Serum concentrations of proangiogenic cytokines (VEGF, bFGF) depending on the histopathological types of Hodgkin lymphoma in children – preliminary report

Surowicze stężenia cytokin proangiogennych (VEGF i bFGF) w zależności od rodzajów histopatologicznych chłoniaka Hodgkina u dzieci – doniesienie wstępne

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SUMMARY

Background. The different histological types of classical Hodgkin Lymphoma (cHL) differ from other percentage share of the Reed-Sternberg cells (R-SC) in the affected lymphoid tissue In the Lymphocyte Depletion cHL (LDcHL) type are present almost only R-SC. In turn, in the Nodular Lymphocyte Predominant Hodgkin lymphoma (NLP-HL) in the structure lymphocytes, histiocytes ora "popcorn" cells are present. Angiogenesis, which is necessary for development neoplasma tissue, is stimulated by proangiogenic cytokines including Vascular-Endothelial Growth Factor (VEGF) and basic Fibroblast Growth Factor (bFGF). In HL these cytokines are produced mainly by the R-SC. The aim of the study was to assess the concentrations of VEGF and bFGF in the serum (sVEGF, sbFGF) in different histopathological types childhood HL.

Procedure. 37 children with HL were studied: group A – 34 children with CHL and group B – 3 children with NCHL. In the control group there were 20 children. Using enzyme-linked immunosorbent assays we quantified VEGF and bFGF in the serum of the children with HL.

Results. The median sVEGF in group A was 657.137 pg/ml (43.777–1210.52) and was significantly higher ($p<0.05$) in comparison with group B (446.182 pg/ml; 234.673–609.68) and group C (44.6 pg/ml; 32.2–734.8). In the LDcHL the sVEGF were the highest (865.220, 1032.665 pg/ml).

Conclusions. Conclusions. sVEGF in children with cHL are significantly higher in relation to the NLP-HL at the moment of diagnosis. There is a tendency to different sVEGF in the histopathological types of CHL.

Key words: Cytokines, Angiogenesis, Hodgkin lymphoma, Pediatric

STRESZCZENIE

Wstęp. Typy histopatologiczne w klasycznym chłoniaku Hodgkina (cHL) różnią się między innymi procentowym udziałem komórek Reed-Sternberga (R-SC) w utkaniu nowotworowym. W typie z deplecją limfocytów (LDcHL) w utkaniu nowotworu obecne są prawie wyłącznie R-SC. Z kolei w guzkowym typie chłoniaka Hodgkina z przewagą limfocytów (NLP-HL) w zajętej tkance węzłowej obecne są komórki limfocytowe i histiocytowe lub o typie „popcorn". Angiogeneza, niezbędna do rozwoju tkanki nowotworowej stymulowana jest przez cytokiny proangiogenne: Vascular-Endothelial Growth Factor (VEGF) i basic Fibroblast Growth Factor (bFGF). Cytokiny te w HL produkowane są głównie przez R-SC. Celem pracy była ocena stężeń VEGF i bFGF w surowicy (sVEGF, sbFGF) w różnych typach histopatologicznych chłoniaków Hodgkina u dzieci.

Materiał i metody. Badaniem objęto 37 dzieci z HL. Wyodrębniono dwie grupy: grupa A – 34 dzieci z cHL i grupa B – 3 dzieci z NLP-HL. Grupę kontrolną (grupa C) stanowiło 20 zdrowych dzieci. Za pomocą zestawów Human VEGF i Human FGF basic Quantikine Colorimetric Sandwich ELISA firmy R&D Systems oznaczono sVEGF i sbFGF u badanych dzieci i w grupie kontrolnej.

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Wyniki. Mediana sVEGF w grupie A wynosiła 657,137 pg/ml (43,777–1210,52) i była znamiennie wyższa ($p<0,05$) w porównaniu z grupą B (422,177 pg/ml; 234,673–609,68) i grupą C (44,6 pg/ml; 32,2–734,8). W typie LDcHL sVEGF były największe (865,220, 1032,665 pg/ml).

Wnioski. W chwili rozpoznania choroby stężenia VEGF w surowicy u dzieci z klasycznym chłoniakiem Hodgkina są istotnie wyższe względem typu guzkowego z dominacją limfocytów. Obserwuje się tendencję do różnic w surowiczych stężeniach VEGF w poszczególnych typach cHL u dzieci.

Słowa kluczowe: cytokiny, naczyniotworzenie, chłoniak Hodgkina, pediatria

Introduction

Hodgkin Lymphomas (HL) account for approximately 5–7% of all cancer diagnoses in the population of children. They are cancers with one of the highest rate of complete remissions after the completion of treatment. According to the Polish data, the 5-year probability of overall survival (OS) is 99%, relapse-free survival (RFS) 93% and event-free survival (EFS) 90% [1, 2]. The HL histopathological classification in accordance with WHO is based on a morphological and immunohistochemical assessment of the cells of the affected lymphoid tissue [3–6]. The WHO classification distinguishes the Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLP-HL), and the classical HL (cHL). In the NLP-HL type in the histopathological structure are single lymphocytic-histiocytic cells or "popcorn" cells surrounded by normal lymphocytes. This type is claimed to have the best prognosis [7–9]. The histopathological types in cHL, cooper others differ percentage of Reed-Sternberg cells In affected tissue. The histopathological picture of Lymphocyte Depletion Classical Hodgkin Lymphoma (LDcHL) type shows almost only Reed-Sternberg cells (RSc) and heavy fibrosis with almost a complete lack of lymphocytes and reactive cells [4, 5].

Angiogenesis is crucial for the development of tumour tissue. The process of angiogenesis in the body is stimulated by cytokines among which the following play the key role: Vascular Endothelial Growth Factor (VEGF) and basic Fibroblast Growth Factor (bFGF). In oncological diseases these cytokines are produced mainly by tumour cells, to a lesser extent by the reactive cells accompanying the carcinogenesis, and also by the stroma [10, 11]. It has been proved that both in adult and children HL these factors are mainly produced by RS cells which additionally show increased expression of receptors for VEGF [10–18]. So, it may seem that the greater the number of tumour cells in affected lymphoid tissue favors the higher possibility of the production proangiogenic cytokines resulting in a better vascularisation of the tumour and thus in a higher tendency to growth [10, 12, 19]. According to the authors of few studies concerning childhood oncology, high serum concentrations of proangiogenic markers assessed at diagnosis are autonomic and unfavourable prognostic factors in Hodgkin and non-Hodgkin Lymphomas in children [15, 20, 21]. Yet there have been no reports on the behaviour of proangiogenic markers in different histopathological types of Hodgkin Lymphomas in children which motivated us to analyse this issue.

Therefore, the aim of the study is the analysis of serum concentrations of VEGF and bFGF in children with Hodgkin Lymphoma (HL) with respect to the histopathological type of the disease as well as its prognostic impact.

Materials and Methods

The study comprised 37 children (21 boys – 58%; 15 girls – 42%), aged 4.5–17 (mean 12.8), with Hodgkin Lymphoma diagnosed and treated in our institution between 2000–2008 years. Among the children included in the study, the two groups analyzed were divided: group A consisting of 34 children (19 boys – 56%, 15 girls – 44%), aged 4.5–17 (mean 12.7), with CHL diagnosed, and group B consisting of 3 children (3 boys), aged 12–14 (mean 13.3) with NLP-HL diagnosed. In the group A were 3 children (8.8%) with Lymphocyte Rich cHL (LRcHL), 22 children (64.7%) with Nodular Sclerosis cHL (NScHL), 7 children (20.6%) with Mixed Cellularity cHL (MCcHL) and 2 children (5.9%) with LDcHL. The control group (Group C) was 20 healthy children (13 boys – 65%, 7 girls – 35%; aged 2–17; mean 11 years). These children presented no abnormalities in physical examination and laboratory tests.

All children were staged according Ann-Arbor classification system. The number and location of initial disease sites were obtained in each case during a full physical examination and chest radiography (x-ray), thorax and abdominal computed tomography (CT), and cervix, abdominal and minor pelvis ultrasonog-

raphy (USG). All patients had bone marrow biopsies. Histopathological subtypes were defined according to WHO criteria. The HL were routinely diagnosed by two independent pathologists based on histology and immunohistochemistry. The material from children who were diagnosed with HL before the validity of the WHO classification in Poland was re-evaluated by histopathologist.

From all children (examinated and from control group) we were taken 2 cm^3 blood for the determination of blood concentrations of proangiogenic cytokines in the serum. From their parents or guardians and children > 16 years of age both HL and from the control group were obtained written consent for blood collection. The consent of the Bioethics Committee of the Medical University of Silesia was obtained in order to conduct this study. Laboratory tests included serum Vascular endothelial growth factor (sVEGF) and basic Fibroblast growth factor (sbFGF) concentrations. In order to evaluate the VEGF and bFGF concentration, an additional amount of 2 cm^3 was taken during routine tests. After coagulation, the blood was centrifuged for 20 minutes at 2000 rpm. The serum obtained was frozen at -80°C. The analyses were performed using the set Human VEGF Quantikine Colorimetric Sandwich ELISA, R&D Systems for VEGF and FGF basic Quantikine Colorimetric Sandwich ELISA, R&D Systems for bFGF. The results of proangiogenic markers concentrations were compared with the control group.

The analysis additionally included the marking at the moment of diagnosis of clinical indicators of known prognostic importance such as: age, clinical stage of disease, the presence of bulky disease, spleen and extra-nodal location of the disease, the presence of B symptoms. All patients received uniform treatment according to Protocol Hodgkin Disease 95 based on 4–8 cycles of MVPP (mechlorethamine, vinblastin, procarbazin, prednisone) and B-DOPA (bleomycin, vincristin, adriamycin, dacarbazin, prednisone) with or without radiation therapy. These changes in subsequent, control radiology images gradually disappear. The partial remission (PR) was defined as the residual tumor volume was less or equal 50% of the initial diameter and reduction of tumor mass in the mediastinum at least 25%. The follow-up ranged from 34 to 132 months after initial diagnosis (median 74 months).

Statistics

Since the distribution of variables was considerably different from the normal distribution (Kołmogorow-Smirnow test), they were described using the median (range); the verification of the x hypothesis was performed by means of non-parametric tests. The differences between the two groups in relation to individual numerical variables were verified using the Mann-Whitney U test. Differences and relations with $p < 0.05$ were regarded as statistically significant.

Table I. The clinical characteristics of groups A and B
Tabela I. Charakterystyka kliniczna grupy A i B

	Classical Hodgkin lymphoma					Nodular Lymphocyte Predominant Hodgkin Lymphoma
	Group A (n=34; 91.9%)	LRCHL (n=3; 8.8%)	NSCHL (n=22; 64.7%)	MCCHL (n=7; 20.6%)	LDCHL (n=2; 5.9%)	Group B (n=3; 8.1%)
age < 10 r.ż. > 10 r.ż.	 6 (17.6%) 28 (82.4%)	 2 (70%) 1 (30%)	 3 (13.7%) 19 (86.3%)	 1 (14.3%) 6 (85.7%)	 – 2 (100%)	 – 3 (100%)
clinical stage of disease I II III	 – 23 (64.7%) 11 (35.3%)	 – 2 (70%) 1 (30%)	 – 15 (68.2%) 7 (31.8%)	 – 5 (71.4%) 2 (28.6%)	 – 1 (50%) 1 (50%)	 1 (30.3%) 2 (60.6%) –
bulky disease	13 (38.2%)	–	11 (50%)	–	2 (100%)	–
A symptoms B symptoms	23 (70.6%) 11 (29.4%)	3 (100%) –	14 (63,6%) 8 (36,4%)	6 (85.7%) 1 (14.3%)	– 2 (100%)	3 (100%) –
response to treatment (evaluated 32 children) CR PR	 28 (88.2%) 4 (11.8%)	 3 (100%) –	 18 (90%) 2 (10%)	 6 (85.7%) 1 (14.3%)	 1 (50%) 1 (50%)	 3 (100%) –
alive death (before ending therapy)	32 (94%) 2 (6%)	3 (100%) –	20 (90.9%) 2 (9.1%)	7 (100%) –	2 (100%) –	3 (100%) –

Results

Clinical characteristics of the examined groups (Table I)

Most children from group A were classified as stage II of clinical advancement (n=22; 64%). Extranodal location of the disease was not observed in any of the patients. The majority of children (n=28; 82%) were in the less favourable age group (> 10 years old). 24 children (70.6%) from group A did not show the B symptoms of the disease. The NScHL type was significantly more common in group A ($p<0.05$). Type LDcHL was represented by two patients with adverse clinical indicators.

Complete remission (CR) was observed in 94% of the group. Two children (6%) died as a result of a generalized infection (Varicella Zoster Virus) in the course of drug-induced neutropenia.

Even though children included in Group B were at a less favourable age, they were classified at low clinical stages and did not present B symptoms at the time of diagnosis. These children achieved complete remission of the disease.

Analysis of serum concentrations of proangiogenic cytokines

The median serum VEGF concentration in group A was 657.137 pg/ml (43.777–1210.52) and was significantly higher ($p<0.05$) in comparison with group B (446.182 pg/ml; 234.673–609.68) and group C (44.6 pg/ml; 32.2–734.8) (Table II). No statistically significant differences between the medians of VEGF serum concentrations in respective histopathological types of CHL were concluded. In the LDcHL type sVEGF were the highest (865.220, 1032.665 pg/ml).

Medians of bFGF concentrations between groups A, B and C were not statistically considerable (Table II). Amid the histopathological types of CHL in LRCHL the lowest serum concentration of bFGF of statistical significance was concluded at the moment of diagnosis (Table II).

Discussion

The generally known main prognostic factors in childhood HL such as the stage of clinical advancement and the presence or lack of B symptoms are beyond doubt but new indicators of the activity of the disease are still being sought [6, 20].

In recent years more attention has been given to the role of cytokines in the development of neoplastic diseases. The serum concentrations of these proteins may reflect both the mass of tumour tissue and the level of malignancy of the tumour pattern which in turn may have a diagnostic and prognostic significance. The unfavourable prognostic significance of high serum concentrations of Interleukin-10, Tumor Necrosis Factor-alfa and Transforming Growth Factor-beta assessed in the process of diagnosing neoplastic diseases, including HL has been proven [4, 6]. Recent reports suggest proangiogenic cytokines (VEGF and bFGF) play a role in the development of HL. These factors, according to the majority of authors are produced by RSc and to a lesser extent by reactive cells accompanying the carcinogenesis [11, 12, 14–18, 22–25]. Observations made by the authors unanimously conclude that in patients with oncologi-

Table II. The serum VEGF and bFGF concentrations in the groups A, B and C
Tabela II. Surowicze stężenia VEGF i bFGF w grupach A,B i C

	Classical Hodgkin Lymphoma						Nodular Lymphocyte Predominant Hodgkin Lymphoma		
	Group A (n=34; 91.9%)	LRCHL (n=3)	NSCHL (n=22)	MCCHL (n=7)	LDCHL (n=2)	p	Group B (n=3; 8.1%)	Group C (n=20)	p
VEGF (pg/ml)	657.137 (43.777 –1210.52)	550.184 (243.45– 733.661)	673.576 (43.777– 1210.52)	445.395 (169.782 –805.209)	865.220, 1032.665	NS	446.182 (234.673 –609.68)	44.6 (32.3– 734.8)	Group A *vs* Group C <0.05 Group B *vs* Group C <0.05 Group A *vs* Group B <0.05
bFGF (pg/ml)	9.5 (3.022 –29.650)	3.867 (3.704– 10.638)	9.530 (3.022 –23.997)	10.424 (4.13– 29.65)	12.336, 24.023	LRCHL *vs* NSCHL<0.05 LRCHL *vs* MCHL<0.05 LRCHL *vs* LDCHL<0.05	8.78 (6.576– 8.76)	10.7 (2.7–48.7)	NS

cal diseases, both adults and children, there are high concentrations of the above-mentioned cytokines at diagnosis and their significant reduction after remission has been achieved. Exceptionally high values at the moment of diagnosis and their slight reduction after the therapy are related with a higher tendency for the recurrence of the disease or its progression [11, 14, 22, 23, 26, 27]. There are single works proving that high serum concentrations of proangiogenic markers assessed at diagnosis are unfavourable prognostic factors in childhood Hodgkin Lymphomas [14, 20, 21, 23].

Recently greater attention has been given to the impact of respective histopathological types on the prognosis. Nodular Lymphocyte Predominant Hodgkin Lymphoma in whose pattern single lymphocytic-histiocytic cells or "popcorn" cells surrounded by normal lymphocytes are observed, was distinguished [4, 5]. Low histopathological malignancy of the NLP-HL according to clinical observations usually results in a low level of clinical advancement and often in a lack of B symptoms. The above-mentioned is reflected in the good results of the therapy as noted by Pellegrino at al. [7], Murphy at al. [8] and Sandowal et al. [9]. The authors emphasize the validity of minimizing the treatment in children with NLP-HL, which has already been observed in new therapeutic protocols for children [28].

Low production of proangiogenic cytokines, especially of VEGF by the tumour tissue poor in tumour cells may be also a factor contributing to a good prognosis. According to our observations, children with NLP-HL had a statistically lower initial concentration of VEGF in the serum. They were classified to low clinical stages of disease, did not present B symptoms and achieved complete remission. The serum concentration of VEGF may constitute both a diagnostic and prognostic marker in new therapeutic protocols. However more research is needed with respect to the above-mentioned issue.

In classical HL four histopathological types have been distinguished. According to the WHO classification the division is based above the others on the assessment of reactive cells such as lymphocytes, eosinophiles, histiocytes, fibroblasts and neutrophils and the stage of the accompanying fibrosis. In the LRcHL type the histopathological picture is dominated by lymphocytes and reactive cells, with minimal participation of R-SC and accompanying fibrosis. In turn, in the LDcHL type, are almost only R-SC and significant fibrosis with nearly complete lack of lymphocytes and reactive cells [4, 5].

In our material we have observed the highest sVEGF in LDcHL which confirms that the cytokines are produced by the tumour cells. Despite the differentiation of clinical stages of disease (II and III), the sVEGF in respective children were high. However more research is needed with respect to the above-mentioned issue. Following chemotherapy children with LDcHL achieved the state of partial remission (25% reduction of mediastinal mass). After a 4-year observation they have a stable tendency to reduce the residual lesions. The children require constant observation which is stressed by Ben Arush et al. [14], who describe the correlation between high initial serum concentrations of VEGF and the relapse of the disease in children with HL.

Both the diagnostic and prognostic values of bFGF in HL seem to be of less significance. In the presented material the invariably lower median of bFGF concentrations in the NLP-HL group in relation to the cHL group and only significantly the lowest median in the LRcHL type may confirm the multidirectional action of this cytokine. According to Khnytkin et al. [29] who examined the expression of the bFGF cytokine and its receptors, only the FGFR3 expression is observed in R-SC. According to the authors oxygen deficiency stimulating angiogenesis in the tumour cell induces the expression of VEGF and not bFGF. The bFGF receptors seem to express only in R-SC of selected phenotypes [28].

Since the presented study is based on a small and piloting group of children, the issues raised warrant further study. However, we would like to stress the tendency to a correlation between the concentrations of proangiogenic cytokines in the serum and histopathological types of Hodgkin Lymphomas in children. The above report confirms the conclusion made by the authors that the research concerning angiogenesis in childhood HL is valid and may be in the future used in antiangiogenic treatment in resistant types of HL [14, 15, 18, 19].

Conclusions

VEGF concentrations in the serum in children with classical Hodgkin Lymphoma are significantly higher in relation to the Nodular Lymphocyte Predominant Hodgkin Lymphoma at the moment of diagnosis which may be an additional prognostic factor in childhood HL.

There is a tendency to differences in serum VEGF concentrations in respective types of classical HL in children.

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