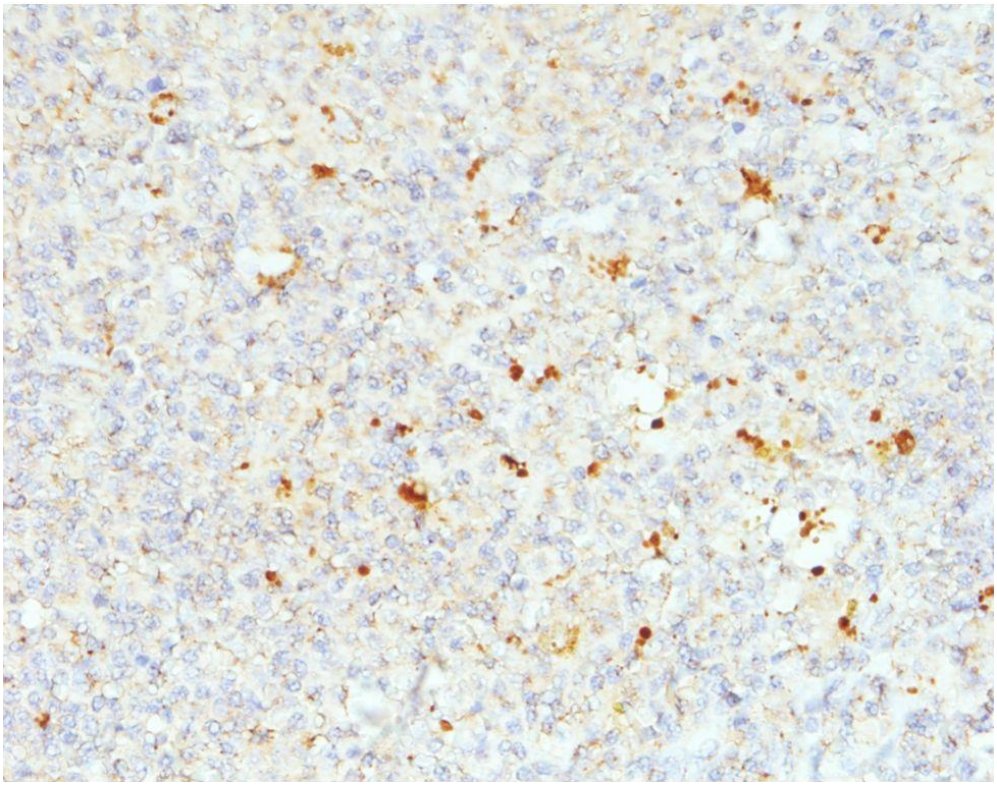

b

Figure 1. Immunostaining for OPN in cytoplasmic components of different pathological degree of intestinal NHL. (a) Intensity score 0 (negative staining); (b) intensity score 3+ (strong staining).

Table 2. *Results from quantitative analysis of OPN IHC scores.*

Sex/age	Cell type	DLBCL	Jejenum (1)	OPN total	OP(1)	Survival time	Alive (1)
F/81	B	0	1	2	0	70	0
F/51	B	1	0	50	0	75	1
F/80	B	1	1	50	0	55	1
M/63	B	1	0	60	1	81	0
M/47	B	1	1	60	1	9	0
M/75	B	0	0	90	1	5	0
M/87	B	0	1	40	1	46	1
M/47	T	0	1	120	1	1.5	0
M/56	T	0	0	60	0	24	1
F/50	B	0	1	120	1	46	1
M/57	T	0	1	180	1	1	0

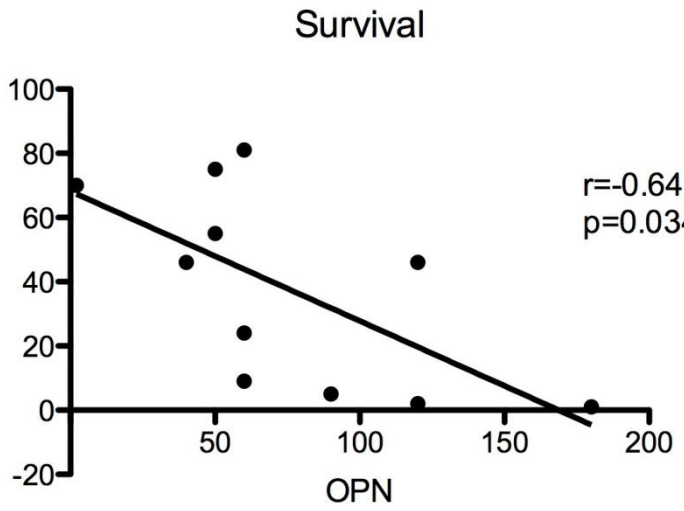

Figure 2. Linear regression confirmed that there was a significant association of score of OPN in intestinal NHL and survival time (P = 0.034, r = -0.641, respectively).

Table 3. Series comparison of emergency surgical rate in primary small intestine.

Article	Small intestine	Jejunum	Complications requiring primary surgery (Jejunum)	Emergency surgical rate (%)
Jeeyun, 2004 (Z3)	21	NA	5	5/21(23.8)
Peter, 2001 (Z4)	32	NA	3	3/32 (9.3)
Severin, 2003 (Z5)	83	30	19	19/83 (22.8)
Mantel, 2001 (Z6)	37	NA	NA	NA
Shinichi, 2009 (Z7)	23	3	3	3/23 (13)
Seok, 2011 (21)	92	17	12	12/92 (13)
Our study	14	8	7 (5)	7/14 (50) 5/8 (62.5)

There are also important limitations to the present study. While this was not a randomized trial, the patient characteristics in the two groups were essentially different. Furthermore, the numbers of patients and duration of follow-up were limited. A longer follow-up might stabilize the trends and permit the drawing of conclusions.

Conclusion

The results reported here may explain, at least in part, OPN and the involved site as predictive factors of requirement to primary surgical intervention. The authors found a similar trend OPN can as a prognostic factor in small intestinal NHL, although in our cohort the subgroups of primary small intestinal lymphoma were too small for individual analysis.

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