Clinical Study

Prognostic Significance of Serum Proangiogenic Molecules in Patients with De Novo Non-Hodgkin Lymphomas

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1. Introduction

Angiogenesis, the formation of new blood vessels, is of great importance in neoplastic growth and progression in both solid and hematologic malignancies. The growing of new capillaries is activated by proangiogenic molecules such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). The VEGF is a soluble 46-kD protein and the bFGF is an 18- to 24-kD polypeptide \cite{1-3}. In solid tumors, the progenetic molecules act as inducers of neovascularization, thereby enhancing tumor growth and metastatic potential \cite{4}.

In hematologic malignancies, roles of angiogenesis were established in multiple myeloma (MM). Various studies had shown that increased microvascular density in bone marrow was associated with poor prognosis \cite{5,6}. Importantly, yet other studies found that antiangiogenic agents such as thalidomide or immunomodulatory drugs were associated with survival advantages in patients with MM \cite{7-9}.

Though predictive values of serum angiogenic factors in non-Hodgkin lymphoma (NHL) have been studied, confirmation studies in different ethnic groups should be conducted. Therefore, this study was undertaken to assess the clinical significance of the serum proangiogenic molecules, VEGF, and bFGF, in Thai patients with de novo NHL.

2. Materials and Methods

A total of 79 adult patients with newly diagnosed stage 2–4 non-Hodgkin lymphomas was enrolled at Songklanagarind Hospital, the major tertiary care center in southern Thailand, between December 30, 2005 and April 9, 2009. Patients with a reactive test for human immunodeficiency virus or primary extranodal lymphomas were excluded. Histological classification was in accordance with the WHO classification system. Monoclonal antibodies targeting CD3, CD5, CD20, and CD79a (Dako, Glostrup, Denmark) were used for the T- or B-lineage determination. This study was approved by the Ethics Committee of Prince of Songkla University.

Clinical staging was evaluated according to the Ann Arbor staging system. Prognostic assessment was performed based on the International Prognostic Index (IPI).

All patients were treated with a standard CHOP regimen including a minimum of six courses of cyclophosphamide, doxorubicin, vincristine, and prednisolone. Rituximab was
not routinely administered in Thailand. Treatment response
was classified as complete remission (CR), undetermined complete
remission (CRu), partial remission (PR), stable
disease (SD), or progressive disease (PD) according to the
standard criteria.

Ten mL of peripheral venous blood samples was collected
from all participants before their treatment was begun. All
samples were centrifuged at 2000 g for 10 minutes and frozen
at −20°C soon after collection. The samples were thawed
and analysed after 12–24 months’ storage. Serum VEGF
and bFGF concentrations were measured by quantitative sand-
wich enzyme immunoassay technique (Quantikine R; R&D
systems, Minneapolis, MN) following the manufacturers’
instructions. All analyses and calibrations were performed
in duplicate. A set of standard wells containing known
quantities of recombinant human VEGF and bFGF were
included in all experiments. Concentrations were recorded
as the mean of duplicate measurements in picograms per
milliliter. The intra- and interassay variations were within the
ranges given by the manufacturers.

### Table 1: Initial characteristics of 79 patients with de novo NHL.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Performance status (ECOG ≥2)</td>
<td>15 (19.0)</td>
</tr>
<tr>
<td>B symptoms</td>
<td>46 (58.2)</td>
</tr>
<tr>
<td>Ann Arbor stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>7 (8.9)</td>
</tr>
<tr>
<td>II</td>
<td>22 (27.8)</td>
</tr>
<tr>
<td>III</td>
<td>10 (12.7)</td>
</tr>
<tr>
<td>IV</td>
<td>40 (50.6)</td>
</tr>
<tr>
<td>Bulky disease</td>
<td>28 (35.4)</td>
</tr>
<tr>
<td>Bone marrow involvement</td>
<td>26 (32.9)</td>
</tr>
<tr>
<td>High serum LDH</td>
<td>46 (58.2)</td>
</tr>
<tr>
<td>Histological subtype</td>
<td></td>
</tr>
<tr>
<td>Diffuse large cell lymphoma</td>
<td>54 (68.4)</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>8 (10.1)</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>3 (3.8)</td>
</tr>
<tr>
<td>Lymphoblastic lymphoma</td>
<td>2 (2.5)</td>
</tr>
<tr>
<td>Burkitt’s lymphoma</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Mucosa-associated lymphoid tissue</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Peripheral T-cell lymphoma, unclassified</td>
<td>6 (7.6)</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma</td>
<td>3 (3.8)</td>
</tr>
<tr>
<td>Angioimmunoblastic T-cell lymphoma</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>International prognostic index</td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>24 (30.4)</td>
</tr>
<tr>
<td>Low-intermediate risk</td>
<td>20 (25.3)</td>
</tr>
<tr>
<td>High-intermediate risk</td>
<td>21 (26.6)</td>
</tr>
<tr>
<td>High risk</td>
<td>14 (17.7)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
</tr>
<tr>
<td>CHOP</td>
<td>44 (55.7)</td>
</tr>
<tr>
<td>R-CHOP</td>
<td>22 (27.8)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (8.9)</td>
</tr>
<tr>
<td>No chemotherapy</td>
<td>6 (7.6)</td>
</tr>
</tbody>
</table>

### Table 2: Factors associated with a poorer complete remission (CR) rate (Ta b l e 2).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy</td>
<td></td>
</tr>
<tr>
<td>CHOP</td>
<td>44 (55.7)</td>
</tr>
<tr>
<td>R-CHOP</td>
<td>22 (27.8)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (8.9)</td>
</tr>
<tr>
<td>No chemotherapy</td>
<td>6 (7.6)</td>
</tr>
</tbody>
</table>

2.1. Statistical Analysis. Frequency tables of baseline char-
acteristics were analyzed with the Chi-square or Fisher’s
exact test. A logistic regression model was used to predict
complete remission (CR). Univariate analysis of survival was
performed with the Kaplan-Meier method. Overall survival
(OS) was calculated as the time interval from the date of
diagnosis to death or last followup. Kaplan-Meier methods
were used to estimate time-to-event endpoints. Survival
data between subgroups were compared using the log rank
test. Multivariate analysis of OS was performed using a
Cox regression model with backward elimination. Critical P
values for entry and removal were 0.2 and 0.4, respectively.
To test the main hypothesis, we forced the serum level of
VEGF and bFGF into the model. Hazard ratios (HR), 95% confi-
dence intervals (95% CI), and P value were obtained
from the best-fit model. All the statistical analyses were
performed using the R program with epicalc package. A
significance level of 0.05 was used throughout all statistical
tests in the study.

### 3. Results

3.1. Patient Characteristics and Histological Subtypes. The
clinical characteristics of the 79 patients are shown in Table 1.
There were 38 males and 41 females with a mean age of 55.2
(range 16–82) years. Regarding immunohistochemistry, the
B-cell phenotype was shown in 69 specimens (87.3%), and
10 tissue specimens (12.7%) expressed the T-cell phenotype.

3.2. Serum VEGF and bFGF at the Time of Diagnosis. At the
time of diagnosis, the serum VEGF concentrations from the
79 patients ranged from 72.0 to 2919.4 pg/mL with a mean of
668.0 pg/dL, median of 516.0 pg/mL, and the third quartile
of 835.5 pg/mL. The serum bFGF concentration ranged from
undetectable to 2919.4 pg/mL with a mean of 12.15 pg/dL,
median of 9.85 pg/mL, and third quartile of 17.60 pg/mL.
Associations between serum VEGF and bFGF and clinical
features at diagnosis were analyzed using mean, median,
and the third quartile of both angiogenetic factors as a cut-
off value. No significant associations were found (data not
shown).

3.3. Prediction of Response Rate by Serum VEGF and bFGF. From
a univariate analyses, the higher levels of serum VEGF
and bFGF using the mean, median, and third quartile
of both angiogenetic factors as cut-off values were not
associated with a poorer complete remission (CR) rate.
However, patients with B symptoms, bulky diseases, ane-
ia, poorer performance status, high serum LDH, and T-
cell immunophenotype had lower CR rates. Considering
the chemotherapy regimens, CHOP and R-CHOP showed
higher CR rates. Multivariate analysis identified higher than
the mean of serum VEGF, B symptoms, bulky diseases, ane-
ia, and treatment with CHOP or R-CHOP as independent
variables influencing CR rate (Table 2).

3.4. Prediction of Survival Rate by Serum VEGF and bFGF. The
median follow-up time was 15.1 (range 0.3–55.2)
months. In univariate survival analyses, there was no association between serum VEGF and OS (Figure 1(a)). However, there was a significant association between shorter OS and B symptoms, more extranodal involvements, poor performance status, anemia, high serum LDH, bFGF (Figure 1(b)), and IPI. In contrast, the CHOP or R-CHOP regimens were predictors for better OS. From a Cox proportional hazards model, variables independently associated with OS were BM involvement, more extranodal involvement, poor performance status, anemia, and higher than the mean of serum bFGF as shown in Table 2.

4. Discussion

In this study, a high pretreatment level of serum VEGF and bFGF were independently associated with poorer CR and OS rates, respectively. Although the role of neovascularization in hematologic malignancies has been extensively explored, few studies have been conducted on the role of angiogenesis in lymphomas [10]. In addition, the predictive value of angiogenesis markers in lymphomas is still controversial due to disease heterogeneity and various detection methods [11]. However, serum proangiogenic markers are a simple method and have the considerable advantage of not requiring an experienced pathologist to reliably assess. There have been only a few published studies on these serum proangiogenic markers in clinical settings. However, confounding variables of known clinical prognostic factors were not considered in some of these reports. Therefore, we conducted this study to determine the independent association between these markers and clinical outcomes.

In earlier studies, Salven et al. [12–14] and Bertolini et al. [15] demonstrated a significant association between these markers and the outcomes of patients with NHL in concordance with our study. The correlation between high VEGF levels and poor CR rate also supported the hypothesis that high VEGF is responsible for an abnormal vessel structure of tumors leading to lowering drug delivery [4]. However, our more recent study found higher levels of pretreatment VEGF and bFGF, probably because the patients in our study had more advanced stages of disease. Ribatti et al. [16] and Crivellato et al. [17] also found that neovascularization was
found frequently in high-grade lymphoma. The different serum VEGF and bFGF levels before treatment in patients with different degrees of disease, or due to other factors, may lead to difficulty in obtaining a single cut-off value for a predictor in all patients with NHL. In addition, the level of bFGF may be elevated due to other conditions associated with increased endothelial activity, infection, or inflammation [18, 19].

Importance roles of angiogenesis in lymphomas have been demonstrated in clinical studies. Tzankov and colleagues [20] performed immunohistochemical and morphometric studies in B-cell lymphomas and found higher microvessel density, and VEGF and COX2 in aggressive lymphomas. This result is in concordance with Ganjoo et al. [21] who reported that patients with negative stained VEGF-A or VEGF-R1 had a superior survival rate. These studies confirmed the potential importance of increased angiogenesis in prognosis and tracking of disease progression in non-Hodgkin lymphomas.

In conclusion, our study suggests that serum VEGF and bFGF are associated with poor prognosis in patients with de novo non-Hodgkin lymphomas. Further studies are needed to determine more clearly whether monitoring of consecutive levels of these molecules during or after therapy could predict CR or relapse. In addition, these markers may play an important role in patient selection for antiangiogenic treatment.

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References


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