Pol J Pathol 2000, 51, 1, 45-50 PL ISSN 1233-9687

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Significance of DNA Ploidy Measurements in Spitz Nevi

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In 28 Spitz nevi DNA content was determined by video-imaging cytometry. The nevi were selected for this study because of difficulties in differentiation from melanoma and heterogeneity of this type of nevus. Morphological features of Spitz nevi and differences helpful for differentiation between Spitz nevi and malignant melanoma were identified. DNA ploidy was measured in paraffin embedded and fresh tissue material from each patient and the results were comparable. The sample preparation process and video-imaging method are presented in this study. Twenty two (78.6%) lesions contained diploid cell populations, 5 (17.9%) aneuploid and 1 (3.6%) tetraploid cell population. A significant correlation was observed between DNA ploidy measured in fresh tissue and retrospective material.

The results indicate the presence of abnormal DNA content in some of the lesions. This observation does not indicate that DNA ploidy cytometry is useful for the differentiation of Spitz nevi from malignant melanoma.

Introduction

DNA content measured by video-imaging cytometry in malignant and premalignant cells becomes a routine adjunct to clinical and histological diagnosis [2, 5, 7, 15, 29, 32]. Many nevi are thought to be associated with a potential risk for developing malignant melanoma - especially dysplastic and congenital nevi [9, 11, 12, 23 - 25, 31].

It seems that the presence of abnormal DNA in many neoplasms is correlated with the poor clinical course [7, 10, 15, 29]. However, diploid cell populations have also been found in such malignant cell transformations as leukemias, lymphomas, whereas aneuploidy in such benign transformations as thyroid adenoma, mastopathy and premalignant cutaneous lesions [2, 29, 32].

It is clear that DNA ploidy is not always an adequate parameter to differentiate benign from malignant lesions [15, 29 - 31].

Spitz nevi (melanoma juvenile) have long been regarded as premalignant lesions [13, 18, 20, 34]. This term recognises the important contribution of Sophie Spitz who published for the first time in 1948 the criteria for the diagnosis of a specific lesion of childhood which, despite some histological resemblance to malignant melanoma, was known to behave in a benign fashion [34]. It is a variant of nevocellular naevus known in the past by the terms: "spindle cell nevus", "epithelioid cell nevus", "nevus of large spindle and/or epithelioid cells" and "benign juvenile melanoma".

Most Spitz nevi occurs in children, at least 25% are diagnosed after 30 years of age. The lesion is usually single and occurs most often on the face (cheeks), arms or thighs. It is usually convex, with its size less than a few millimetres, it is not hairy, salmon or pink in color, rarely brown or black. Numerous widened blood vessels (teleangiectasiae) frequently occur on the surface. Sometimes it has a tendency to relapse, but most often remains for many years without undergoing malignant transformation. Spitz nevi have been reported to arise in normal skin, lightly pigmented patches and hypopigmented patches. There are some reports of Spitz nevi arising in hyperpigmented patches [14].

Spitz nevus is a lesion frequently posing diagnostic difficulties. Clinically it is misdiagnosed with haemangioma or melanoma, pathologists often have difficulties differentiating of Spitz nevi from melanoma. The measurement of additional features such as DNA ploidy could be helpful in correct diagnosis of Spitz nevi.

The aim of this study is to present the results of DNA ploidy measurements in Spitz nevi removed surgically in out-patients in comparison with clinical and histopathological data. The video-imaging method and process of retrospective paraffin tissue block preparation for DNA measurement are described.

Material and Methods

The study material contained 28 Spitz nevi preventively removed in the GreatPoland Cancer Center between 01.01.93 and 31.12.97. Histopathology was assessed in the Department of Oncological Pathology, University of Medical Sciences in Poznań.

Sample preparation

Fresh cell smears delivered from the operation theatre and retrospective paraffin blocks were evaluated by video-imaging cytometry. The smears were supplied on the microscopic glass covered with gel and then with human lymphocytes used as positive diploid internal control tissue. The microscopic glass was rinsed in Carnoy's solution which consisted of absolute alcohol (15 parts), formalin (4 parts), acetic acid (1 part) which, as it was proved in other studies, is a sufficient chromatine fixer and permits examination of DNA content [16, 28]. Fixation time was 3min. Then the sample was stained with Feulgen method.

The preparation of paraffin blocks was more complicated and consisted of the following steps:

a. 2 - 3 50μm sections were cut from each paraffin embedded block,
b. excessive tissue was removed with the scalpel,
c. sections were dewaxed in xylene baths for 1 - 2h,
d. tissue was rehydrated in 96% alcohol and xylene (1:1) for 1h, alcohol 96% for 0.5h, alcohol 85% for 0.5h, alcohol 70% for 24h,
e. rinsed with destillated water,
f. digested with 0.5% pepsine (SIGMA) in 0.9% NaCl at pH=1.5 and temp 37°C (0.5h),
g. centrifuged 3x10min in 0.9% NaCl.

Cell suspension was placed on the microscopic glass and stained with Feulgen method.

DNA measurement

DNA content in the consecutive phases of the cell cycle determined by video-imaging cytometry was reffered to the cells of control population with diploid (2n) standard DNA content. To do this the DNA index was defined:

$$\text{DNA index} = \frac{\text{DNA content in G0/G1 study population}}{\text{DNA content in G0/G1 control population}}$$

For the normal diploid population the DNA index was 1. The DNA index higher or lower than 1 was classified as aneuploid (the index of 2 was interpreted as tetraploidy). The results were received as histograms. The histograms were scaled using diploid references where diploid is equal 2n and is defined by the presence of cells differing from diploid by less than 10%. The tumor was considered as aneuploid when the histograms showed a distinctive peak differing from standard by at least 10%. Histograms were classified according to Auer classification [1].

Image cytometry quantitation was performed using Magical (Joyce-Loebl) analyser. Till 1000 nevi cells and 100 control cells - human lymphocytes were measured each time. GENIAS and RESULTS programs were used. The examinations were carried out in constant conditions of: magnification 640x, individually provided light, analysed area - 0.04mm^2, mistake range - 2.58.

Figure 1 shows a histogram of diploid Spitz nevi, whereas figure 2 a histogram of aneuploid Spitz nevi.

Results

In the whole population of Spitz nevi aneuploid DNA content (Auer type III) was identified in 5 nevi (17.9%) and diploid (Auer type I) in 22 nevi (78.6%). In one Spitz nevus the tetraploid profile of DNA was found (Auer type II). Diploid DNA pattern was found in 9 of 13 (69.2%) Spitz nevi in men and in 13 of 15 (86.7%) Spitz nevi in women. In 4 of 8 (50.0%) nevi with diameter between 6 and 10mm DNA content was aneuploid or tetraploid. Other correlations between DNA ploidy and age, sex, location and size were not found (Tab. 1).

The results obtained from fresh tissue material and retrospective material from the paraffin embedded blocks were identical. The image analysis equipment used in this study was accurate, simple to use, produced results rapidly and was economic.

Discussion

It was found that 6.5% of all melanomas referred were in fact Spitz nevi and that Spitz nevi represented the majority of pathologically misdiagnosed melanomas [20]. The Spitz nevi were more likely to develop on the lower extremities and were, on average, considerably smaller than melanomas. Patients with Spitz nevi were more likely to be younger, female, have fewer dysplastic nevi and have brown eyes. This study [20] concludes that Spitz nevi that are pathologically misdiagnosed as melanomas retain the clinical characteristics of Spitz nevi and that better clinicopathological communication may reduce the frequency of diagnostic error.

Peters et al. reviewed 33 cases of Spitz nevus in comparison with 19 malignant melanomas [22]. The histological findings were studied prior to the review of clinical data. There were no malignant melanomas in patients less than 9 years old; almost half (15/33) of the Spitz nevi were in this age group. Among the 25 histological criteria evaluated in the 52 lesions, the most striking differences between malignant melanomas and Spitz nevi were a higher degree of pagetoid spread, cellular pleomorphism, nuclear hyperchromasia and mitotic activity in the malignant melanomas, and a more prominent spindle cell component in Spitz nevi. As the clinical and histological differential diagnosis between Spitz nevus and cutaneous melanoma may be very difficult some indications may be helpful for their differentiation (Tab. 2).

TABLE 1

Comparison between clinical features and DNA ploidy content

Clinical features	Diploid DNA	Aneuploid DNA	Tetraploid DNA
Age:			
<10	6	1	
10 - 19	9	2	1
20 - 29	2	1	
30 - 39	5	1	
Sex:			
Man	9	3	1
Woman	13	2	
Size:			
<6mm	10	1	
6 - 10mm	4	3	1
11 - 20mm	1		
>20mm	1	1	
Location:			
Face	2	1	
Torso	7	2	1
Forearm	1		
Arm	3	1	
Thigh	2	1	
Shank	4		
Hand	2		
Foot	1		

TABLE 2

Differentiation between Spitz nevus and malignant melanoma [acc. 4]

Feature	Spitz Nevus	Malignant Melanoma
Symetry	Usually present	Usually absent
Border artefact (gap)	Present	Absent
Presence of single melanocytes in epidermis	Rarely	Frequently
Cell cohesion	Absent	Present
Kamino bodies	Frequently present	Usually absent
Cytoplasmic pleomorphism	Present	Present
Nuclear pleomorphism	Absent	Present
Giant cells	Homogenous nuclei	Nuclear pleomorphism
Mitotic activity	Usually weak	Often considerable
Atypical mitoses	Never	Frequently
Melanin	Frequently absent	May be plentiful
Broadening of vessels	Frequently present	Usually absent

So far DNA ploidy results have been contradictory. The role of DNA was studied by in situ hybridisation - the result suggests that this method may contribute to the positive identification of histologically equivocal pigmented lesions [7]. In another report the technique of DAPI-DNA microfluorometry was used for cellular DNA content measurements [21]. The diploid pattern in Spitz nevi was similar to that of histogically confirmed Spitz nevi and of acquired nevi but different from the aneuploid pattern of malignant melanoma. According to the authors this method provided confirmatory evidence for the diagnosis of Spitz nevus. The method appears to reflect sensitively the biological behaviour of tumour cells, and is a useful aid to the diagnosis of uncertain Spitz nevi [21].

Other authors confirmed that diploid pattern of DNA help in differentiating the lesion from malignant melanoma [19].

Rode et al. carried out a study to define the means of positive identification of Spitz nevi of doubtful malignancy [26]. The nuclear DNA content distribution in 35 Spitz nevi was measured using image analysis cytometry. As compared with the malignant cases (19 melanomas), the Spitz nevi showed significantly lower staining intensity for both S100 protein ($p<0.0001$) and NSE ($p<0.0001$). The nuclear DNA content in Spitz nevi proved to be a normal diploid pattern in 31 cases, in four cases showed a small proportion of hyperdiploid nuclei. The results show that normal diploid DNA content distribution appears to be typical of Spitz nevi.

LeBoit et al. compared nuclear DNA content in 13 Spitz nevi and nodular malignant melanomas [17]. In this study Spitz nevi showed a lower DNA content in the deepest dermal cells as compared with upper dermal cells, suggesting that some Spitz nevi have an admixture of diploid and hyperdiploid cells in their upper portions, but mostly diploid cells in their deep portions. Only samples of nodular malignant melanoma showed higher mean DNA content in deep dermal cells as opposed to superficial dermal cells, suggesting that some nodular melanomas may either have clones of cells in their deep portions that have higher levels of ploidy, or more cells in the deep portions of melanomas may be in active phases of the cell cycle. According to the authors their results suggested that there are important cytometric differences between Spitz nevi and nodular melanoma. Vogt et al. evaluated nuclear DNA distribution and nuclear size using image analysis cytometry in 28 Spitz nevi and 34 malignant melanomas [33]. In multivariate analysis five features of DNA distribution proved to be most important for objective discrimination between melanomas and Spitz nevi: 2c deviation index, 5c exceeding rate, standard deviation of the nuclear DNA content, and both the 85th and the 95th percentiles of DNA distributions. This data demonstrated that diagnostically misleading large nuclei in Spitz nevi

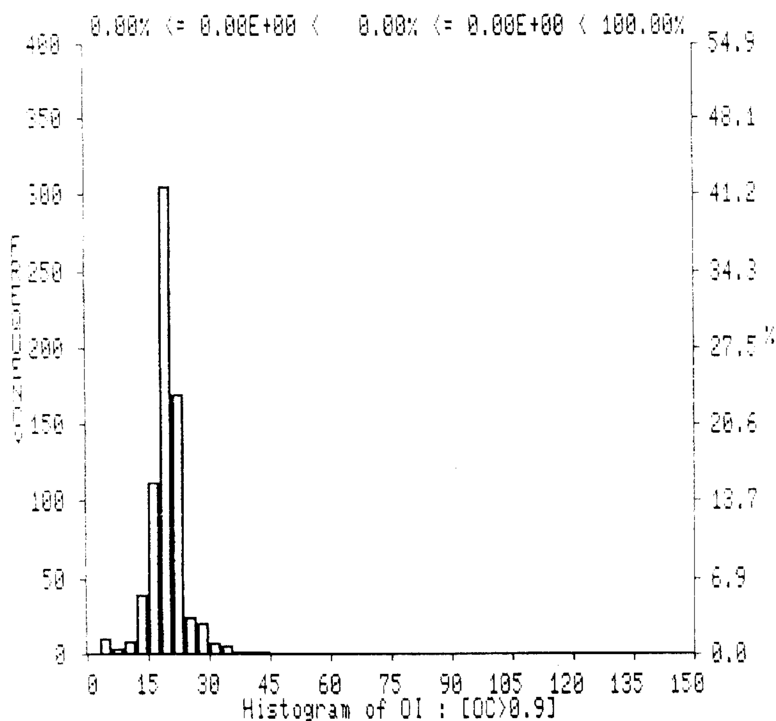

Fig. 1. Diploid histogram of Spitz nevi - correlation between DNA content (OI) and frequency.

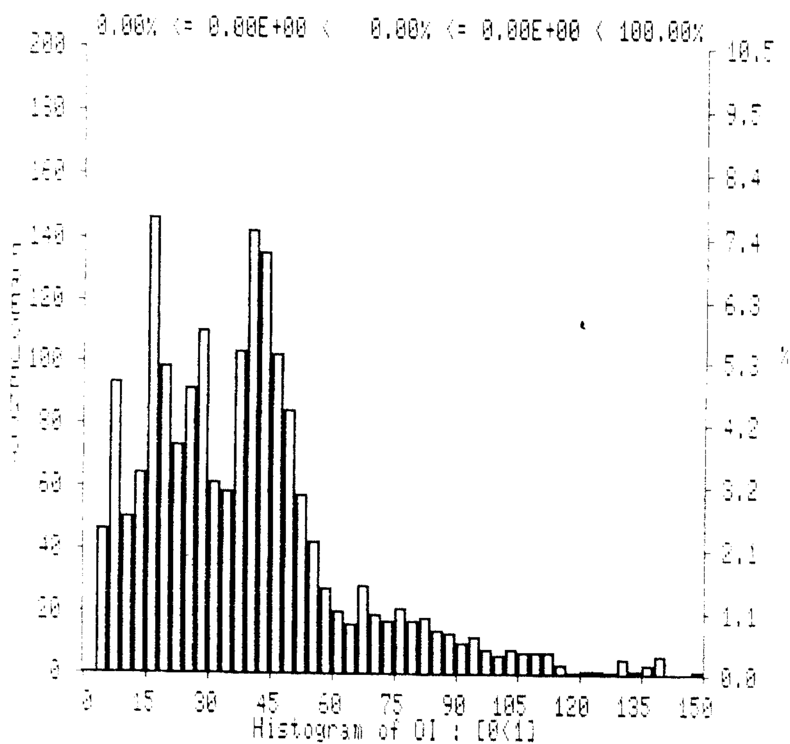

Fig. 2. Aneuploid histogram of Spitz nevi - correlation between DNA content (OI) and frequency.

are euploid, in contrast to melanoma. It is thus possible to discriminate Spitz nevi and melanoma with high accuracy using DNA cytometry [33].

In another study Sangueza et al. measured DNA ploidy in 11 Spitz nevi - one of them was tetraploid, 10 were diploid [27]. They used interactive image analysis using nuclear extracts of paraffin-embedded tissue.

Chi et al. measured cellular DNA content for the purpose of differentiating Spitz nevus from malignant melanoma [6]. Among 20 Spitz nevi, 18 showed a diploid

DNA content, other two had a few polyploid cells with the major population of cells containing diploid DNA. In contrast all malignant melanomas showed an aneuploid DNA distribution. The results suggest that cytofluorometric analysis of cellular DNA content reflects the biologic behavior more sensitively than conventional clinical or histological criteria [6].

Bjornhagen et al. [3] found significant differences between benign melanocytic lesions and melanomas in terms of DNA 2.5c and 5c exceeding rates.

DNA aneuploidy was found in thick malignant melanomas and melanomas metastases rather than in benign melanocytic lesions and thin malignant melanomas. DNA content seems to reflect different stages in tumor progression of malignant melanoma.

The present study confirmed the usefulness of video-imaging cytometry method in routine DNA content analysis in Spitz nevi cells. The techniques of preparing samples from paraffin blocks for DNA ploidy assessment and the preparation of cell smears from fresh tissue material are relatively simple and reproducible.

Our own results indicated the occurrence of a certain amount of aneuploidy and tetraploidy in Spitz nevi. Clinical and histopathological data were similar to those reported by other authors. 19/28 (67.9%) of examined patients were below 19 years, 15 of them were women (53.6%). 25/28 nevi were less than 10mm in diameter. We did not find any significant correlation between the clinical and histological data and DNA ploidy. Those results confirmed the findings of other authors. A group of 5 aneuploid patterns among benign nevi can indicate an increased tendency towards proliferation and associated increased risk of malignant transformation. This suggests more careful prophylactic examination in this group of patients. We conclude that Spitz nevi cannot be used as diploid control in DNA ploidy measurements.

Conclusions

1. The determination of cellular DNA ploidy alone does not permit on distinction of Spitz nevi from malignant melanoma.

2. In some Spitz nevi abnormal aneuploid DNA pattern can be measured.

3. There are no correlations between choosen clinical, histological data and DNA ploidy.

References

1. *Auer GU, Caspersson TO, Wallgren AS:* DNA content and survival in mammary carcinoma. Anal Quant Cytol 1980, 2, 161-165.
2. *Barlogie B, Raber MC, Schumann J, Johnson TS, Drewinko B, Swartzendruber DE, Gohde W, Andreff M, Freireich EJ:* Flow cytometry in clinical cancer research. Cancer Res 1983, 43, 3982-3997.
3. *Bjornhagen V, Bonfoco E, Brahme EM, Lindholm J, Auer G:* Morphometric, DNA, and profilerating cell nuclear antigen measurements in benign melanocytic lesions and cutaneous malignant melanoma. Am J Dermatopathol 1994, 16, 615-23.
4. *Bręborowicz J, Bręborowicz D:* Diagnostyka histopatologiczna czerniaka i łagodnych rozrostów melanocytów skóry. W: Skowronek J, Mackiewicz A, Zygulska-Mach H: Czerniak złośliwy. Podręcznik dla lekarzy i studentów. Termedia Poznań 1998, 53.
5. *Buchner T, Hiddemann W, Wormann B, Kleinemeier B, Schumann J, Gohde W, Ritter J, Muller KM, von Bassewitz DB, Roessner A, Grundmann E:* Differential pattern of DNA-aneuploidy in human malignancy. Pathol Res Pract 1985, 179, 310-317.
6. *Chi HI, Ishibashi Y, Shima A, Mihara I, Otsuka F:* Use of DAPI cytofluorometric analysis of cellular DNA content to differentiate Spitz nevus from malignant melanoma. J Invest Dermatol 1990, 95, 154-157.
7. *Czerniak B, Eppich EM, Gorczyca W, Herz F, Koss JG:* Cytometryczne pomiary ploidii DNA w rakach jelita grubego. Pat Pol 1988, XXXIX, 1, 1-12.
8. *De Wit PE, Kerstens HM, Poddighe PJ, Van Muijen GN, Ruiter DJ:* DNA in situ hybridization as a diagnostic tool in the discrimination of melanoma and Spitz naevus. J Pathol 1994, 173(3), 227-233.
9. *Elder DE, Greene MH, Guerry DIV, Kraemer KH, Clark WHJr:* The dysplastic nevus syndrome: our definition. Am J Dermatopathol 1982, 4, 455-460.
10. *Friedlander ML, Hedley DW, Taylor JW:* Clinical and biological significance of aneuploidy in human tumours. J Clin Pathol 1984, 37, 961-974.
11. *Gołębiowska A, Szymańczyk J, Michalska I, Jabłońska S:* Zespół znamion dysplastycznych. Klinika 1994, IX, 41-43.
12. *Greene MH, Clark WHJr, Tucker MA, Kraemer KH, Elder DE, Fraser MC:* High risk of malignant melanoma in melanoma-prone families with dysplastic nevi. Ann Intern Med 1985, 102, 458-462.
13. *Helm KF, Schwartz RA, Janniger CK:* Juvenile melanoma (Spitz nevus). Cutis 1996, 58(1), 35-39.
14. *Herd RM, Allan SM, Biddlestone L, Buxton PK, McLaran KM:* Spitz naevi arising on hyperpigmented patches. Clin Exp Dermatol 1994, 19(6), 483-486.
15. *Hermann CJ:* Cytometric DNA analysis in the management of cancer. Cancer 1992, 69, 1553-1556.
16. *Krygier-Stojałowska A:* Zasady cytofotometrii. W: Topochemiczne metody badań komórek i tkanek. Warszawa 1987, 111-134.
17. *LeBoit PE, Van Fletcher MD:* A comparative study of Spitz nevus and nodular malignant melanoma using image analysis cytometry. J Invest Dermatol 1987, 88, 753-757.
18. *Liebhart M:* Melanoma juvenile (naevus Spitz) dzieci i młodzieży w 6-letniej obserwacji Zakładu Patomorfologii Instytutu Matki i Dziecka. Ped Pol 1986, LXI, 500-505.
19. *Nogita T, Nagayama M, Kawashima M, Hidano A, Kasori J, Morishima T:* Spitz naevus of the toe. Br J Dermatol 1992, 126(5), 520-522.
20. *Orchard DC, Dowling JP, Kelly JW:* Spitz naevi misdiagnosed histologically as melanoma: prevalence and clinical profile. Austral J Dermatol 1997, 38(1), 12-14.
21. *Otsuka F, Chi HI, Umebayashi Y:* Successful differentiation of Spitz naevus from malignant melanoma by microfluorometric analysis of cellular DNA content. Clin Exp Dermatol 1993, 18(5), 421-424.
22. *Peters MS, Goellner JR:* Spitz naevi and malignant melanomas of childhood and adolescence. Histopathology 1986, 10(12), 1289-1302.
23. *Reimer RR, Clark MH, Greene MH, Ainsworth AM, Fraumeni JFJr:* Precursor lesions in familial melanoma. A new genetic preneoplastic syndrome. JAMA 1978, 239, 744-746.

24. *Rhodes AR, Wood WC, Sober AR, Mihm MCJr:* Non-epidermal origin of malignant melanoma associated with a giant congenital nevocellular nevus. Plast Reconstr Surg 1981, 67, 782-790.
25. *Rhodes AR, Sober AJ, Day CL, Melski JW, Harrist TJ, Mihm MC, Fitzpatrick TB*: The malignant potential of small congenital nevocellular nevi: an estimate of association based on a histologic study of 234 primary cutaneous melanomas. J Am Acad Dermatol 1982, 6, 230-241.
26. *Rode J, Williams RA, Jarvis LR, Dhillon AP, Jamal O:* S100 protein, neurone specific enolase, and nuclear DNA content in Spitz naevus. J Pathol 1990, 161(1), 41-45.
27. *Sangueza OP, Hyder DM, White CRJr, Pallander J, Perkins M, Remple N, Bakke A:* Comparison of image analysis with flow cytometry for DNA content analysis in pigmented lesions of the skin. Anal Quant Cytol Histol 1992, 14, 55-59.
28. *Skowronek J:* Analiza zawartości DNA metodą video-imaging w komórkach czerniaków złośliwych. Nowotwory 1997, 47, 89-99.
29. *Skowronek J:* Ploidia DNA jako czynnik rokowniczy w nowotworach. Nowotwory 1997, 47, 343-353.
30. *Skowronek J, Adamska K, Filipiak K, Karaś Z, Krenz RM, Rutkowski R, Warchoł JB:* DNA ploidy in malignant melanoma, skin cancer and pigmented nevi. Neoplasma 1997, 44, 282-288.
31. *Skowronek J, Filipiak K, Karaś Z, Krenz RM, Włodarczyk H:* Estimation of DNA-ploidy in melanocytic nevi cells using video-imaging cytometry. Pol J Pathol 1997, (48)1, 37-41.
32. *Skowronek J, Filipiak K, Warchoł JB:* Znaczenie pomiarów cytometrycznych w onkologii na przykładzie czerniaka złośliwego. Nowiny Lekarskie 1998, 67, 508-514.
33. *Vogt T, Stolz W, Glassl A, Abmayr W, Hohenleutner U, Schmoeckel C, Schiffner R, Landthaler M:* Multivariate DNA cytometry discriminates between Spitz nevi and malignant melanomas because large polymorphic nuclei in Spitz nevi are not aneuploid. Am J Dermatopathol 1996, 18, 142-150.
34. *Weedon D:* The Skin. Systemic Pathologic. 3rd ed, vol 9. Churchill Livingstone 1992.

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