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ABNORMALITIES OF CENTROMERES AND CARCINOGENESIS OF NON-HODGKIN'S LYMPHOMA IN CHILDREN

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Abstract. This article presents the results of long-term studies (1991–2023) of the relationship between abnormal behavior of the centromere, in particular, the phenomena of premature centromere division of metaphase chromosomes (PCD) and premature anaphase of metaphase chromosomes (C-anaphase), and the pathogenesis of non-Hodgkin lymphomas (NHL) in children. 17 children with B-form non-Hodgkin's lymphoma were studied before the start of treatment in the first acute period. The studies were aimed at the manifestation and levels of these phenomena during the first acute period of B-form non-Hodgkin's lymphoma in peripheral blood and bone marrow cell culture in children. The study found compelling evidence, supported by statistical analysis, indicating that C-anaphase occurs with an extremely high frequency in children diagnosed with non-Hodgkin's lymphoma, both in the bone marrow and in the peripheral blood. C-anaphase levels in children with non-Hodgkin's lymphoma were 51.27 ± 1.99% in peripheral blood and 67.47 ± 2.19% in red bone marrow. At the same time, this phenomenon did not occur at all in the control group - normal healthy donors and healthy children neither in the peripheral blood nor in the red bone marrow (0%). These data allow us to propose the use of C-anaphase as an additional diagnostic criterion for the detection of non-Hodgkin's lymphoma, especially in the early stages of the disease. Further study of the mechanisms underlying centromere abnormalities may reveal new mechanisms of the appearance, development and pathogenesis of non-Hodgkin's lymphoma, paving the way for targeted therapeutic interventions and personalized treatment strategies, and elucidate the mechanisms of new forms of programmed cell death in various pathologies. Our studies allow us to draw a conclusion about the relationship between centromere abnormalities and the pathogenesis of non-Hodgkin's lymphoma, about the importance of determining the level of C-anaphase as a diagnostic indicator, and its potential role in the study of both pathogenesis and clinical practice related to this hematological malignancy.

Key words: non-Hodgkin's lymphoma, C-anaphase, premature centromere division, metaphase chromosomes, treatment strategies, pediatric cases.

1. INTRODUCTION

Non-Hodgkin's lymphoma (NHL) are group of oncological diseases of the blood – forming system, the cancer cells of which are developed from lymphocytes. In lymphomas, a clone of oncotransformed lymphocytes spreads through the lymphatic system forming a tumor mass. At the same time, this malignant clone can enter the red bone marrow leading to the leukemisation of lymphoma. Among lymphomas, Hodgkin's disease is distinguished with specific Reed-Sternberg giant cells and non-Hodgkin's lymphoma. In the four main forms of non-Hodgkin's lymphoma, 60 main types of lymphomas are identified [18]. Each type includes indolent, aggressive and highly aggressive forms. Although medicine has made significant progress in the treatment of non-Hodgkin's lymphoma over the past decades, non-Hodgkin's lymphoma still remain a difficult and complex medical problem. The question of early diagnosis and the problem of carcinogenesis of non-Hodgkin's lymphoma is crucial. Carcinogenesis of non-Hodgkin's lymphoma may involve chromosomal mutations, including translocations such as t(8;14), t(2;8), t(8;22), etc. [2, 10, 13, 17, 18].

Our work is devoted to finding new diagnostic criteria for non-Hodgkin's lymphoma and investigating the connection between centromere abnormalities – the phenomena of premature centromere division of metaphase chromosomes (PCD) and premature anaphase (C-anaphase) and the pathogenesis of non-Hodgkin's lymphoma. The molecular mechanisms and biological significance of these phenomena have not yet been investigated, although these phenomena were described as early as the 1960s, for some time these phenomena were considered to be the effect of cell culture, but then it was discovered and demonstrated in many works that these phenomena are associated with oncological diseases, including acute myeloblastic and lymphoblastic leukemias [11] and a number of other pathologies, in particular, anemias of various genesis, Alzheimer's disease [9, 16], etc. Today, it is believed that these phenomena are not accidental and are related to the pathogenesis of various pathologies [11, 12, 14].

2. MATERIALS AND METHODS

A study of 17 children with non-Hodgkin's lymphoma, aged from 2 to 17 years, was conducted. All patients suffered from the B-form of non-Hodgkin's lymphoma, with one patient experiencing leukemization. Among the patients, 14 were male and 3 were female. There were most patients in the age group from 11 to 14 years – 10 in number. The research focused on patients from the Lviv Regional Children's Specialized Hospital. It was in 1991–2023.

Fig. 1. The phenomenon of C-anaphase in peripheral blood cells of a patient with non-Hodgkin's lymphoma

Chromosomal preparations of peripheral blood and bone marrow cell cultures were examined. Cell cultures were establised using standard media, including Eagle's medium and RPMI-1640 from "Life Technologies" and "Sigma", fetal bovine serum from "Biomark Ink," and L-glutamine from "Sigma". Peripheral blood cells were stimulated with phytohemagglutinin (PHA) from Difco. When red bone marrow cells were cultivated, no mitogen was added. Cells were cultured for 72 hours at a temperature of 37ºC, treated with a hypotonic solution of KCl, and fixed with a mixture of ethanol and acetic acid (3:1).

Chromosome preparations were routinely stained with Giemsa stain. They were analyzed microscopically using a Leitz microscope. Blood and bone marrow donors who underwent examination were used as a control group. The phenomenon of PCD is defined as a phenomenon in which from 1 to 10 chromosomes per cell show premature centromere division. And C-anaphase refers to a phenomenon in which all chromosomes of a cell show premature separation (Fig. 1). [5, 7, 1, 3, 4, 7].

3. RESULTS AND DISCUSSION

There were observed high levels of the premature centromere division (PCD) phenomenon in patients with B-form of non-Hodgkin's lymphoma. In peripheral blood cells, the average was 6.13 ± 1.25%, in bone marrow cells it was 6.87 ± 0.74%. This statistically significantly different from the levels of PCD in the control group: for peripheral blood on average 1.7 ± 0.3%, for bone marrow 3.0 ± 0.3%. An abnormally high level of C-anaphase was found in patients with non-Hodgkin's lymphomas: 51.37 ± 1.99%, and in the culture of red bone marrow cells it was 66.47 ± 2.19%, while in the control group the phenomenon of C-anaphase was entirely absent (0%) both in peripheral blood cells and bone marrow cells (Tables 1, 2, Fig. 2).

Tab. 1. Average frequency of PCD and C-anaphase in non-Hodgkin's lymphomas and in the control group. NHL - non-Hodgkin's lymphoma; PB – peripheral blood; BM - bone marrow; PCD - premature centromere division

№	Diagnosis	PCD (%)		C-anaphase (%)	
		PB	KM	PB	BM
1.	Control group	1,7 ± 0,3	3,0 ± 0,3	0	0
2.	nHL	6,13 ± 1,25	6,87 ± 0,74	51,27 ± 1,99	66,47 ± 2,19

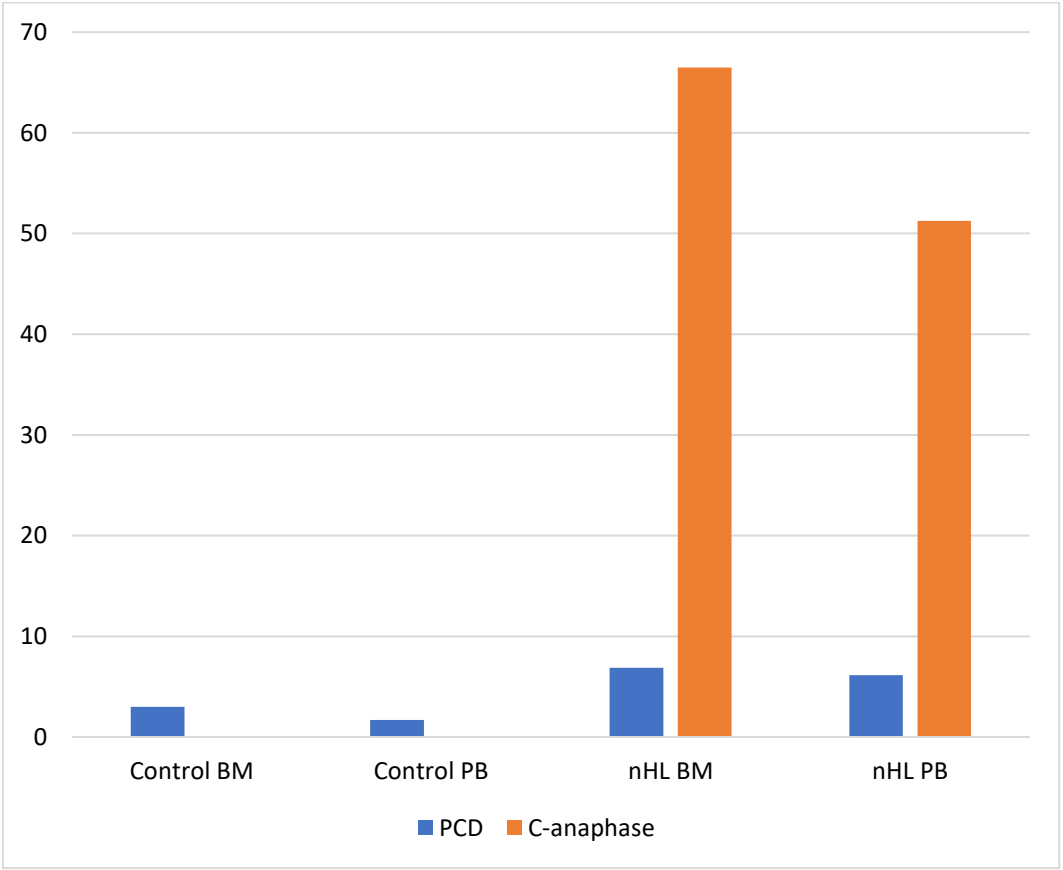

Fig. 2. Levels of PCD and C-anaphase in patients with NHL and in the control group

Clinical indicators of patients with non-Hodgkin's lymphoma are shown in tables. 2, 3. Correlation analysis showed no correlation between the phenomena of PCD and C-anaphase, between these phenomena and clinical parameters (levels of leukocytes, hemoglobin, platelets in the blood) in patients with non-Hodgkin's lymphomas. The results of the correlation analysis are presented in Figures. 3–7. The correlation between various clinical parameters of patients with NHL (including the levels of leukocytes, hemoglobin level, etc.) and the phenomena of PCD and C-anaphase was investigated. No correlation was found between these indicators. Only a correlation was found between the level of PCD in peripheral blood and the level of leukocytes in peripheral blood ($r = 0.850$) (Fig. 8, 9).However this correlation, both linear and non-linear, is due to the process of leukemia in one of the examined patients. Obviously, the phenomenon of PCD is seen to a greater extent in malignant cells that enter PB and BM during leukemization. The studied correlation between the level of PCD in the bone marrow (BM) and this indicator in peripheral blood: $r = –0.193$ – there is no correlation. The explored correlation between the level of C-anaphase in PB and BM: $r = 0.306$ – there is no correlation. Also, no correlation was found between the levels of PCD and C-anaphase in peripheral blood: $r = 0.324$.

Tab. 2. The frequency of PCD and C-anaphase phenomenon in the peripheral blood and bone marrow of children with non-Hodgkin's lymphoma (NHL)

№	Patient	Sex	A form of nHL	Blast in PB (%)	PCD (%)		C-anaphase (%)	
					PB	BM	PB	BM
1	BL	♂	B	0	7	9	51	68
2	TE	♂	B	0	12	8	42	63
3	PA	♂	B	0	3	5	35	72
4	MA	♂	B	81	21	2	65	84
5	TO	♂	B	0	5	8	52	61
6	CL	♂	B	0	5	7	49	55
7	DD	♀	B	0	9	11	54	68
8	KT	♂	B	0	3	7	59	72
9	RV	♂	B	0	4	5	41	54
10	LO	♂	B	0	2	7	59	51
11	TV	♀	B	0	4	12	48	69
12	LV	♂	B	0	5	7	55	72
13	KN	♀	B	0	4	3	57	68
14	MK	♂	B	0	5	9	50	69
15	PR	♂	B	0	3	3	52	71
16	NV	♂	B	0	7	8	57	72
17	MR	♂	B	0	5	4	45	60
Average value				5,4	6,13	6,87	51,27	66,47
Std. Err.					± 1,25	± 0,74	± 1,99	± 2,19

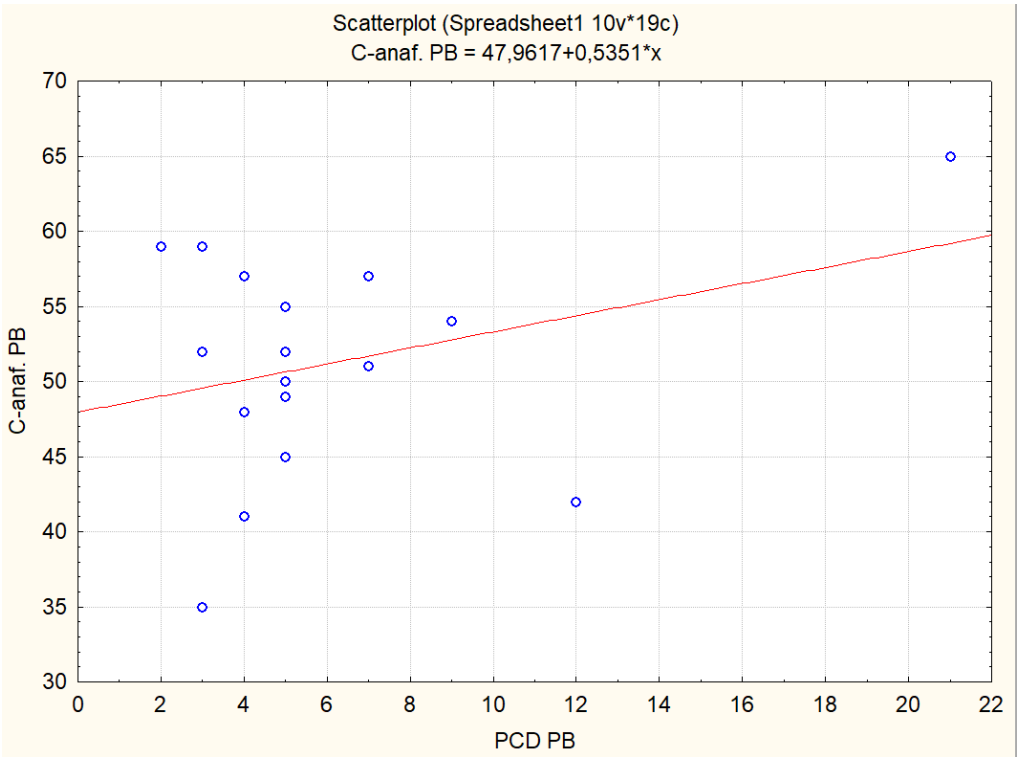

Fig. 3. Correlation analysis of the relationship between PCD levels in the peripheral blood and C-anaphase in the peripheral blood of children with non-Hodgkin's lymphoma. No correlation (r = 0.324)

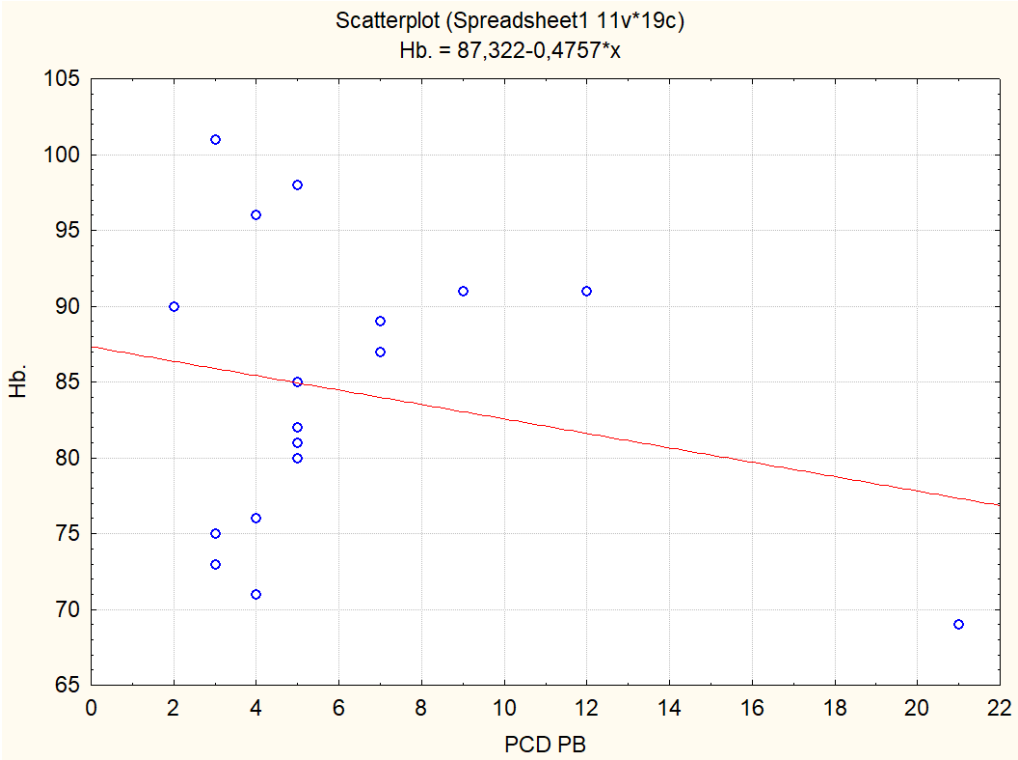

Fig. 4. Correlation analysis between PCD levels in peripheral blood and hemoglobin levels in the blood of patients with non-Hodgkin's lymphoma. No correlation (r = – 0.225)

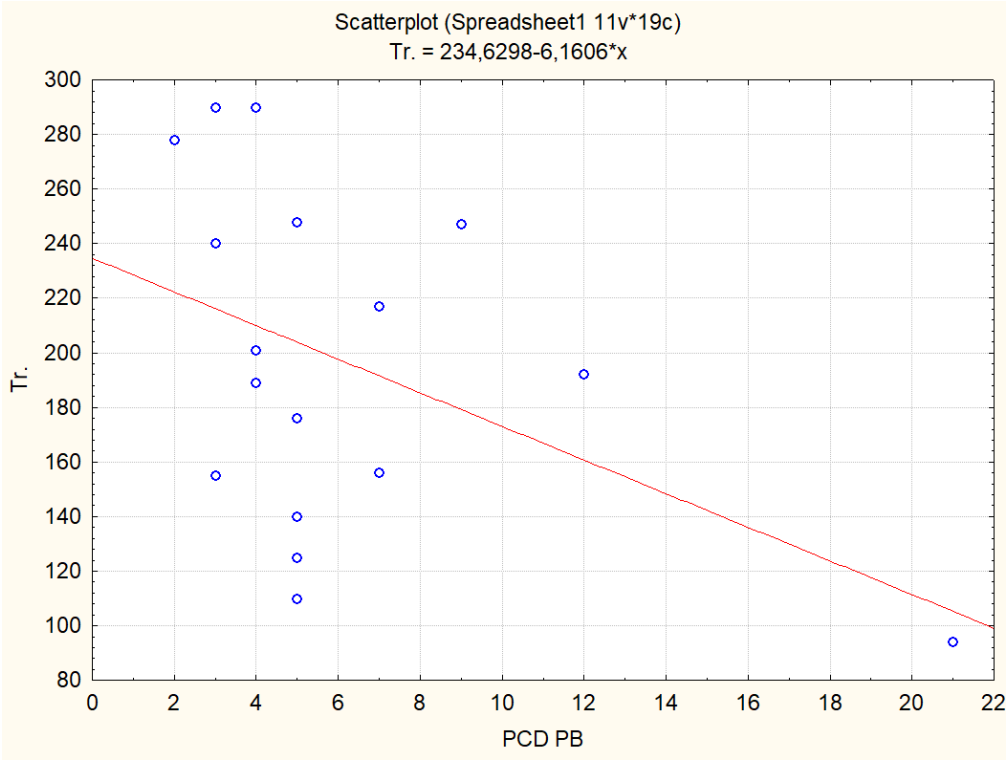

Fig. 5. Correlation analysis between PCD levels in peripheral blood and platelet levels in peripheral blood of patients with non-Hodgkin's lymphoma. No correlation (r= –0.451)

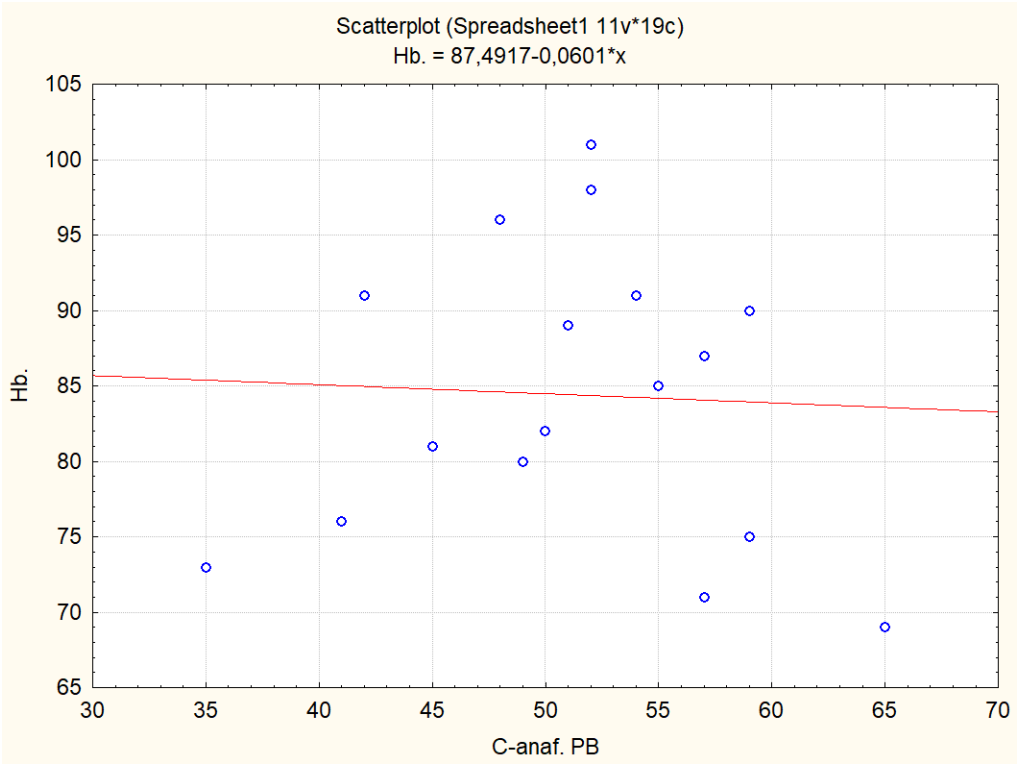

Fig. 6. Correlation analysis between C-anaphase levels in peripheral blood and hemoglobin levels in the peripheral blood of patients with non-Hodgkin's lymphoma. No correlation (r = – 0.047)

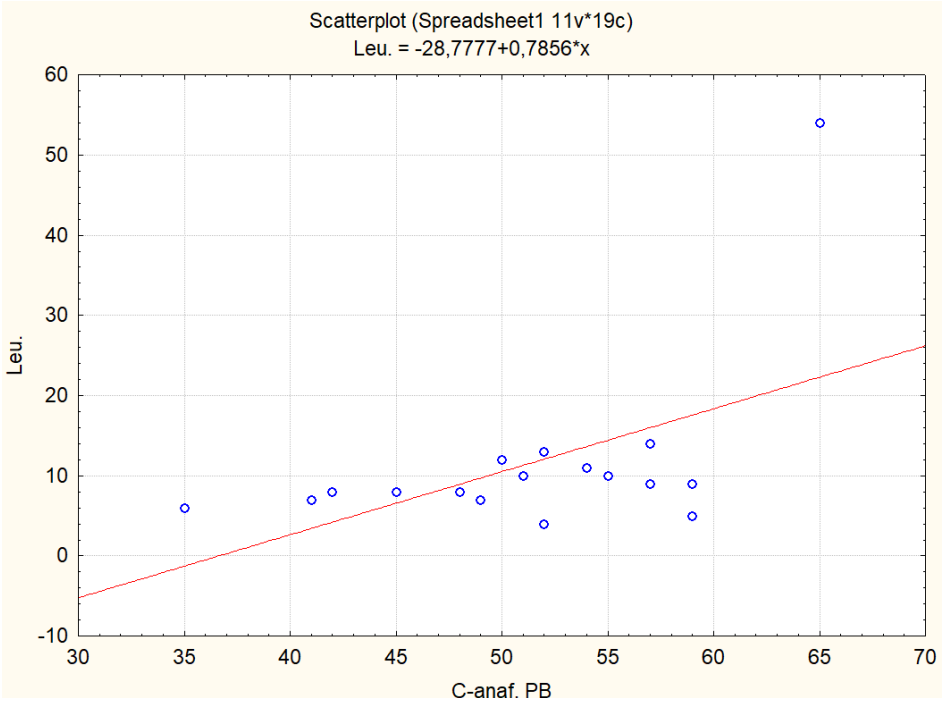

Fig. 7. Correlation analysis between the levels of C-anaphase in the peripheral blood and the levels of leukocytes in the peripheral blood of patients with non-Hodgkin's lymphoma.No correlation (r = 0.524)

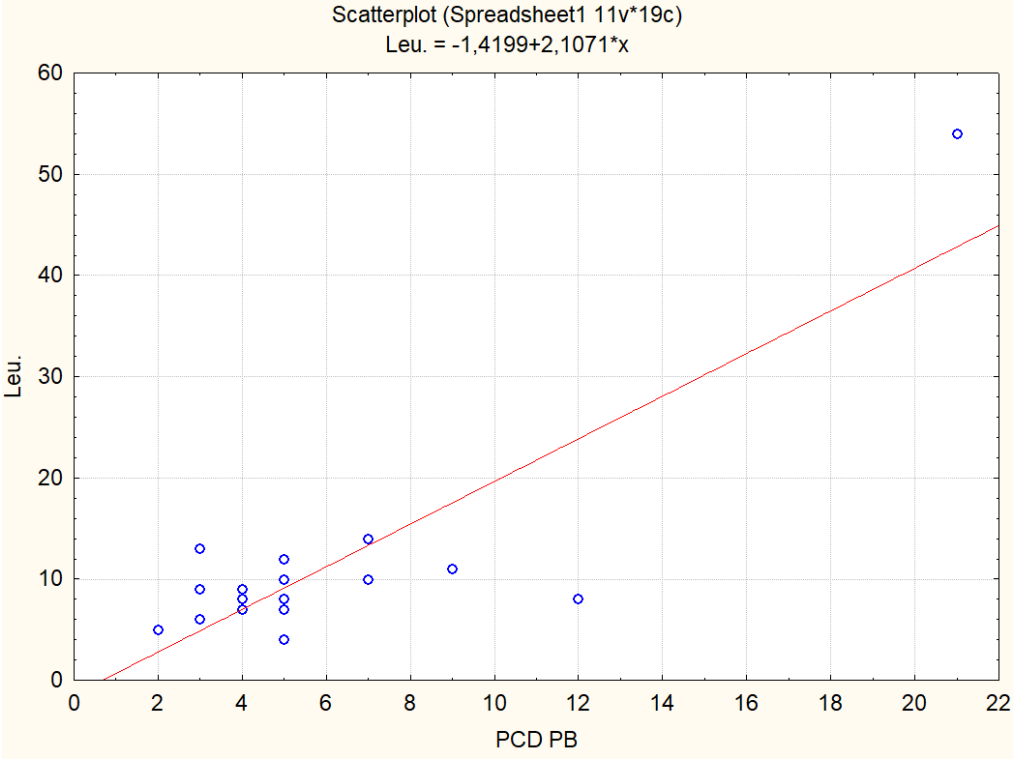

Fig. 8. Correlation analysis between PCD levels in peripheral blood and leukocyte levels in peripheral blood of patients with non-Hodgkin's lymphoma. Correlation is present due to 1 patient with leukemization (r = 0.850)

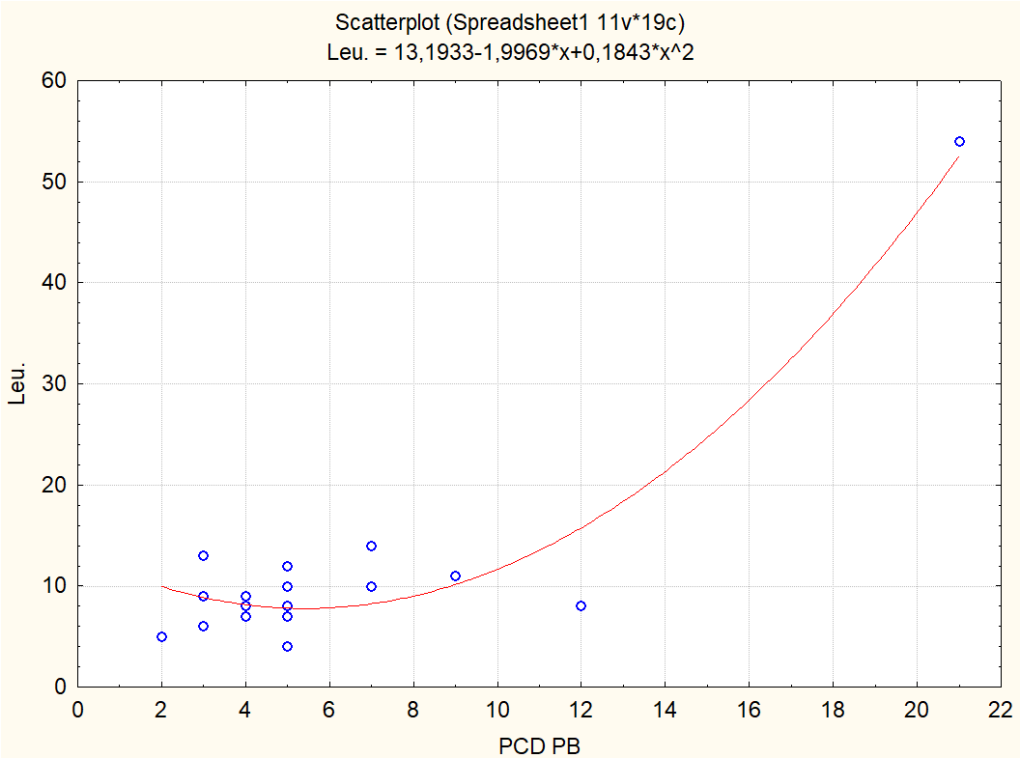

Fig. 9. Nonlinear (polynomial) correlation analysis between PCD levels in peripheral blood and leukocyte levels in peripheral blood of patients with non-Hodgkin's lymphoma

Tab. 3. Clinical indicators of the studied patients with non-Hodgkin's lymphoma (NHL). Hb. – hemoglobin; Leu. - leukocytes; Tr. - thrombocytes

№	Patient	Sex	A form of nHL	Hb. (g/л)	Leu. (thousand/мм³)	Tr. (thousand/мм³)
1	BL	♂	B	89	10	156
2	TE	♂	B	91	8	192
3	PA	♂	B	73	6	240
4	MA	♂	B	69	54	94
5	TO	♂	B	98	4	140
6	CL	♂	B	80	7	125
7	DD	♀	B	91	11	247
8	KT	♂	B	75	9	155
9	RV	♂	B	76	7	189
10	LO	♂	B	90	5	278
11	TV	♀	B	96	8	290
12	LV	♂	B	85	10	248
13	KN	♀	B	71	9	201
14	MK	♂	B	82	12	110
15	PR	♂	B	101	13	290
16	NV	♂	B	87	14	217
17	MR	♂	B	81	8	176
Average value				84,47	11,53	197,0
Std. Err.				± 2,64	± 3,10	± 17,04

The obtained results suggest that the phenomena of PCD and C-anaphase are distinct phenomena and play different roles in the pathogenesis of oncological diseases in general and lymphoma in particular. Based on the obtained results, we do not exlude the possibility that the PCD phenomenon and the C-anaphase phenomenon can be generated by the body itself in response to the appearance of oncotransformed cell clones. The correlation between leukemization and PCD levels in peripheral blood suggests that the phenomenon of PCD is more specific to oncotransformed cells than to normal blood cells.

4. CONCLUSIONS

1. The phenomena of C-anaphase and PCD could be used as an additional nonspecific criterion for non-Hodgkin's lymphoma in children.

2. The phenomena of C-anaphase and PCD have a different nature and play a fundamentally different role in the pathogenesis and course of non-Hodgkin's lymphoma.

3. The proposed new diagnostic marker of non-Hodgkin's lymphoma - C-anaphase is not highly specific.

4. The phenomenon of PCD is more characteristic of oncotransformed cells than of normal blood cells.

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REFERENCES

[1] Arieta, M. I., Martinez, B., Nunez, M. (1995). Premature centromere division: a cytogenetic study. *Cytologia (Tokyo).* 60 (2). 159–165.

[2] Bajalica, S., Sorensen, A., Pedersen, T. at all. (1993). Chromosome painting as a supplement to cytogenetic banding analysis in non-Hodgkin's lymphoma. *Genes Chromosomes & Cancer*, 7, 231–239.

[3] Bakhoum, S. F., Silkworth, W. T., Nardi, I. K., Nicholson, J. M., Compton, D. A., Cimini, D. (2014) The mitotic origin of chromosomal instability. *Current Biology*, 24, 148–149.

[4] Bloom, K., Constanzo, V. (2017). Centromere structure and function. *Progress in Molecular and Subcellular Biology*, 56. 515–539.

[5] Chamla, Y. (1988) C-anaphases in lymphocyte cultures versus premature centromere division syndromes. *Human Genetic*, 78 (2). 111–114.

[6] Chenevix-Trench, G., Brown J. A., Tyler, G. B. (1988). Chromosome analysis of 30 cases of non-Hodgkin's lymphoma. *Medical Oncology and Tumor Pharmacotherapy*, 5 (1), 17–32.

[7] Fitzgerald, P. (1992). Premature centromere division. *Human genetic*, 90 (1–2), 190–191.

[8] Keser, I., Gunduz, G. (1996). Premature centromere division in three unrelated families. *Annales de Genetique*, 39(2), 87–90.

[9] Korman-Bortolotto, M., de Arruda Cardoso, S. (1993). Alzheimer's disease and ageing: a chromosomal approach. *Gerontology*, 39(1). 1–6.

[10] Levine, E. G., Bloomfield, C. D. (1990). Cytogenetics of non-Hodgkin's lymphoma. *The Journal of the National Cancer Institute Monographs*, 10, 7–12.

[11] Littlefield, L., Joiner, E., Sayer, A. et al. (1985). Premature separation of centromeres in marrow chromosomes from an untreated patients with acute myelogenous leukemia. *Cancer Genetic and Cytogenetic*, 16 (2), 109–116.

[12] Luo, J., Craver, A., Bahl, K., et al. (2022) Etiology of non-Hodgkin lymphoma: A review from epidemiologic studies. *Journal of the National Cancer Center,* 2 (4), 226–234.

[13] Madan, K., Lindhout, D., Palan, A. et al. (1987). Premature centromere division (PCD): a dominantly inherited cytogenetic anomaly. *Human Genetic*, 77 (2), 193–196.

[14] Mathew, P., Sanger, W. G., Weisenburger, D. D. (1997). Detection of the t(2;5)(p23;q35) and NPM-ALK fusion in Non-Hodgkin's lymphoma by two color fluorescence in situ hybridization. *Blood*, 89, 1678–1685.
[15] Mehes, K., Buhler, E. (1995). Premature centromere division: a possible manifestation of chromosome instability. *American Journal of Medical Genetic*, 56(1), 76–79.
[16] Mehes, K., Kosztolanyi, G. (1990). Premature centromere division of a translocation-carrier autosome. *Human Genetic*, 85 (3), 379–380.
[17] Mehrotra, S., Mittra, I. (2020). Origin of genome instability and determinants of mutational landscape in cancer cells. *Genes*, 11(9), 1101.
[18] Moorhead, P. S., Heyman, A. (1993). Chromosome studies of patients with Alzheimer disease. *American Journal Medical Genetic*, 14 (3), 545–556.
[19] Mrozek, K., Bloomfield, C. D. (1996). Cytogenetics of non-Hodgkin's lymphoma and Hodgkin's disease. *Neoplastic Diseases of Blood. New York: Churchill Livingstone*, 835–862.
[20] Murphy, S. B. (1980). Classification, staging and end results of treatment of childhood non-Hodgkins-lymphomas: dissimilarities from lymphomas in adults. *Seminar Oncology*, 7, 332.
[21] Pua, K., Chew C., Lane, D., et al. (2020). Inflammation-associated genomic instability in cancer. *Genome Instability & Disease.* 1, 1–9.
[22] Singh, R.,1 Shaik S., Singh-Negi, B., et al. (2020) Non-Hodgkin's lymphoma: A review. *Journal of Family Medicine and Primary Care.* 9(4), 1834–1840.

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Олександр Онуфрієв, Артур Сіренко. Аномалії центромер та канцерогенез неходжкінських лімфом у дітей. *Журнал Прикарпатського університету імені Василя Стефаника*, 10 (2023), 96–106.

У цій статті наведені результати багаторічних досліджень (1991–2023) зв'язку між аномальною поведінкою центромери, зокрема, феноменами передчасного розділення центромер метафазних хромосом (ПРЦ) та передчасною анафазою метафазних хромосом (С-анафазою), та патогенезом неходжкінських лімфом (НХЛ) у дітей. Досліджено 17 хворих дітей на В-форму неходжкінської лімфоми до початку лікування в першому гострому періоді. Дослідження були спрямовані на прояв та рівні цих феноменів під час першого гострого періоду В-форми неходжкінської лімфоми в культурі клітин периферійної крові та кісткового мозку у дітей. Дослідження виявило переконливі факти, підтверджені статистичним аналізом, які вказують на те, що С-анафаза проявляється з надзвичайно високою частотою у дітей з діагнозом неходжкінська лімфома як в кістковому мозку так і в периферійній крові. Рівні С-анафази у дітей хворих на неходжкінську лімфому складали 51,27 ± 1,99 % в периферійній крові та 67,47 ± 2,19% в червоному кістковому мозку. У той же час цей феномен не зустрічався взагалі в контрольній групі – нормальних здорових донорів та здорових дітей ні в периферійній крові, ні в червоному кістковому мозку (0%). Ці дані дозволяють пропонувати використання С-анафази як додаткового діагностичного критерію для виявлення неходжкінської лімфоми, особливо на ранніх стадіях захворювання. Подальше вивчення механізмів, що лежать в основі аномалій центромери, можуть розкрити нові механізми появи, розвитку і патогенезу неходжкінської лімфоми, прокладаючи шлях для цілеспрямованих терапевтичних втручань та персоналізованих

стратегій лікування та вияснити механізми нових форм запрограмованої смерті клітин при різних патологіях. Наші дослідження дозволяють зробити висновок про взаємозв'язк між аномаліями центромери та патогенезом неходжкінської лімфоми, про важливість визначення рівня С-анафази як діагностичного показника, та її потенційну роль у досліджені як патогенезу, так і клінічної практики, пов'язаної з цим гематологічним злоякісним новоутворенням.

Ключові слова: неходжкінська лімфома, С-анафаза, передчасне розділення центромер, метафазні хромосоми, клітини червоного кісткового мозку, кореляційний аналіз.