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Changes of blood count, lymphocyte subpopulations and immunoglobulin levels in nephroblastoma long term survivors

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The aim of this study was to investigate the frequency of blood count, lymphocyte subpopulations, and immunoglobulin levels alterations in a group of healthy nephroblastoma long-term survivors. The group included 122 nephroblastoma long-term survivors who were at least five years post anticancer therapy and free of any sign of recurrence The proportion of lymphocyte subpopulations was analyzed by flow cytometry using antibodies anti CD45 FITC/CD14 PE, anti CD3 FITC/ CD16+CD56 PE, anti CD4 FITC/ CD8 PE and anti CD20 FITC. Immunoglobulin G, A, and M levels were evaluated by immunoturbidimetry. Total blood count was also examined. The occurrence of decreased immunoglobulin levels, leukocytes, lymphocytes, and granulocytes count, proportion of T lymphocytes and their CD4+ subpopulation are not frequent. The most frequently decreased lymphocyte subpopulation was CD8 (15.5%). The most frequent abnormal findings were increased proportion of NK cells (38.5 %), B lymphocytes (38,52 %), decreased number of erythrocytes (25.2 %), hemoglobin levels (41.7 %) and hematocrit (13.9 %). The only significant differences between results of immunological examination and course of the disease were more frequently decreased proportion of CD4+ lymphocytes in recurrent disease survivors and lower IgA levels in survivors after radiotherapy. We found decreased at least one immunological parameter in one fifth of the survivors. The most frequently altered parameter was hemoglobin, which was decreased in 41.7 % of survivors. Decraesed hemoglobin may worsen quality of survivors´ life.

Key words: nephroblastoma long-term survivors, blood count, lymphocyte subpopulations, immunoglobulin G, A, M serum levels

The success of therapies used in the treatment of childhood cancer has brought a group of maturing individuals, pursuing the normal experiences of life. Out of 1,000 twenty-year-olds, there is one who is a survivor of childhood cancer [1]. Nearly one half of young adult survivors of childhood cancer have at least one major adverse outcome of their health status resulting from cancer therapy [2]. Chemotherapy and radiotherapy are the cause of immunodeficiency and pancytopenia because almost all cytostatic drugs and irradiation of lymphatic tissue and bone marrow have immunosuppressive and myelosuppresive effect. The long-term immunological and hematological offspring of anticancer therapy have not been equally studied until now.

Nephroblastoma caused approximately seven percent of all childhood malignant tumors and its incidence rate is about eight cases per million in Caucasian children who are less than fifteen years of age [3]. Developments in surgery, recognition of its sensitivity to irradiation, and the availability of several active chemotherapeutic agents have led to a change in the prognosis for nephroblastoma patients with this once incurable malignant tumor [4]. Survival rate of nephroblastoma patients in our department between the years of 1996–2000 was 90 % [5].

The aim of this study was to investigate the frequency of blood count, lymphocyte subpopulations, and immunoglobulin levels alterations in a group of long-term survivors of nephroblastoma.

Patients and methods

One hundred twenty two long- term survivors of nephroblastoma treated at the University Hospital Motol, be-

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tween April 1980 and April 2001 were examined. They were treated according to therapeutic protocols SIOP-6 (years 1980–1986), SIOP-9 (years 1987–1993) and SIOP93-01GPHO (1994– 2001). None of them underwent megatherapy with subsequent hematopoietic progenitor cells transplantation. Examination for this study was done at least five years after finishing of anticancer therapy and all survivors were free of any sign of tumor recurrence. Survivors' characteristics are shown in Table 1.

At the time of blood collection for immunological examination survivors were examined by pediatric oncologist (J.R) and were found to be without any sign of recurrence or any acute disease. None of the survivors had recurrent infections, new allergic disease or allergic symptoms in history. Proportions of lymphocyte subpopulations were analyzed in all 122 survivors by flow cytometry (FACSCalibur, BD, San Jose, CA) using antibodies produced by Immunotech (Beckmann Coulter, Nyon, Switzerland). Antibodies used were anti CD45 FITC/CD14 PE (for correct lymphocyte gating), anti CD3 FITC/ CD16+CD56 PE (all T and NK cells), anti CD4 FITC/ CD8 PE (T helper and T cytototoxic/supressor cells) and anti CD20 FITC (all B cells). Immunoglobulin G, A, and M serum levels were examined in 99 survivors by immunoturbidimetric assay by analyzer ADVIA 1650 (Bayer, Tarrytown, NY) with reagents produced by Dako (Dako, Glostrup, Denmark). Total blood count was examined in 115 survivors.

Results of an immunological examination and of the blood count were compared with our reference values (see Table 2.) and correlated with anamnestic data concerning nephroblastoma and anticancer therapy. After classifying the results in a frequency, a 2 x 2 table chi square test with Danderar´s correction was performed to study the relationship between different categories. All numerical data were presented as mean ± standard deviations and analyzed statistically using Student's t-test**.** Correlations between the immunological parameters were evaluated by nonparametric Spearman's coefficient. For the statistical examination, SPSS version 10.1 software was used.

Table 1. Survivors characteristics. (y- years; m- month)

		N	%
Whole group		122	100.0
Sex	M	54	44.3
	F	68	55.7
Age at the time of examination		18y 7 m (12 – 34 y)	/
Clinical stage	1	87	71.3
	2	6	4.9
	3	8	6.6
	4	15	12.3
	5	6	4.9
Histological typ	cystical	2	1.6
	necrosis	2	1.6
	standard	113	92.6
	blastem	2	1.6
	anaplastic	3	2.5
Therapeutic protocol	SIOP-6	22	18.0
	SIOP-9	50	41.0
	SIOP93-GPO 01	50	41.0
Radiotherapy		34	27.9
Age at the time of diagnosis		3y 11 m (1 m- 17 y)	/
Interval after finishing therapy		14y 8 m (6 y.-26 y 3 m)	/

Results

The percentage of blood count parameters, immunoglobulin levels, and lymphocyte subpopulations which are out of reference values and average values are shown in Table 3. Decreased immunoglobulin levels were not frequent, and decreases were rare in the number of leukocytes, granulocytes and lymphocytes and proportion of T lymphocytes and their CD4+ subpopulation. The most frequently decreased lymphocyte subpopulation was CD8+, which was under reference values in 19 (15.5 %) survivors. Twenty five (20.5 %) survivors had at least one immunological parameter (immunoglobulin levels and/or

Table 2. Reference values of immunological examination and blood count. Age 12 – 55 years.(M- male; F- female)

Parameters	Reference values
CD3	55- 80 %
CD3- CD16+CD56+	2- 10 %
CD4	25- 56 %
CD8	20- 45 %
CD20	5- 15 %
CD4/CD8	0.9- 3.1
IgG	6,7- 15 g/l
IgA	0.9- 3.7 g/l
IgM	0.5- 2.0 g/l
Leucocytes	4- 10x 10^9/ l
Lymphocytes	1- 3 x 10^9/ l
Granulocytes	2.3- 6.8x 10^9/ l
Erythrocytes	M 4.5- 6.3x 10^{12}/ l F 4.2- 5.4 x 10^{12}/ l
Hemoglobin	M 14- 18 g/dl F 12- 16 g/dl
Hematocrit	M 0.38- 0.52 F 0.37- 0.46

Table 3. Results of immunological and hematological examination.

	Average	Standard deviation	% decreased	% increased
Leukocytes x10^9/ l	6.5	1.6	3.3	2.5
Lymphocytes x10^9/ l	2.1	0.6	1.7	1.7
Granulocytes x10^9/ l	3.7	1.3	10.4	2.5
Erythrocytes x10^{12}/ l	4.8	0.5	25.2	0
Hemoglobin g/dl	14.4	1.3	41.7	0
Hematocrit %	0.42	0.04	13.9	0
CD3 %	67.1	8.4	9.8	2.5
CD3- CD16+CD56+ %	11.2	6.7	0	38.5
CD4 %	39.3	7.4	3.3	1.6
CD8 %	25.6	5.6	15.6	0
CD20 %	13.8	4.5	0.8	38.5
CD4/CD8 %	1.6	0.5	4.9	2.5
IgG g/l	11.5	2.2	1.0	3.0
IgA g/l	1.8	0.7	4.0	3.0
IgM g/l	1.1	0.5	4.9	4.9

percentage of any lymphocyte subpopulation) that was decreased. The most frequent abnormal finding was an increased proportion of NK cells (38.5 %) and B lymphocytes (38.5 %).

Decreased values were seen frequently in numbers of erythrocytes (25.2 %), hemoglobin levels (41.7 %) and hematocrit (13.9 %). Granulocytes were decreased in 12 survivors (10.4 %) and there were decreased lymphocytes in two (1.7 %). Those parameters were decreased only slightly. Four cases (3.5 %) of decreased hemoglobin were hypochromic, with the rest of cases (38.3 %) found to be normochromic.

The only significant differences between the results of immunological examination and the disease course were more frequently decreased proportion of CD4+ lymphocytes in recurrent disease survivors ($p < 0.05$, chi square test with Danderar´s correction as 2 x 2 table) and lower IgA levels in survivors who were treated by radiotherapy ($p<0,05$, t-test). There were no significant differences in the results of immunological and hematological examinations between survivors treated according to different protocols and lenght of interval after finishing of anticancer therapy . There were no significant differences between the course of the disease and any parameter of the blood count.

The correlation of immunological parameters and total blood count is shown in Table 4. There were significant positive correlations between the percentages of CD3+ and CD4+ cells, IgG and IgA, and IgA and IgM levels. We found significant negative correlations between percentages of NK cells and CD3+ and CD4+ cells, and between percentages of CD20+ and CD3+ and CD8+ cells. The percentage of CD3+ and CD4+ negatively correlate, while percentages of NK cells positively correlate with erythrocyte count, hemoglobin levels and hematocrit.

Discussion

Our results showed that rarely is there a decrease of lymphocyte subpopulations or immunoglobulin G, A or M levels in healthy, long-term nephroblastoma survivors. The most frequent abnormalities were increased proportions of NK cells (CD3- CD16+ CD56+) and B lymphocytes (CD20+), which were found in more than one third of these long-term nephroblastoma survivors. One fifth of the survivors in our group had at least one immunological parameter decreased. This is less than in group of Hodgkin's disease survivors in which we found 38 % to have decreased CD4/CD8 ratio and/or percentage of CD3+ lymphocytes [6] while in this group of nephroblastoma survivors it was only 14.8 %. We suppose that in Hodgkin's disease, the defects in the immune system are inherent [7] but in nephroblastoma survivors it is mainly caused by the anticancer therapy. Limited information is available about the changes of immunity in untreated patients suffering from nephroblastoma since this was described in only one study from 1986, which found a weakly decreased percentage of T lymphocytes with high affinity receptors for sheep red blood cells and lower response of lymphocyte to phytohemaglutinin in the patients with nephroblastoma at the time of diagnosis [8]. Decreased proportion of CD4+ lymphocytes in recurrent nephroblastoma survivors is more likely caused by the more intensive and longer therapy used to cure the recurrence.

Decrease of erythrocytes, hematocrit and hemoglobin were only slightly (>100 g/l in female, >110 g/l in male) subnormal and asymptomatic. The majority of cases of anemia were normochromic with hemoglobin levels higher than 100 g/l in female and 110 g/l in male. We suppose that this decrease is more probably caused by the anticancer therapy. Bone marrow examination was not performed because of ethical reason. Serum erythropoietin levels, which were examined only in six survivors from technical reason, were normal in all examined survivors. One of them had decreased hemoglobin level (male, hemoglobin levels 129 g/l). There were described that erythropoietin and hemoglobin levels were normal by the fourth month after unilateral nephrectomy in kidney donors

Table 4. Correlation of immunological parameters and blood count. + postive correlation p< 0,05; ++ postive correlation p< 0,01; – negative correlation p< 0,05; – negative correlation p< 0,01.

CD3-CD16+CD56	CD4	CD8	CD20	CD4/CD8	IgG	IgA	IgM	Leu	Lympho	Neutroph.	Hb	Ery	Hct	
--	++	+	-								-	-	-	CD3
	--			-							+	+	+	CD3-CD16+CD56
		-		++	-						-	-	-	CD4
			-	--										CD8
												+		CD20
														CD4/CD8
						+								IgG
							+							IgA
														IgM
														Leu
														Lympho
														Neutroph.
														Hb
														Ery

[9]. We found decreased levels of hemoglobin in 78.8 % of 260 Hodgkin's disease survivors (unpublished results). Therefore we suppose that lower hemoglobin levels are not consequence of nephrectomy. Also, a decreased granulocyte count was only borderline, with the lowest count being 1,4 x 10^9/ l. There is only limited knowledge about erythrocytes counts and hemoglobin levels in long-term cancer survivors, even though myelotoxicity is one of the most frequent side effect of chemotherapy, approximately 80 % of children treated for malignant tumors have severe anemia at the time of chemotherapy [10]. As we know just Polish authors described late hematological complication only in one survivor from the group of thirty five adult people, who received aggressive chemotherapy, and were observed 3, 5 and 10 years after finishing of therapy [11].

Immunological abnormalities in nephroblastoma survivors were symptom-free. On the other hand, one may speculate that defects of cellular immunity in childhood cancer survivors may contribute to the occurrence of secondary malignancies. There was described that nephroblastoma survivors treated according to protocol SIOP 1, 2, 5, and 6 suffering four times more likely from secondary cancer [12]. This is significantly less than in the group of all childhood cancer survivors, where there is a risk of secondary cancer six times higher than in a normal population [13]. There were no cases of secondary malignancy in our group of long-term nephroblastoma survivors. This difference may be caused by using less cancerogenic chemotherapy in nephroblastoma.

Negative correlations between percentage of NK cells and CD4+, and between CD20+ and CD8+ cells indicate that increase of the percentage of NK cells is to the prejudice of CD4+, while an increase of CD20+ goes along with a decrease in the percentage of CD8+ cells. Negative correlations of erythrocyte count and hemoglobin with CD3+ and CD4+ cells percentage is difficult to explain.

In conclusion, we found at least one immunological parameter decreased (the percentage of lymphocyte subpopulations and/or immunoglobulin G, A and M levels) in one fifth of long-term nephroblastoma survivors. More frequently, what was found was a decreased proportion of CD4+ lymphocytes in survivors with recurrent disease and lower IgA levels in survivors who were treated by radiotherapy. The most frequently altered parameter was hemoglobin level which was decreased in 41.7 % of survivors. Decraesed hemoglobin levels may worsen quality of survivors´ life because symptoms of mild anemia (tiredness, decreased labour efficiency) may be noteless by patients.

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References

[1] Schwartz CL. Long-term survivors of childhood cancer: The late effects of therapy. Oncologist 1999; 4: 45–54.

[2] Oeffinger KC, Hudson MM. Long-term complications following childhood and adolescent cancer: Foundations for providing risk-based health care for survivors. CA Cancer J Clin 2004; 54: 208–236. doi:10.3322/canjclin.54.4.208 PMid:15253918

[3] Green DM, D'Angio GJ, Beckwith JB et al. Wilms tumor. CA Cancer J Clin 1996; 46: 46–63. doi:10.3322/canjclin.46.1.46 PMid:8548525

[4] Metzger ML, Dome JS. Current therapy for Wilms' tumor. Oncologist 2005; 10: 815–826. doi:10.1634/theoncologist.10-10-815 PMid:16314292

[5] Smelhaus V, Koutecky J. Patient summary of the paediatric oncology in teaching hospital in Motol and the development of the results of their therapy in the years 1976–2000. /in czech/ Klinicka onkologie 2003, Suppl: 85–92

[6] Eckschlager T, Prusa R, Hladikova M et al. Lymphocyte subpopulations and immunoglobulin levels in Hodgkin's disease survivors. Neoplasma 2004; 51: 261–264.

[7] Weinshel EL, Peterson BA. Hodgkin's disease. CA Cancer J Clin 1993; 43: 327–346. doi:10.3322/canjclin.43.6.327 PMid:8242436

[8] Kowalczyk D, Szymik S, Grochowski J et al. Immunological reactivity in children with Wilms' tumor. Neoplasma 1986; 33: 509–518.

[9] Romero RR, Alberu J, Correa-Rotter R et al. Serum erythropoietin levels in kidney donors after renal transplantation. Transplantation 2000; 70: 386–387. doi:10.1097/00007890-200007270-00027 PMid:10933169

[10] Michon J. Incidence of anemia in pediatric cancer patients in Europe: Results of a large, international survey. Med Pediatr Oncol 2002; 39: 448–450. doi:10.1002/mpo.10183 PMid:12203661

[11] Pawlicki M, Zuchowska-Vogelgesang B, Ziobro M et al. Late hematologic status after chemotherapy observed in patients 3 to 10 years after treatment. Przegl Lek 1995; 52: 344–346. (in Polish)

[12] Carli M, Frascella E, Tournade MF et al. Second malignant neoplasms in patients treated on SIOP Wilms tumour studies and trials 1, 2, 5, and 6. Med Pediatr Oncol 1997; 29: 239–244. doi:10.1002/(SICI)1096-911X(199710)29:4<239::AID-MPO1>3.0.CO;2-N PMid:9251727

[13] Hawkins MM, Draper GJ, Kingston JE. Incidence of second primary tumours among childhood cancer survivors. Br J Cancer 1987; 56: 339–347.