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High serum angiogenin at diagnosis predicts for failure on long-term treatment response and for poor overall survival in non-Hodgkin lymphoma

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ABSTRACT

Background: Angiogenin is a potent inducer of angiogenesis. We prospectively evaluated the prognostic significance of serum angiogenin from 204 consecutive non-Hodgkin lymphoma (NHL) patients diagnosed and treated in a single institution.

Methods: Serum angiogenin, VEGF, and bFGF concentrations at diagnosis were determined using a quantitative sandwich enzyme immunoassay technique. Kaplan–Meier survival curves were compared by the log-rank test. Multivariate survival analyses were performed using the parametric model of Weibull and the non-parametric proportional hazards model of Cox.

Results: Patients with a high serum angiogenin at diagnosis (>median; 401 ng/ml) had significantly lower 5-year survival rate than those with a low (⩽median) angiogenin (42% versus 63%, respectively; $P = 0.0073$). Serum angiogenin provided additional information to the International Prognostic Index (IPI) identifying a subgroup (serum angiogenin >median and IPI > 1) with very poor prognosis (5-year survival 19%, $P < 0.0001$). In receiver operating characteristic (ROC) analyses the accuracy of the IPI to correctly classify patients with favourable or poor survival was improved from fair to good by complementing the IPI with serum angiogenin concentration. With patients who initially achieved complete response (CR) after chemotherapy, a high angiogenin at diagnosis (>median; relative risk (RR) 2.38; $P = 0.0077$) and an advanced tumour stage (III–IV; RR 2.41; $P = 0.0087$) were the only independent predictors for patients with unfavourable outcome although first responding well to therapy.

Conclusions: We conclude that elevated serum angiogenin surfaced as an independent predictor for failure in long-term treatment response and for poor overall survival in a series of 204 NHL patients, and might thus also complement the IPI in identifying the patients with particularly aggressive and/or treatment resistant disease.

1. Introduction

Angiogenesis is considered to depend on the balance of inhibitors and stimulators of endothelial cell proliferation, endothelial cell migration, and capillary formation molecules.[1] Like in solid tumours, angiogenesis plays a role also in the pathogenesis of haematologic malignancies.[2–8] Expression of angiogenic growth factors such as vascular endothe-

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lial growth factor (VEGF) and basic fibroblast growth factor (bFGF) correlate with clinical characteristics in lymphoma and leukaemia[9–11] and their serum concentrations may serve as prognostic markers and tools for monitoring response to treatment and recurrence in haematological malignancies.[12–22]

Human angiogenin is a single chain, 123 amino acid non-glycosylated polypeptide that exhibits both ribonuclease activity and special biological actions.[23] Angiogenin, which was originally isolated from the medium conditioned by HT-29 human colon adenocarcinoma cells, is one of the most potent known angiogenic factors *in vivo*.[24,25] Angiogenin may promote tumour growth, as suggested by studies of breast cancer in a mouse model in which anti-angiogenin antibodies inhibit the formation of human breast cancer xenografts by reducing tumour neovascularisation.[26] The mechanism of the angiogenic activity is not known but it may involve interactions between angiogenin molecule itself and a variety of non-receptor protein molecules. These molecular partners include heparin, plasminogen, elastase, and actin.[27,28] Angiogenin levels are evident in normal human serum in ng/ml concentrations[29,30] and elevated in patients with solid tumours including pancreatic cancer,[30] ovarian cancer[31] endometrial cancer,[32] cervical cancer,[33] colorectal cancer,[34] urothelial carcinoma[35] and malignant melanoma.[36] We wanted to study the possible prognostic value of circulating angiogenin in haematologic malignancies, and measured angiogenin concentrations by enzyme-linked immunosorbent assay (ELISA) in serum taken from 204 consecutive non-Hodgkin lymphoma (NHL) patients at the time of diagnosis.

2. Methods

2.1. Patients

Serum angiogenin concentrations were measured in 204 consecutive adult patients with NHL diagnosed and treated in the Department of Oncology, Helsinki University Central Hospital (Helsinki, Finland), from 1981 to 1987, for whom frozen serum taken at the time of diagnosis but before lymphoma treatment was available. The study was performed after approval by a local institutional review board. Forty-seven lymphomas (23%) had been classified as low grade, 97 (48%) intermediate grade and 50 (25%) as high grade lymphoma according to the Working Formulation Scheme.[37] Ten cases (5%) were considered as unclassifiable. The histologic types of lymphomas in the series according to the Working Formulation Scheme were small lymphocytic, consistent with chronic lymphocytic leukaemia (CLL; $n = 12$; 6%); small lymphocytic, plasmocytoid ($n = 2$; 1%); follicular, predominantly small cleaved cell ($n = 24$; 12%); follicular, mixed small and large cell ($n = 4$; 2%); follicular, predominantly large cell ($n = 5$; 2%); diffuse, small cleaved cell ($n = 14$; 7%); diffuse, mixed small and large cell ($n = 8$; 4%); diffuse, large cell ($n = 61$; 30%); large cell immunoblastic ($n = 24$; 12%); lymphoblastic ($n = 6$; 3%); small non-cleaved cell, non-Burkitt ($n = 6$; 3%); small non-cleaved cell, Burkitt's type ($n = 3$; 1%); other ($n = 35$; 17%). Clinical staging was done according to the Ann Arbour classification system. The examination of clinical status, routine laboratory tests including serum lactate dehydrogenase (LDH) concentration, chest x-ray, computerised tomography scans of the mediastinum and the abdomen, and a bone marrow biopsy were performed as staging examinations. Seventy-four (36%) of the patients had stage I, 51 (25%) stage II, 35 (17%) stage III, and 44 (22%) stage IV disease at diagnosis. Thirty-seven (18%) had B-symptoms (weight loss, unexplained fever, or night sweats). The International Prognostic Index (IPI)[38] was determined for each patient. A total of 132 patients were treated with standard combination chemotherapy. Briefly, the patients with intermediate or high grade lymphoma and disseminated disease were treated usually with bleo-CHOP (bleomycin, cyclophosphamide, doxorubicin, vincristine, prednisone), M-BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone) or another anthracycline containing combination chemotherapy regimen. Low grade lymphomas were usually treated with a single agent chlorambucil if symptomatic. Seventy patients received megavoltage radiotherapy. The patients were regularly followed-up for a minimum of five years with intervals of a few months in an outpatient department. During the follow-up time 109 patients died.

2.2. Venous blood samples

Peripheral venous blood samples were collected in sterile test tubes a few hours or a few days before starting lymphoma therapy and centrifuged at 2000*g* for 10 minutes. Serum was then collected and stored at −20 °C.

2.3. Serum immunoassays for angiogenin, VEGF, and bFGF

Angiogenin concentrations were determined as serum angiogenin immunoreactivity using a quantitative sandwich enzyme immunoassay technique (Quantikine® Human Angiogenin Immunoassay, R&D Systems, Minneapolis, MN). Serum VEGF concentrations were determined as serum VEGF immunoreactivity using a quantitative sandwich enzyme immunoassay technique (Quantikine Human VEGF Immunoassay; R&D Systems, Minneapolis, MN) as described earlier.[39,40] Serum bFGF concentrations were determined as serum bFGF immunoreactivity using a quantitative sandwich enzyme immunoassay technique (Quantikine High-Sensitivity Human FGF Basic Immunoassay; R&D Systems) as described earlier.[13] All analyses and calibrations were carried out in duplicate. The calibrations on each microtiter plate included recombinant human standards. Optical densities were determined using a microtiter plate reader. The blank was subtracted from the duplicate readings for each standard and sample. A standard curve was created by plotting the logarithm of the mean absorbance of each standard versus the logarithm of the cytokine concentration. Concentrations are reported as ng/ml.

2.4. Statistical methods

Statistical analyses were performed and the graphs were created using the software packages StatView 5.01 and JMP 7.01 (SAS Institute Inc., Cary, NC). The Mann–Whitney test, the Spearman rank correlation coefficient, and Pearson

product-moment correlations were used to calculate associations between serum angiogenin concentration and various other parameters. Cumulative survival was computed according to the product-limit method of Kaplan–Meier from the date of the diagnosis. The logrank (Mantel–Cox) test was used to compare survival of the different subgroups of patients. The relative influence of different variables on survival was studied in multivariate survival analyses using the parametric model of Weibull and the non-parametric proportional hazards model of Cox. Receiver operating characteristic (ROC) curves were created to test the accuracy to predict the 5-year survival of the 204 NHL patients. The prognostic factors introduced in the models (age, stage, serum LDH, the WHO performance status, and the number of extranodal tumour sites) are commonly accepted and constitute the components of the International Prognostic Index (IPI) for NHL.[38] All *P*-values are 2-tailed.

3. Results

3.1. Association between serum levels of angiogenin at diagnosis and clinicopathologic features in 204 NHL patients

Serum angiogenin concentrations at diagnosis amongst the 204 patients with NHL ranged from 201 ng/ml to 684 ng/ml (median, 401 ng/ml; mean, 404 ng/ml). Women had slightly lower serum angiogenin than men (median, 390 ng/ml versus 424 ng/ml, respectively; $P = 0.036$). Patients with lower than the median serum angiogenin had somewhat lower serum LDH concentration at diagnosis than the patients with higher than the median serum angiogenin (median LDH, 359 U/l versus 396 U/l, respectively; $P = 0.038$). A high serum angiogenin was associated with a high peripheral blood platelet count ($P = 0.0031$; Spearman rank correlation) but not with peripheral blood leukocyte count ($P = 0.07$). The patients with low grade lymphoma ($n = 47$) had lower angiogenin concentrations than those with intermediate or high grade lymphoma (median, 369 ng/ml versus 412 ng/ml, respectively; $P = 0.047$). When patients with different histologic types of lymphomas (according to the Working Formulation Scheme) were compared, patients with large cell diffuse or immunoblastic lymphoma ($n = 85$) had higher pretreatment serum angiogenin concentrations than did the rest of the patients (median, 435 ng/ml versus 380 ng/ml, respectively; $P = 0.0057$). No significant differences were found when the angiogenin levels of the other histologic types were compared to the rest of the patients, possibly because of low numbers of patients in these subgroups ($P > 0.1$ for all comparisons). No significant associations were found between angiogenin concentration and the age at diagnosis, WHO performance status, the Ann Arbour stage, the number of extranodal tumour sites, or the presence of B-symptoms ($P > 0.1$ for all comparisons).

3.2. Serum angiogenin in univariate survival analyses

The ability of pretreatment angiogenin concentrations and the components of the International Prognostic Index (IPI) to predict overall survival was first studied in univariate survival analyses (Table 1). Patients with a high serum angiogenin concentration at diagnosis had inferior overall survival in comparison to those with a lower serum angiogenin. The 5-year survival rate of the patients with higher than the median serum angiogenin (>401 ng/ml) was only 42% in comparison to the 63% 5-year survival rate found amongst patients with lower than the median serum angiogenin ($P = 0.0073$; Fig. 1A, Table 1). A high serum angiogenin concentration was associated with poor prognosis also when the highest tertile (450 ng/ml) was used as a cut-off value ($P = 0.0086$: Table 1).

Table 1 – Univariate survival analyses of 204 NHL patients.

Variable	5-year survival (%)	P
Serum angiogenin at diagnosis		
⩽the median (401 ng/ml)	63	0.0073
>the median	42	
⩽the highest tertile (450 ng/ml)	59	0.0086
>the highest tertile	40	
Age at diagnosis		
⩽60	64	<0.0001
>60	39	
WHO performance status		
0–1	59	<0.0001
2–4	21	
Ann Arbour stage		
I–II	62	<0.0001
III–IV	37	
Serum LDH at diagnosis		
Normal	63	<0.0001
Abnormal	27	
No. of extranodal sites		
0–1	59	0.0083
>1	28	
IPI		
⩽1	72	<0.0001
>1	30	

Abbreviations: IPI, International Prognostic Index; WHO, World Health Organization; LDH, lactate dehydrogenase.

The association between serum angiogenin concentration and survival was also studied separately in the subgroup of large cell diffuse and immunoblastic lymphomas. This was the largest histologic subgroup ($n = 85$) in the present series. In this group, the 5-year survival rate of the patients with a high (>median; 435 ng/ml) was 33% in comparison to the 56% 5-year survival rate of those patients with a lower serum angiogenin ($P = 0.0022$).

3.3. Particularly poor prognosis predicted by a combination of high serum angiogenin at diagnosis and a high IPI score

In univariate survival analysis the patients with two or more adverse features in the International Prognostic Index (IPI) had only a 30% 5-year survival rate in contrast to a 72% 5-year survival rate of the patients with 0–1 adverse features in the IPI ($P < 0.0001$; Table 1; Fig. 1B). We next striated the patients with 0–1 or 2–5 adverse features in the IPI by serum

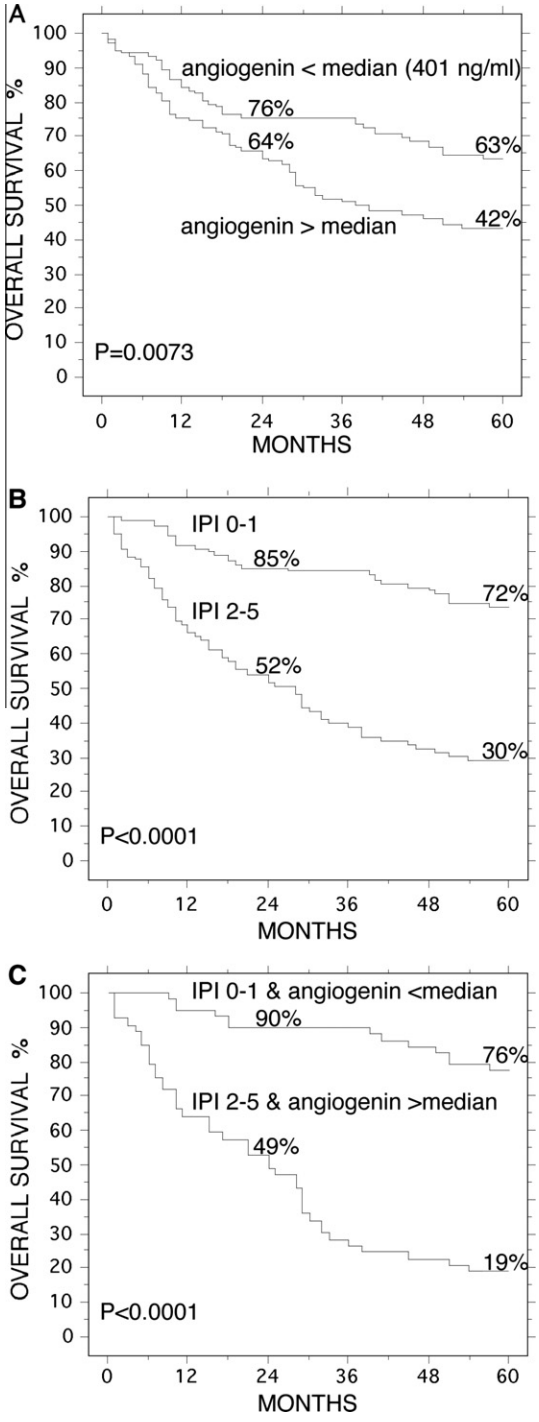

Fig. 1 – Overall survival of 204 patients with non-Hodgkin's lymphoma. (A) Overall survival by serum angiogenin concentration at diagnosis. The median was used as the cut-off value. Survival rates at 24 and 60 months are given. (B) Survival by the International Prognostic Index (IPI; 0–1 versus 2 or more adverse features). (C) Survival by combining the IPI (0–1 versus 2 or more adverse features) and serum angiogenin at diagnosis (median). The combination of a high IPI score and high serum angiogenin identified a subgroup of patients with particularly poor prognosis (5-year survival only 19%).

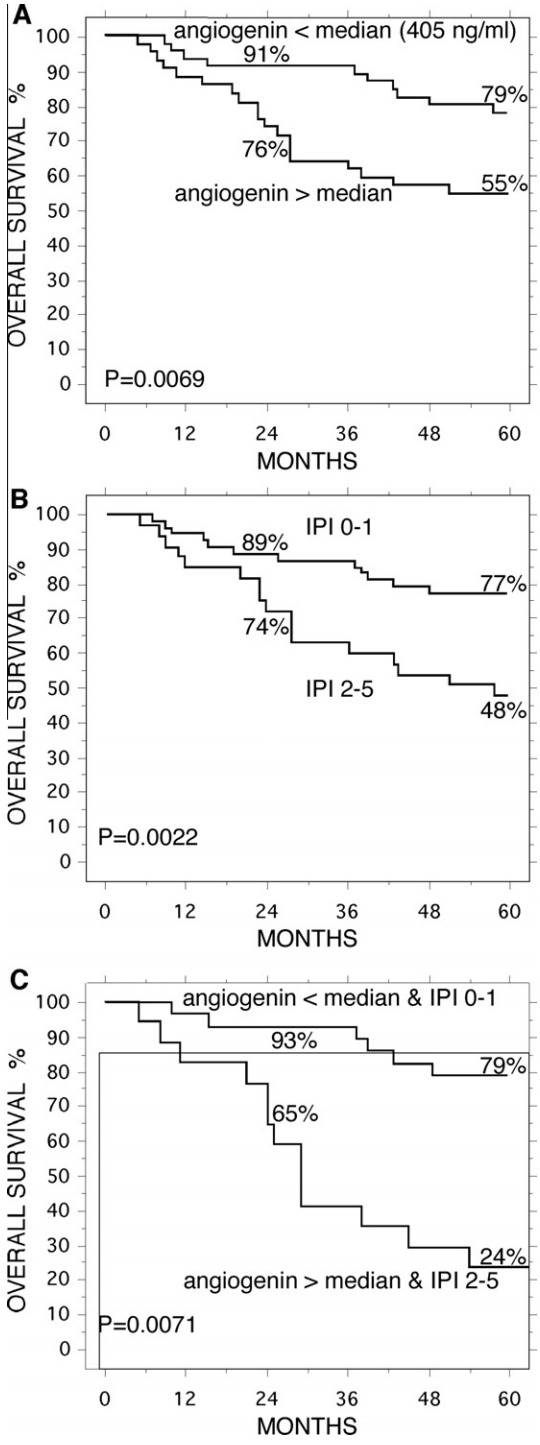

Fig. 2 – Overall survival in the subgroup of 84 patients with disseminated NHL who achieved complete response (CR) after chemotherapy. (A) Survival by serum angiogenin concentration at diagnosis (median). (B) Survival by the International Prognostic Index (IPI; 0–1 versus 2 or more adverse features). (C). Survival by combining the IPI (0–1 versus 2 or more adverse features) and serum angiogenin at diagnosis (median). Combining serum angiogenin and IPI enabled the identification of those patients with particularly poor prognosis (only 24% 5-year survival) although initially responding well to primary chemotherapy and achieving CR.

angiogenin. The 5-year survival rate of the patients with both a high IPI score (2–5) and high serum angiogenin (>median) was as low as 19% ($P < 0.0001$; Fig. 2B). In contrast, the 5-year survival rate of those patients with a low IPI score (0–1) and a low serum angiogenin (<median) was 76%. Thus, serum angiogenin concentration provided additional information to the IPI, and enabled us to identify patients with particularly poor prognosis.

3.4. *Serum angiogenin and the components of the IPI in multivariate survival analyses*

In the present series, the relative risk (RR, e^{β}) of death of the patients having an IPI score of 2 or greater was 5.78 (95% confidence interval (CI) 3.32–10.08; $P < 0.0001$). In order to find out if a high serum angiogenin level at diagnosis has an independent influence on survival, it was entered in multivariate analyses together with the components of the IPI. In multivariate analyses angiogenin concentration surfaced as an independent prognostic factor. Serum angiogenin had an independent influence on survival ($P = 0.0006$; Table 2) in the multivariate model when the angiogenin concentration and the other variables were entered to the model as continuous variables. The relative risk of death of the patients having a high (>median) serum angiogenin was using the Weibull model estimated to be 1.72 (95% CI 1.09–2.71; $P = 0.020$; Table 2) when compared to the patients with a low (<median) serum angiogenin. In multivariate analyses angiogenin had a stronger prognostic value than the number of extranodal tumour sites, which is a component in the IPI. When the same data were entered to the proportional hazards model of survival the results were comparable to those obtained using the Weibull model. In the proportional hazards model the estimated RR for high serum angiogenin (>median) was 1.54 (95% CI 1.03–2.32; $P = 0.037$).

Table 2 – Serum angiogenin concentration and the components of the International Prognostic Index (IPI) in multivariate analysis.

Variable	Relative risk (RR, e^{β})	95% CI for RR	P
Serum angiogenin at diagnosis			
>the median (401 ng/ml)	1.72	1.09–2.71	0.020
Continuous			0.0006
WHO performance status			
>1	3.45	2.01–5.92	<0.0001
Continuous			<0.0001
Age at diagnosis			
>60	2.34	1.49–3.69	0.0002
Continuous			0.0004
Serum LDH at diagnosis			
Abnormal	2.26	1.39–3.68	0.0010
Continuous			0.058
Ann Arbour stage			
III–IV	1.95	1.22–3.11	0.0052
Continuous			0.0006
No. of extranodal tumor sites			
>1	1.66	0.83–3.34	0.15
Continuous			0.89

Abbreviations: CI, confidence interval; WHO, World Health Organization; LDH, lactate dehydrogenase.

3.5. *Survival analyses for predictive indicators in the subgroup of patients with disseminated disease who achieved complete response (CR) after chemotherapy*

A total of 132 patients with intermediate or high grade lymphoma and disseminated disease were treated with standard combination chemotherapy. After chemotherapy, 84 of the treated patients (64%) achieved complete response (CR). However, after five years 31% of these patients had died. We therefore wanted to find predictive indicators for unfavourable outcome amongst these patients who initially responded well to primary chemotherapy and achieved CR. When the components of IPI were tested separately in univariate analyses, an advanced stage ($P < 0.0001$), a poor WHO performance status ($P = 0.0022$) and an elevated LDH ($P = 0.041$) predicted for an unfavourable outcome (Table 3). However, two other IPI components (age at diagnosis, the number of extranodal tumour sites) did not have prognostic power to predict the outcome for these patients ($P > 0.05$ for both; Table 3). With these

Table 3 – Univariate survival analyses for predictive indicators in the subgroup of NHL patients with disseminated disease who achieved complete response (CR) after chemotherapy ($n = 84$).

Variable	5-year survival (%)	P
Serum angiogenin at diagnosis		
<the median (405 ng/ml)	79	0.00669
>the median	55	
⩽the highest tertile (342 ng/ml)	82	0.016
>the highest tertile	59	
Ann Arbour stage		
I–II	77	<0.0001
III–IV	44	
WHO performance status		
0–1	71	0.0022
2–4	33	
Serum LDH at diagnosis		
Normal	71	0.041
Abnormal	50	
Age at diagnosis		
⩽60	70	0.15
>60	62	
No. of extranodal sites		
0–1	68	0.29
>1	50	
IPI		
⩽1	77	0.0022
>1	48	

Abbreviations: IPI, International Prognostic Index; WHO, World Health Organization; LDH, lactate dehydrogenase

84 patients, the 5-year survival rate of the patients with higher than the median serum angiogenin (>405 ng/ml) was 55% in comparison to the 79% 5-year survival rate found amongst patients with lower than the median serum angiogenin ($P = 0.0069$; Fig. 2A, Table 3). The IPI score provided comparable prognostic power, predicting a 48% 5-year survival rate for the patients with an IPI > 1 and 77% amongst patients with IPI 0–1 ($P = 0.0022$; Fig. 2B). Again, combining serum angiogenin and IPI enabled the identification of those patients with particularly poor prognosis (only 24% 5-year survival rate; $P = 0.0071$; Fig. 2C) although first achieving CR.

Finally, we incorporated serum angiogenin concentration at diagnosis and the IPI components into the Weibull model multivariate survival analysis. As a result, only a high serum angiogenin concentration at diagnosis (RR 2.38; 95% CI 1.26–4.50; $P = 0.0077$) and an advanced stage (RR 2.41; 95% CI 1.25–4.64; $P = 0.0087$) had independent influence on survival (Table 4). In conclusion, serum angiogenin level at diagnosis and tumour stage was the only independent predictors that could identify those patients with unfavourable outcome although first responding well to therapy.

3.6. Serum angiogenin and two other circulating angiogenic growth factors VEGF and bFGF and overall survival

We have earlier observed that high serum concentrations of the angiogenic growth factors VEGF and bFGF associate with poor overall survival in NHL.[12,13,15] In the present series serum angiogenenin concentration did not associate with serum VEGF concentration (S-VEGF; $P = 0.49$; Pearson product-moment correlations) or with serum bFGF concentration (S-bFGF; $P = 0.58$) at diagnosis. However, a high S-VEGF and a high S-bFGF at diagnosis were strongly associated ($P < 0.0001$). The pretreatment serum concentrations of all three angiogenic growth factors were associated with poor overall survival when studied in logrank (Mantel-Cox) test. The 5-year survival rate of the patients with higher than the median S-VEGF (>263 ng/ml) was 44% in comparison to the 61% 5-year survival rate found amongst patients with lower than the median S-VEGF ($P = 0.0097$) while for S-bFGF (median 3.3 pg/ml) the corresponding survival rates were 47% and 61% ($P = 0.0290$). Thus, in the present series the predictive power of a high serum angiogenin concentration (42% versus 63% 5-year survival rates using median; $P = 0.0073$; Fig. 1B, Table 1) was slightly stronger that that of S-VEGF or S-bFGF. The highest predictive power was achieved when the serum angienin, S-VEGF, and S-bFGF were used in univariate analyses as a single combined parameter ($P < 0.0001$). Those patients with pretreatment angienin, S-VEGF, and S-bFGF all over their respective median had a 5-year survival rate of only 39% while patients with all three angiogenic factors below their medians had a 5-year survival rate of 71% ($P = 0015$).

Table 4 – Multivariate survival analysis model for the predictive power of serum angiogenin concentration and the International Prognostic Index (IPI) component variables of 84 patients with disseminated NHL who achieved complete response (CR) after chemotherapy.

Variable	Relative risk (RR, e^{β})	95% CI for RR	P
Serum angiogenin at diagnosis			
>the median (405 ng/ml)	2.38	1.26–4.50	0.0077
Continuous			0.0070
Ann Arbour stage			
III–IV	2.41	1.25–4.64	0.0087
Continuous			0.0049
Age at diagnosis			
>60	1.61	0.90–2.89	0.11
Continuous			0.31
WHO performance status			
>1	2.00	0.77–5.18	0.16
Continuous			0.02
Serum LDH at diagnosis			
Abnormal	1.18	0.55–2.54	0.67
Continuous			0.45
No. of extranodal tumor sites			
>1	0.98	0.34–2.84	0.98
Continuous			0.48

Abbreviations: CI, confidence interval; WHO, World Health Organization; LDH, lactate dehydrogenase.

3.7. Receiver operating characteristic (ROC) analyses of the accuracy of prognostic factors in NHL

We created receiver operating characteristic (ROC) curves for the IPI components and for the pretreatment serum concentrations of angiogenin, VEGF, and bFGF describing the accuracy of these individual parameters in predicting the 5-year survival of the 204 NHL patients. The accuracy of these predictive parameters, measured by the area under the ROC curve, was fair for only WHO performance status (0–1 versus 2–4; area = 0.78) and pretreatment serum LDH (normal versus abnormal; area 0.75). For the IPI components age ($\leqslant$60 versus >60), stage (I–II versus III–IV), and the number of extranodal sites (0–1 versus >1) as well as for the pretreatment serum concentrations of angiogenin and VEGF (both tested $\leqslant$ the median versus >median) the accuracy of the test was poor (area = 0.60–0.70). For the pretreatment serum concentration of bFGF ($\leqslant$the median versus >median) the accuracy of the test was worthless (area = 0.59).

The 5 IPI components together were able to predict the 5-year survival rates of the 204 NHL patients with only a fair accuracy when the IPI scores of 0–1, $\geqslant$2 and $\geqslant$3 were used as the cutpoints (area = 0.74–0.79; Fig. 3A). The accuracy of the prediction was clearly improved when the pretreatment serum concentration of angiogenin ($\leqslant$the median versus >median) was combined to the IPI: the cutpoint of the IPI score $\geqslant$3 plus a high (>median) angiogenin provided a good predictive accuracy with the area under the ROC curve = 0.84 (Fig. 3B). Combining the IPI score with the elevation of any of the three angiogenic growth factor studied (all 3 factors under their respective medians versus one or several of the factors over median) provided a further improvement when using IPI score $\geqslant$2 as the cutpoint (area = 0.82), but did not increase the accuracy of the prediction when the other cutpoints were used (Fig. 3 C).

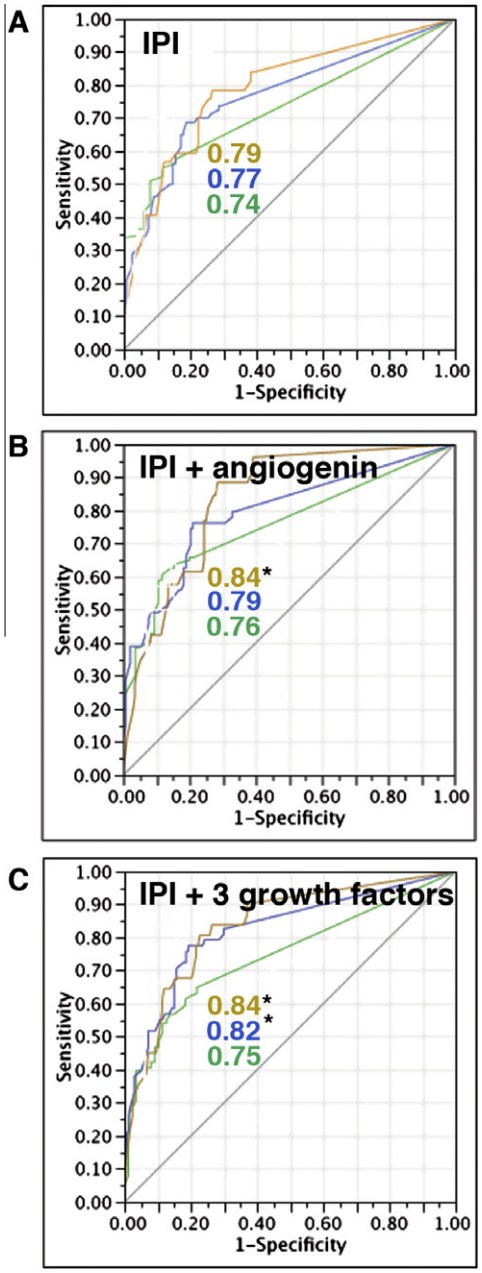

Fig. 3 – Receiver operating characteristic (ROC) analyses of the accuracy of prognostic factors in NHL. The accuracy of the predictive parameters is measured by the area under the ROC curve which is shown for each curve. The area measures discrimination, that is, the ability of the test to correctly classify patients with favourable or poor 5-year overall survival. A good accuracy (area under the curve > 0.80) is indicated with an asterix (*). (A) Survival by the International Prognostic Index (IPI; 0–1, ⩾2, or ⩾3 adverse features). (B) Survival by the combination of the IPI score (0–1, ⩾2, or ⩾3 adverse features) and serum angiogenin concentration at diagnosis (median). C. Survival by combining the IPI (0–1 versus 2 or more adverse features) and the elevation of any of the three angiogenic growth factors studied (all three factors under their respective medians versus one or several of the factors over median).

4. Discussion

Angiogenin has strong angiogenic potential.[41] However, its role in a variety of haematological malignancies is ambiguous. In some solid tumours increased angiogenin serum levels are associated with aggressive disease or poor prognosis.[30,34,35,42] Elevated serum angiogenin may predict disease progression in early chronic lymphocytic leukaemia[43] while in acute myeloid leukaemia and advanced myelodysplastic syndrome increased angiogenin levels are associated with longer survival.[44] Yet in other studies in patients with malignant lymphoproliferative diseases the levels of circulating angiogenin, have failed to provide any predictive power.[45–47] In 65 patients with NHL, pre-treatment serum levels of angiogenin were significantly lowered than in healthy controls and did not predict the outcome.[48] However, in the same study when the post-treatment angiogenin levels of 28 patients were analysed, a positive association was found between lower levels of post-treatment angiogenin and better survival.[48] In the present study, we aimed to better understand the possible clinical significance of serum angiogenin in NHL by prospectively evaluating the prognostic significance of angiogenin in a large series of 204 consecutive NHL patients diagnosed and treated in a single institution.

A high serum angiogenin concentration at diagnosis was associated with poor overall survival in the present series. Importantly, serum angiogenin concentration proved to be an independent prognostic factor in multivariate analyses. Indeed, angiogenin had in the multivariate model a stronger prognostic value than the number of extranodal tumour sites at diagnosis, one of the five variables presently included in the IPI. We have earlier observed that also high serum concentrations of the angiogenic growth factors VEGF and bFGF associate with poor overall survival in NHL.[12,13,15] Also in the present series the serum concentrations of VEGF and bFGF were associated with poor overall survival. The highest predictive power was achieved when the serum angionin, S-VEGF, and S-bFGF were used as a single combined parameter.

While serum angiogenin and also S-VEGF and S-bFGF had a statistically significant power in predicting outcome univariate analyses of survival, none of these factors alone could very efficiently classify patients with favourable or poor 5-year overall survival in ROC analyses of the test accuracy. However, this was also the case for the IPI components age, stage, and the number of extranodal sites, and even the combined IPI score provided only a fair accuracy in detecting patients with divergent outcomes. A good predictive accuracy in ROC analyses was achieved only when the IPI was complemented with serum angiogenin concentration, or serum angiogenin, VEGF, and bFGF together. Widely used clinical scoring systems such as the IPI aid in the identification of specific patient risk groups amongst the patients with NHL. Clinical models also aid comparison of the efficacy of different therapeutic approaches. In this context it is of particular interest that angiogenin concentration appeared to provide additional prognostic information to the IPI, and also enabled us to identify those patients with unfavourable outcome although first responding well to chemotherapy.

The significance of angiogenin in angiogenesis and in NHL progression remains controversial and circulating angiogenin levels have been reported to have predictive power in some studies while not in others. Therefore, the results of the present study, although designed to address the issue in a large series of consecutive patients from a single institution, should be interpreted with circumspection. Taken together, the results of the present series suggest that elevated serum angiogenin at diagnosis might be used as a predictor for failure on long-term treatment response and for poor survival in NHL, and it might also complement the IPI to identify the patients with particularly aggressive and/or treatment resistant disease.

Conflict of interest statement

None declared.

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