Prognostic Significance of DNA Cytometry in Cutaneous Malignant Lymphomas

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The current classification of cutaneous malignant lymphomas (ML) into low-grade and high-grade lymphomas was found to be of limited reproducibility and permitted only a rough prediction about outcome. With this in mind, the relationship between nuclear DNA content and both prognosis and histologic grading according to the Kiel classification was evaluated on Feulgen-stained imprint specimens. In all, 49 cases of malignant non-Hodgkin's lymphoma, primary of the skin or with an involvement of the skin as one of the first symptoms, were studied using a computerized high-resolution image analysis system. The 2c deviation index (2cDI), which reflects the variation of the nuclear DNA values around the normal diploid peak, was found to be the best prognostically relevant criterion. Using the 2cDI, a significant discrimination (*P* less than 0.001 in the U test) between low-grade and high-grade ML was achieved. The prognostic benefit of the 2cDI was well documented by a significant inverse correlation between the 2cDI and the period of time until the patients progressed at least into one higher stage or died of lymphoma (*r* equals −0.63, *P* less than 0.05). In addition, the 2cDI enabled prognosis of the course of disease. In the group with low 2cDI values (2cDI, less than 0.5), no progression of the disease was observed after 1 year. In the groups presenting with a 2cDI between 0.5 and 1.0 and higher than 1.0, a progression was found in 57% and 64% of the cases studied, respectively. In conclusion, these measurements indicate that the determination of DNA distribution patterns in imprint specimens allows a precise and objective prognostic evaluation of cutaneous ML. *Cancer* 68:1095–1100, 1991.

THE CORRECT GRADING of skin infiltrates in cutaneous malignant lymphomas (ML) is an important prerequisite for selection of the most appropriate therapy. Currently, morphologic classification systems, such as the Kiel classification,[1] allow only a rough discrimination between low-grade and high-grade ML, which may not reflect the full range of biologic variation.[2–4] Grading of lymphomas according to morphologic features alone also is subjective and was shown to be insufficiently reproducible.[5,6] Moreover, approximately 15% of cutaneous ML do not fit into common classification schemes.[7]

This was demonstrated in one study[4] where there was identification of four prognostically relevant subgroups in mycosis fungoides (MF) alone; some of these cases had the same poor prognosis as high-grade lymphomas. In addition to histologic and immunohistologic findings, more objective and reliable criteria are necessary for an accurate prognostic evaluation of individual cases of ML.

It is widely accepted that malignant tumors often have abnormal nuclear DNA values and that DNA measure-

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ments are very helpful for the prognostic and diagnostic evaluation of neoplasms of many tissues.[8-13] To our knowledge, measurements of Feulgen-stained (Merk, Darmstadt, Germany) nuclei were used only for the diagnosis of cutaneous ML,[14-17] not for the determination of prognostically relevant markers. Therefore, we used high-resolution image analysis[18,19] of imprint preparations of cutaneous ML to examine the prognostic significance of DNA cytometry and its relationship to histologic grading.

Materials and Methods

Patients

The DNA measurements were done on imprint preparations of skin lesions of 49 cases of malignant non-Hodgkin's lymphoma, primary of the skin or with an involvement of the skin as one of the first symptoms. The diagnoses were established by histologic, immunohistologic, and clinical criteria before DNA analysis. Because the Kiel classification[1] was not found to be reliable for grading of all low-grade malignant lymphomas,[4] cases with a predominance of medium-sized or large lymphocytes in the infiltrate were classified by two independent well-trained histopathologists into a group of intermediate grades of malignancy (Table 1). According to this modified Kiel classification,[1] 23 of 49 cases were classified as low-grade malignant lymphomas (12 of B-cell type and 11 of T-cell type); ten cases, as intermediate-grade (six of T-cell type, one of B-cell type, and three unclassified cases), and 16 as high-grade lymphomas (two of T-cell type, eight of B-cell type, four of neither B-cell nor T-cell types, and two unclassified cases; Table 1).

Detailed staging procedures were done, including chest radiography, computed tomography, sonography, and if necessary, radionuclide imaging, and biopsies were taken of the bone marrow, liver, and lymph nodes. Stages were defined according to the tumor–node–metastasis (TNM) system.[7] Detailed data concerning diagnosis, histologic type, grading, age, and sex are given in Table 1.

The interval between imprint preparation and progression to the next higher stage in the TNM system or death was known for 18 of the 49 patients. In these cases, it was possible to study the correlation between DNA data and progression.

Preparation of Imprints

Imprints were made by touching clean glass slides with the freshly cut surface of a skin biopsy specimen. Fixation of the imprints with methanol (100%), formaldehyde (37%), and glacial acetic acid (96%) 85:10:5 (v/v/v) and Feulgen staining were done according to Böhm.[20] The imprints were hydrolyzed with 5 N HCl for 50 minutes at a constant temperature of 28°C and stained with Schiff's reagent for 60 minutes.

DNA Measurements

DNA analysis was done on the imprints using the high-resolution video image-analysis system IPS (Kontron, Eching, Germany). The routine for DNA measurements (described in detail previously)[21] allows a fully automatic segmentation of the nuclear area by using different steps of image processing such as gray-scale normalization and high-pass filtering.

Variables Calculated

For each nucleus, the optical density (OD) and the area were measured. The integrated optical density (IOD) was obtained by multiplying the OD by the area. In Feulgen-stained nuclei, the IOD is correlated linearly with the DNA content. Twenty chicken erythrocytes were used as an internal reference on each slide. In a previous pilot study, a comparison of nuclei of chicken erythrocytes and normal human lymphocytes revealed that:

$$IOD_{ery} * 3.26 = 2c\ DNA_{lymphocytes} \quad (1B)$$

The 2c DNA represents the DNA content of a normal human diploid cell. A euploid cell in G2 contains 4c DNA. In this study, the relative DNA content of 100 randomly selected cells was calculated in each case as follows:

$$\text{Relative DNA content} = IOD_{nucleus}/IOD_{1cDNA} \quad (2B)$$

Algorithms for Data Evaluation

The discriminating power of the following features of the DNA histogram was evaluated using the Kontron software package:

The 2c deviation index (2cDI): The 2cDI was defined according to Böcking *et al.*[22] as the ratio between the sum of the squares of the differences between the DNA values of individual cells (c_i) and the 2c value and the number of cells measured (N).

$$2cDI = \frac{1}{N} \sum_{i=1}^{N} (c_i - 2c)^2 \quad (3B)$$

Roughly, the 2cDI reflects the variation of the nuclear DNA values around the 2c value for an individual patient.

The 5c exceeding rate (5cER): The 5cER was defined according to Böcking *et al.*[22] as the percentage of cells with a DNA content equal to or more than 5c. Because euploid-polyploid cells should not contribute to the 5cER, all cells with DNA values that are integer-valued exponents of 2c ± 12.5% are ignored. The maximum error of measurement was set at 12.5%.[22] Thus, the 5cER identifies aneuploid cells only.

TABLE 1. Patient Characteristics

Grade of malignancy*	Patient no.	Age (yr)/sex	Histologic diagnosis	2cDI	4cER (%)	5cER (%)
Low-grade	1	76M	CLL-B	0.05	0	0
	2	52F	MF I	0.05	0	0
	3	40M	IC	0.09	1	0
	4	58F	IC	0.11	9	0
	5	65M	CBCC	0.14	4	0
	6	69M	SS	0.24	2	0
	7	62M	MF III	0.28	2	0
	8	59F	MF II	0.29	2	1
	9	76F	CBCC	0.31	0	0
	10	59M	MF I	0.31	3	0
	11	75F	MF II	0.32	3	0
	12	63M	IC	0.32	3	1
	13	59M	CBCC	0.34	2	1
	14	45M	MF I	0.37	5	0
	15	75M	CLL-T	0.37	1	1
	16	71M	MF I	0.39	5	1
	17	34M	SS	0.40	6	0
	18	53F	MF-II	0.41	4	1
	19	36M	CBCC	0.50	4	1
	20	73M	CBCC	0.50	4	1
	21	75M	IC	0.57	6	0
	22	37F	CBCC	0.77	5	4
	23	65F	CBCC	0.81	12	0
Intermediate-grade	24	56M	uc-ML	0.43	4	1
	25	65F	MF III	0.62	7	1
	26	71M	uc-ML	0.65	9	0
	27	58M	CBCC	0.85	6	0
	28	66M	MF III	0.96	5	0
	29	79M	uc-ML	1.05	12	2
	30	70M	MF III	1.07	10	1
	31	73M	MF III	1.15	10	2
	32	40F	MF II	1.18	12	4
	33	72F	MF III	1.68	15	0
High-grade	34	67M	uc-ML	0.53	10	0
	35	73F	LBL	0.55	7	0
	36	72F	CBL	0.56	7	0
	37	77F	LBL-B	0.68	8	0
	38	78F	IBL-B	0.73	1	0
	39	52M	CBL	1.00	7	0
	40	64M	LBL	1.03	6	0
	41	67M	IBL	1.31	8	0
	42	37M	CBL	1.39	8	0
	43	46M	IBL	2.01	17	2
	44	43F	IBL-T	2.09	21	0
	45	37M	uc-ML	2.97	37	1
	46	75F	LBL-B	3.84	8	3
	47	66F	CBL	5.99	27	10
	48	70M	LBL-T	10.50	45	7
	49	57M	CBL	11.73	84	5

2cDI: 2c deviation index; 4cER: 4c exceeding rate; 5cER: 5c exceeding rate; CLL-B: chronic lymphatic leukemia, B-cell type; MFI: mycosis fungoides, premycotic lesion; IC: immunocytoma; CBCC: centroblastic-centrocytic lymphoma; SS: Sezary syndrome; MF III: mycosis fungoides, tumor lesion; MF II: mycosis fungoides, plaque lesion; CLL-T: chronic lymphatic leukemia, T-cell type; uc-ML: unclassified malignant lymphoma; LBL: lymphoblastic lymphoma; CBL: centroblastic lymphoma; LBL-B: lymphoblastic lymphoma, B-cell type; IBL-B: immunoblastic lymphoma, B-cell type; IBL: immunoblastic lymphoma; IBL-T: immunoblastic lymphoma, T-cell type; LBL-T: lymphoblastic lymphoma, T-cell type.

* According to a modified Kiel classification.

The 4c exceeding rate (4cER): For the classification of benign and malignant lesions, van Vloten *et al.*[14] used the percentage of cells with a DNA value higher than 4c. They classified a lesion as malignant if more than 5% of the cells contained 4c DNA or more.

Data Analysis

Statistical evaluation of the data was based on the Mann–Whitney's U test and the correlation coefficient.[23] Significance in both tests was defined as P less than 0.05.

The reproducibility of DNA measurements was tested four times during the study by the demonstration of the different ploidy peaks in rat liver imprints.

Results

The best discrimination between low-grade and high-grade lymphomas was possible using the 2cDI (Fig. 1). In the group of low-grade lymphomas, the 2cDI ranged from 0.05 to 0.81; in high-grade lymphomas, 2cDI values from 0.53 to 11.73 were observed. In the intermediate group, the 2cDI was between 0.43 and 1.68. Statistically significant differences were found between low-grade and high-grade ML (P less than 0.001), between intermediate-grade and low-grade ML (P less than 0.001), and between the intermediate-grade and high-grade ML (P less than 0.05). No significant differences were found between B-cell and T-cell lymphomas in either the low-grade or the intermediate-grade or high-grade groups of ML (P more than 0.05). The determination of the 4cER also allowed significant discrimination between low-grade and intermediate-grade ML (P less than 0.001), but not between the intermediate-grade and high-grade groups (P more than 0.05). The 4cER values ranged from 0 to 12 in low-grade, 4 to 15 in intermediate-grade, and 1 to 84 in high-grade ML. Significant differences were not observed for the 5cER. Values between 0 and 4 were found in the low-grade and intermediate-grade groups and between 0 and 10, in high-grade ML.

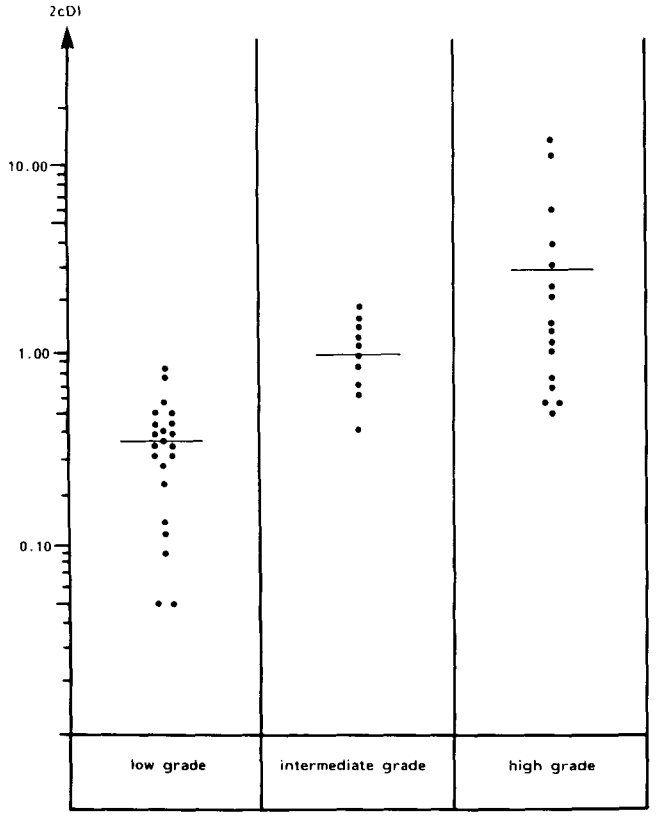

FIG. 1. Correlation between grading into low-grade, intermediate-grade, and high-grade malignant lymphomas and 2c deviation index.

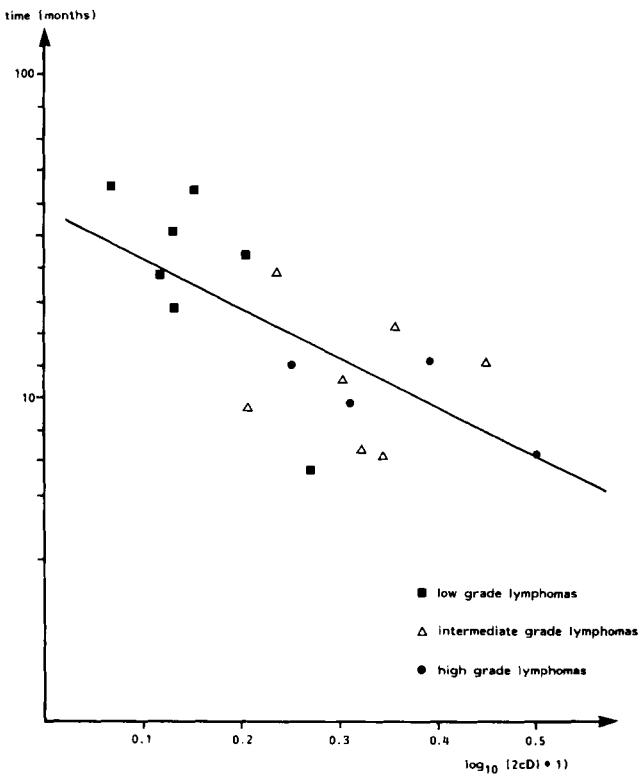

FIG. 2. Significant correlation ($r = -0.63$) between the period ($\log_{10}$ months) until progression into the next higher TNM stage or death and the 2c deviation index.

Not only was 2cDI a useful marker for differentiating between low-grade and high-grade ML; but the formula $\log_{10}(2cDI+1)$ showed a significant inverse correlation (r equals -0.63, P less than 0.05) to $\log_{10}$(time) until progression to the next higher stage of the TNM scheme or death occurred. This also was found for low-grade lymphomas (Kiel) alone (r equals 0.68, P less than 0.05); for high-grade lymphomas (Kiel), the correlation was not significant. For B-cell lymphomas alone, the correlation coefficient was even higher (r equals -0.75, Fig. 2).

In addition, the 2cDI was a predictor of the course of the disease. None of the patients with a 2cDI lower than 0.5 had tumor progression within 1 year, whereas 57% and 64% of the patients with a 2cDI from 0.5 to 1.0 or higher than 1.0, respectively, had to be assigned to a higher TNM stage after 1 year. Simultaneously, the percentage of patients with a remission under therapy decreased in the groups with higher 2cDI values (Fig. 3).

The 2cDI also was useful for the prediction of mortality. In the group with initial 2cDI values below 0.5, all patients were still alive after 2 years, whereas the mortality rate

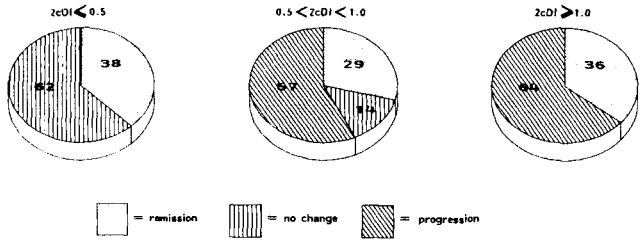

FIG. 3. Percentage of patients with remission, no change, and progression of the disease after 1 year of adequate therapy for the three prognostic groups defined according to the 2c deviation index.

was 30% in the group with a 2cDI between 0.5 and 1.0 and 45% in patients with a 2cDI exceeding 1.0.

The 2cDI showed a significant correlation with both the 4cER (r equals 0.92, P less than 0.05) and the 5cER (r equals 0.79, P less than 0.05). However, the 5cER and the 4cER did not provide additional prognostic information.

Discussion

The most impressive result in this cytometric study was the finding that calculation of the 2cDI allowed a significant discrimination between low-grade, intermediate-grade, and high-grade lymphomas using a modified Kiel classification (P less than 0.05, by two-sided U test, Fig. 1). Applying the 4cER as a criterion for grading, a difference was obtained between low-grade and high-grade lymphomas, but not between intermediate-grade and high-grade lymphomas because the zone of overlap between the groups was higher than using the 2cDI. The 5cER, which represents the percentage of cells containing more than 5c DNA and is therefore an indicator for definite aneuploidy, was found to be an unreliable marker for grading. The 5cER seems to be more useful for the differentiating between benign and malignant lesions, as shown for cutaneous pseudolymphomas and malignant lymphomas,[21] and for tumors of the breast, bone, prostate, and cervix.[11,22]

A prerequisite for grading strategies in the Kiel classification[1] or the New Working Formulation for clinical usage of the National Cancer Institute[24] is that histomorphologically similar lymphomas have the same malignant potential. However, morphologically defined subgroups of malignant lymphomas often are heterogeneous in their clinical course and do not always have the same survival probability.[2-4] Different prognostically relevant subgroups of MF were defined because, with respect to survival probability, cases with many large and medium cells were more likely to represent high-grade than low-grade lymphomas.[4] However, a major handicap for the broad application of such detailed classification strategies[4] is the low interobserver reproducibility.[6] In contrast to morphologic grading, the 2cDI is a more objective and reproducible parameter.[2,3]

Moreover, the currently applied histopathologic classification schemes, such as the Kiel classification or the New Working Formulation, allow discrimination only between two or three prognostic relevant groups. The 2cDI provides a continuous scale for grading of malignancy of individual lesions and has been established as a sensitive feature for grading and diagnosis of tumors of the bone, cervix, breast, prostate, and nodular lymphomas.[2,3,22] To our knowledge, the prognostic impact of DNA distribution pattern has not been demonstrated in cutaneous ML.

The calculation of the 2cDI using imprint investigation with an image-analysis system does not require a well-trained histopathologist with great experience in the field of ML; it can be done with the same accuracy by a technician. However, the procedure is more time consuming than detailed histopathologic examination.

The relevance of this newly described grading parameter for cutaneous ML has to be demonstrated by its clinical impact. We found a significant correlation between the $\log_{10}(2cDI+1)$ and the $\log_{10}$ of the time elapsed until the next higher TNM stage was reached or death occurred. This correlation also was significant in the low-grade group alone. This indicates a heterogeneous prognosis in a morphologically defined subgroup and stresses the advantage of the 2cDI for prognostic evaluation. In these low-grade cases, 5cER was always lower than 5%, and the benefit of the 2cDI was not based on the presence of numerous aneuploid nuclei with DNA values higher than 5c, but on the increase of proliferative nuclei with relative DNA values between 2.25c and 3.5c. Because of the low number of high-grade lymphomas with sufficient prognostic data, a significant correlation could not be calculated in these cases. Using the regression line in Figure 2, the individual risk of the patients can be determined more precisely than by applying the rough discrimination into low-grade, intermediate-grade, and high-grade lymphomas. Moreover, without respect to histomorphology, the 2cDI enabled discrimination between three groups of patients with different progression rates (Fig. 3).

The nuclear area frequently is well correlated with the nuclear DNA content,[25] and morphometric techniques alone (which can be done more rapidly already using low-cost image analysis systems or point sampling methods as described recently for malignant melanomas)[26] also might be clinically beneficial. In the Netherlands, a multicenter study was begun to evaluate the benefits of morphometric measurements in histologic sections of breast cancer.[27] These authors showed that about 22% of patients received a more appropriate therapy with than without morphometric data.

Cytochemical and immunologic cell typing[28-35] also might allow a more detailed prognostic analysis in cuta-

neous ML. One study described the use of the monoclonal antibody Ki-67, reported to be well correlated with the growth fraction of cell populations, which therefore might be an additional predictor of prognosis in human tumors.[31] Using the Ki-1 antibody, a new and distinct entity among the cutaneous lymphomas was defined that could not be characterized by morphologic features alone.[33] However, for most of the cutaneous malignant lymphomas, prognostically significant antibodies are of limited value, and further experience must be gained with this technique.[34] For evaluating the full benefit of DNA measurements for predicting prognosis in cutaneous ML, as indicated by our study, long-term prospective and retrospective studies of more patients are necessary. Such investigations already have been done for mammary adenocarcinoma[13] and nodular lymphoma.[2,3]

The development of techniques for the production of single-cell specimens from paraffin-embedded material[36] now has made these studies easier. High-resolution image analysis of the chromatin structure, altered even in preneoplastic cells,[37,38] therefore may be superior to pure ploidy analysis and may improve the grading of ML.

REFERENCES

1. Lennert K, Mohri N. Malignant lymphomas. Berlin: Springer, 1978.
2. Böcking A, Chatelain R, Auffermann W *et al.* DNA grading of malignant lymphomas: 1. Prognostic significance, reproducibility, and comparison with other classifications. *Anticancer Res* 1986; 6:1205–1216.
3. Böcking A, Chatelain R, Auffermann *et al.* DNA grading of malignant lymphomas. 2. Correlation with clinical parameters. *Anticancer Res* 1986; 6:1217–1224.
4. Kerl H, Hödl S, Smolle J, Konrad K. Klassifikation und Prognose kutaner T-Zell -Lymphome. *Z Hautkr* 1986; 61:63–67.
5. Baak JPA, Oort J. A Manual of Morphometry in Diagnostic Pathology. Berlin: Springer, 1983; 88–95.
6. Krüger GRF. New Working Formulation für Non-Hodgkin Lymphome: Eine klinisch-pathologische Korrelation. In: Diehl V, Sack H, eds. Aktuelle Onkologie Bd 12: Diagnostik und Therapie der Non-Hodgkin Lymphome. München: W. Zuckschwert, 1983.
7. Burg G, Braun-Falco O. Cutaneous Lymphomas, Pseudolymphomas, and Related Disorders. New York: Springer, 1983; 81–93.
8. Böhm N, Sandritter W. DNA in human tumors: A cytophotometric study. *Curr Top Pathol* 1975; 60:152–219.
9. Barlogie B. Abnormal cell DNA content as a marker of neoplasia: Perspectives and commentaries. *Eur J Cancer Clin Oncol* 1984; 20:749–752.
10. Hall TL, Fu YS. Biology of disease: Applications of quantitative microscopy in tumor pathology. *Lab Invest* 1985; 53:5–21.
11. Friedlander ML, Hedley DW, Taylor IW. Clinical and biological significance of aneuploidy in human tumors. *J Clin Pathol* 1984; 37: 961–974.
12. Joensuu H, Söderström K-O, Klemi PJ, Errola E. Nuclear DNA content and its prognostic value in lymphoma of the stomach. *Cancer* 1987; 60:3042–3048.
13. Auer G, Eriksson E, Azavedo E, Caspersson T, Wallgren A. Prognostic significance of nuclear DNA content in mammary adenocarcinomas in humans. *Cancer Res* 1984; 44:394–396.
14. van Vloten WA, van Duijn P, Schaberg A. Cytodiagnostic use of Feulgen-DNA measurements in cell imprints from the skin of patients with mycosis fungoides. *Br J Dermatol* 1974; 91:365–371.
15. Van Vloten WA, Schaberg A, van der Ploeg M. Cytophotometric studies on mycosis fungoides and other cutaneous reticuloses. *Bull Cancer (Paris)* 1977; 64:249–258.
16. Hagedorn M, Kiefer G. DNA content of mycosis fungoides cells. *Arch Dermatol Res* 1977; 258:127–134.
17. Lessin SR, Grove GL, Diamond LW, Au FC, Nowell PC, Vonderheid EC. DNA cytophotometry of lymph node touch imprints in cutaneous T-cell lymphoma. *J Invest Dermatol* 1988; 90:425–429.
18. Bibbo M, Bartels PH, Dytch HE, Wied GL. Ploidy measurements by high resolution cytometry. *Anal Quant Cytol Histol* 1985; 7:81–87.
19. Jenkinson G. Image analysis takes off: Recent software developments are taking image analysis into new and uncharted areas of science. *Nature* 1985; 316:87–91.
20. Böhm N. Einfluß der Fixierung und der Säurekonzentration auf die Feulgenhydrolyse bei 28°C. *Histochemie* 1968; 14:201–211.
21. Stolz W, Vogt T, Braun-Falco O *et al.* Differentiation between lymphomas and pseudolymphomas of the skin by computerized DNA image cytometry. *J Invest Dermatol* 1990; 94:254–260.
22. Böcking A, Adler C-P, Common HH, Hilgart M, Granzen B, Auffermann W. Algorithm for a DNA cytophotometric diagnosis and grading of malignancy. *Anal Quant Cytol* 1984; 6:1–8.
23. Sachs L. Angewandte Statistik: Anwendung statistischer Methoden. Berlin: Springer, 1984; 230–240.
24. National Cancer Institute Sponsored Study of Classification of Non-Hodgkin's Lymphomas. Summary and description of a working formulation for clinical usage. *Cancer* 1982; 49:2112–2135.
25. Dörmer P, Abmayr W. Correlation between nuclear morphology and the rate of DNA synthesis in a normal cell line. *J Histochem Cytochem* 1979; 27:188–192.
26. Sörensen FB. Objective histopathologic grading of cutaneous malignant melanomas by stereologic estimation of nuclear volume: Prediction of survival and disease-free period. *Cancer* 1989; 63:1784–1798.
27. Van der Linden JC, Baak JPA, Lindeman J, Hermans J, Meyer CJLM. Prospective evaluation of prognostic value of morphometry in patients with primary breast cancer. *J Clin Pathol* 1987; 40:302–306.
28. Kaudewitz P, Burg G, Smolle J, Braun-Falco O. Immunologische Phänotypisierung kutaner Lymphome. *Hautarzt* 1986; 37:647–655.
29. Haynes FB, Hensley LL, Jegasothy BV. Phenotypic characterization of skin-infiltrating T cells in cutaneous T-cell lymphoma: Comparison with benign cutaneous T-cell infiltrates. *Blood* 1982; 2:463–473.
30. Turbitt ML, Mackie RM. An assessment of the diagnostic value of the monoclonal antibodies Leu 8, OKT 9, OKT 10 and Ki 67 in cutaneous lymphocytic infiltrates. *Br J Dermatol* 1986; 115:151–158.
31. Gerdes J, Lemke H, Baisch H, Wacher HH, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki 67. *J Immunol* 1984; 133:1710–1715.
32. Ralfkiaer E, Saati T, Bosq J, Delsol G, Gatter KC, Mason DY. Immunocytochemical characterisation of cutaneous lymphomas other than mycosis fungoides. *J Clin Pathol* 1986; 39:553–563.
33. Kaudewitz P, Stein H, Burg G. Atypical cells in lymphomatoid papulosis express the Hodgkin cell-associated antigen Ki 1. *J Invest Dermatol* 1986; 86:350–354.
34. Brodell RT, Santa Cruz DJ. Cutaneous pseudolymphomas: Symposium on cutaneous T-cell lymphoma and related disorders. *Dermatol Clin* 1985; 4:719–734.
35. Wain SL, Braylan RC, Borowitz MJ. Correlation of monoclonal antibody phenotyping and cellular DNA content in non-Hodgkin's lymphoma: The Southeastern Cancer Study Group experience. *Cancer* 1987; 60:2403–2411.
36. van Driel-Kulker AMJ, Mesker WE, van Velzen I, Tanke HJ, Feichtinger J, Ploem JS. Preparation of monolayer smears from paraffin-embedded tissue for image cytometry. *Cytometry* 1985; 6:268–272.
37. Vonderheid EC, Fang S-M, Helfrich MK, Abraham SR, Nicolini CA. Biophysical characterization of normal T-lymphocytes and Sézary cells. *J Invest Dermatol* 1981; 76:28–37.
38. Romen W, Rüter A, Saito K, Harms H, Aus HM. Relationship of ploidy and chromatin condensation in the rat liver. *Histochemistry* 1980; 67:249–256.
39. Vogt T. DNA-Zytometrie bei kutanen Lymphomen (Dissertation). Ludwig-Maximilians Universität München, München, Germany (in press).