DNA-Cytophotometry of Lymph Node Touch Imprints in Cutaneous T-Cell Lymphoma

Stuart R. Lessin, M.D., Gary L. Grove, Ph.D., Lawrence W. Diamond, M.D., Francis C. Au, M.D., Peter C. Nowell, M.D., and Eric C. Vonderheid, M.D.

Departments of Dermatology (ECV), Pathology (LWD), and Surgery (FCA), Temple University Health Sciences Center; The Skin Study Center, Simon Greenberg Foundation (GLG); and the Departments of Dermatology (SRL), and Pathology and Laboratory Medicine (PCN), University of Pennsylvania, Philadelphia, Pennsylvania, U.S.A.

Scanning DNA-cytophotometry was performed on touch imprints of 26 lymph nodes (LN) obtained from 25 patients with cutaneous T-cell lymphoma (CTCL), stained by the Feulgen technique, and interpreted without knowledge of histopathologic diagnosis. Four patterns of DNA distribution were identified, but only histograms that demonstrated cells containing nuclei with more than 4C DNA content (hypertetraploidy) reliably distinguished LN involved with CTCL from LN with reactive changes; for example, dermatopathic lymphadenitis. An abnormal DNA histogram with evidence of hypertetraploidy was demonstrated in 9 of 12 LN showing histopathologic evidence of involvement compared with no abnormal histograms in 14 LN without histopathologic involvement. One LN that was diffusely involved with CTCL had a DNA distribution characteristic of a relatively high level of cell proliferation, but without definite hypertetraploidy. Cytogenetic studies on the blood of this patient, who had Sézary syndrome, demonstrated a clone of lymphocytes with a pseudodiploid karyotype without a related polyploid subline. The remaining two histopathologically involved LN had normal DNA histograms; these LN were only focally involved with CTCL. These observations indicate that DNA-cytophotometry correlates well with the histopathologic findings in LN diffusely involved with CTCL, but may be normal in LN with focal involvement or in those that contain cytogenetically abnormal cells with a near-diploid DNA content. *J Invest Dermatol 90:425–429, 1988*

Cutaneous T-cell lymphoma (CTCL), a term that encompasses mycosis fungoides and Sézary syndrome, constitutes a malignant proliferation of thymic-derived lymphocytes that involve the skin initially, but later lymph nodes, blood, and other visceral organs [1,2]. The neoplastic nature of CTCL has been substantiated further by DNA-cytophotometric and cytogenetic studies [3–11]. Clinically, the prognosis of the patient with CTCL and the selection of modalities used to treat the disease are influenced greatly by the extent of the disease; that is, cutaneous versus extracutaneous involvement [12]. Thus, in the staging evaluation, a lymph node (LN) biopsy is recognized to be of major importance in the assessment of a patient with CTCL. Unfortunately, CTCL involvement of LN may be very difficult to distinguish from dermatopathic lymphadenitis and other reactive changes unless there is considerable effacement of nodal architecture by malignant T cells [13–15]. For this reason, investigators have attempted to augment standard histopathologic methods with special studies such as electron microscopy [16], DNA-cytophotometry [13,17], cytogenetic analysis [4,9,10], immunophenotyping [18,19], and molecular probing for T-cell-receptor gene rearrangement [20] in an effort to detect small populations of neoplastic T cells.

Quantitative DNA-cytophotometry is a relatively simple and sensitive technique that takes advantage of the fact that the malignant cells of CTCL often have an abnormal content of DNA. van Vloten and co-workers [5,17] used touch imprint DNA-cytophotometry to study skin and LN specimens from patients with CTCL and presented data showing that an earlier diagnosis was enhanced by this procedure, but their work has not been confirmed elsewhere. In this report, we present evidence that, although touch imprint DNA-cytophotometry is usually abnormal in LN diffusely involved with CTCL, the technique often fails to detect focal involvement or involvement by disease characterized by a lack of marked polyploidy.

MATERIALS AND METHODS

Touch imprints from LN specimens were obtained at the time of initial staging or restaging evaluation of 25 patients (20 males, 5 females, mean age 59 years) with histopathologically confirmed CTCL. The clinical data for these patients are presented in Table I. Ten LNs were acquired during a 6-month period from October 1977 to April 1978, with a portion of each specimen processed concurrently for dual-parameter flow microfluorimetry (FMF) and/or cytogenetic studies using methods described previously [8,9]. The remaining 16 LN were processed for touch imprint DNA-cytophotometry alone during a 1-year period from April 1982 to May 1983. Touch imprints likewise were obtained as controls from 5 patients without lymphomatous disease. Each imprint was prepared on an acid-washed clear glass slide by gently touching

Manuscript received May 1, 1987; accepted for publication November 5, 1987.

This study was supported by a generous donation from Leonard Rosenstein in memory of his father Harry Rosenstein.

Reprint requests to: E. C. Vonderheid, M.D., Skin and Cancer Hospital, 3322 North Broad Street, Philadelphia, PA 19140.

Dr. Diamond is currently at the Department of Pathology, Mesa Lutheran Hospital, Mesa, Arizona.

Abbreviations:
CTCL: cutaneous T-cell lymphoma
FMF: flow microfluorimetry
LN: lymph node

0022-202X/88/$03.50

Table I. Clinical Summaries of Patients With Cutaneous T-Cell Lymphoma (CTCL)[a]

Patient	Age	Race	Sex	Year Onset of Symptoms	Date of Diagnosis	Date of Lymph Node Biopsy	Stage at Last Contact[b]	Status at Last Contact	Observation Time (months)
1	39	W	M	1974	07/76	11/77	IA (TINOBOMO)	D; progression of CTCL	88
2	63	W	M	1954	09/77	02/78	IA (TINOBOMO)	A; CR	106+
3	51	W	M	1972	10/77	02/78	IA (TINOBOMO)	A; CR	106+
4	53	W	M	1955	02/78	04/78	IA (TINOBOMO)	A; CR	98+
5	56	B	F	1954	11/77	01/78	IB (T2NOBOMO)	A; CR	107+
6	58	W	M	1978	05/81	04/82	IB (T2NOBOMO)	A; PR, CTCL stable	50+
7	56	W	M	1974	02/82	04/82	IB (T2NOBOMO)	A; PR, CTCL stable	49+
8	66	W	M	1968	01/81	05/82	IIA (T2N1BOMO)	D; PR, CTCL stable	52
9	57	W	M	1978	04/82	06/82	IIA (T2N1BOMO)	A; CR	52+
10	76	B	M	1980	01/82	06/82	IIA (T2N1BOMO)	A; PR, CTCL stable	54+
11	66	W	M	1980	12/82	01/83	IIA (T2N1BOMO)	A; PR, CTCL stable	41+
12	54	W	M	1963	03/82	04/82	IIB (T3N1BOMO)	A; PR, CTCL stable	52+
13	65	W	M	1968	03/83	03/83	IIB (T3N1BOMO)	A; progression of CTCL	36+
14	67	W	M	1981	08/82	09/82	III (T4N1BOMO)	A; CR	40+
15	50	B	F	1975	07/77	01/78	IVA (T4N3BOMO)	D; CTCL stable	17
16	53	W	M	1968	11/68	01/78	IVA (T4N3B1MO)	D; progression of CTCL	54
17	59	W	M	1976	01/78	02/78	IVA (T3N3BOMO)	D; CR	21
18	82	W	M	1975	04/77	03/78	IVA (T4N3B1MO)	D; progression of CTCL	62
19	69	W	F	1973	09/76	04/78	IVA (T3N3BOMO)	D; progression of CTCL	54
20	73	W	M	1960	09/72	04/80	IVA (T2N3BOMO)	D; progression of CTCL	60
21	67	W	M	1981	03/82	04/82	IVA (T3N2BOMO)	D; progression of CTCL	13
22	48	B	F	1971	05/74	05/82	IVA (T2N2BOMO)	D; progression of CTCL	51
23	52	W	F	1965	05/78	02/83	IVA (T2N3BOMO)	D; progression of CTCL	20
24	46	W	M	1975	12/76	04/83	IVA (T3N3BOMO)	D; progression of CTCL	38
25	51	W	M	1977	10/82	05/83	IVA (T2N3BOMO)	A; PR, CTCL stable	38+

[a] W, white; B, black, M, male; F, female; A, alive; D, dead; CR, complete remission; PR, partial remission.
[b] Staging according to Mycosis Fungoides Cooperative Group criteria [21].

the freshly cut surface of the LN onto the slide. Touch imprints prepared on the first 10 specimens were fixed in 100% methanol for 1 h at room temperature, allowed to air-dry, and stored until Feulgen staining. Touch imprints from the last 16 LN were fixed in a mixture of 100% methanol and glacial acetic acid (3:1 v/v) for 1 h at room temperature and 24 h at 4°C before being air-dried. Use of the methanol–acetic acid solution was adopted as the preferred method of fixation, as use of 100% methanol alone resulted in some broadening of the DNA histograms.

The Feulgen staining procedure was modified from the method of DeCosse and Aiello [22] and utilized a hydrolysis time of 25 min in 5 N HCl at 37°C and a staining time of 2 h in freshly prepared basic fuchsin–Schiff reagent (CI #42510) at room temperature. Specimens were mounted in equal volumes of Permount. All reagents were purchased from Fisher Scientific Company, Philadelphia, Pennsylvania.

A Vickers M-85 scanning-integrating microspectrophotometer interfaced with a Hewlett–Packard 9825A computer was used to obtain cytophotometric data on Feulgen-stained specimens [23]. One hundred lymphocyte nuclei per specimen were scanned randomly at a wavelength of 540 nm. The integrated density per nucleus, which is directly related to the dye uptake per nucleus, gives a measurement of the nuclear DNA content in arbitrary units. Chicken erythrocyte nuclei were fixed, stained, and measured with each specimen to standardize the arbitrary DNA units to a standard reference and to minimize fixation and staining artifacts. The values obtained for each specimen were plotted in a histogram of number of cells versus DNA content (ploidy level).

Paraffin sections of all lymph node specimens were routinely processed and stained with hematoxylin and eosin and reviewed by one of us (LWD) without knowledge of the cytophotometric data.

Statistical analysis was performed using BMDP statistical software (University of California Press, Berkeley, 1983) in a Cyber 170-750 computer (Control Data Corporation). Fisher's exact test was used to compare proportions [24] and the statistic described by Breslow [25] was used to determine the significance of differences in Kaplan–Meier survival curves.

RESULTS

Four distinct histogram patterns were identified by DNA-cytophotometry on LN specimens obtained from patients with CTCL (Fig 1 *A–D*). One pattern (type 1 histogram) consists of a unimodal distribution of cells centered around the 2C (diploid) DNA value in seven specimens from patients with CTCL and in two specimens from controls; this pattern was considered to indicate a quiescent population of diploid cells (Fig 1*A*). The second or type 2 histogram similarly shows a single peak of cells with 2C DNA content plus an additional small number of cells (<5% of the total number of cells) with DNA values ranging from above 2C to 4C (Fig 1*B*); this histogram was observed in eight LNs from patients with CTCL and in two control specimens. Although the possibility that a small component of aneuploid cells with hyperdiploid to tetraploid DNA content within the sample cannot be excluded, the type 2 histogram more likely is the result of a low degree of cellular proliferation of a diploid population of cells. The type 3 histogram consists of a continuous distribution of cells ranging from 2C to 4C, a DNA histogram characteristic of a high proportion of diploid cells undergoing proliferation (Fig 1*C*); this pattern was encountered in two CTCL samples and in one control specimen which was interpreted histologically as florid lymphoid hyperplasia. The final pattern (type 4 histogram) is distinguished from the other patterns by multiple peaks of cells, some with DNA values located beyond 4C (hypertetraploidy). This pattern, which often appeared to be a composite of hyperproliferation and aneuploidy, was observed only in nine samples from patients with CTCL. It was the cytophotometric pattern considered to be clearly abnormal in terms of involvement by CTCL (Fig 1*D*).

The results of DNA-cytophotometry, histopathologic, and cytogenetic studies on patients with CTCL are presented in Table II. Fourteen of the 26 LN showed only reactive changes, usually diagnosed as dermatopathic lymphadenitis, and the remaining 12 specimens showed involvement with CTCL manifested as some degree of effacement of nodal architecture by atypical lymphocytes containing cerebriform nuclei. Of the lymph nodes considered to be

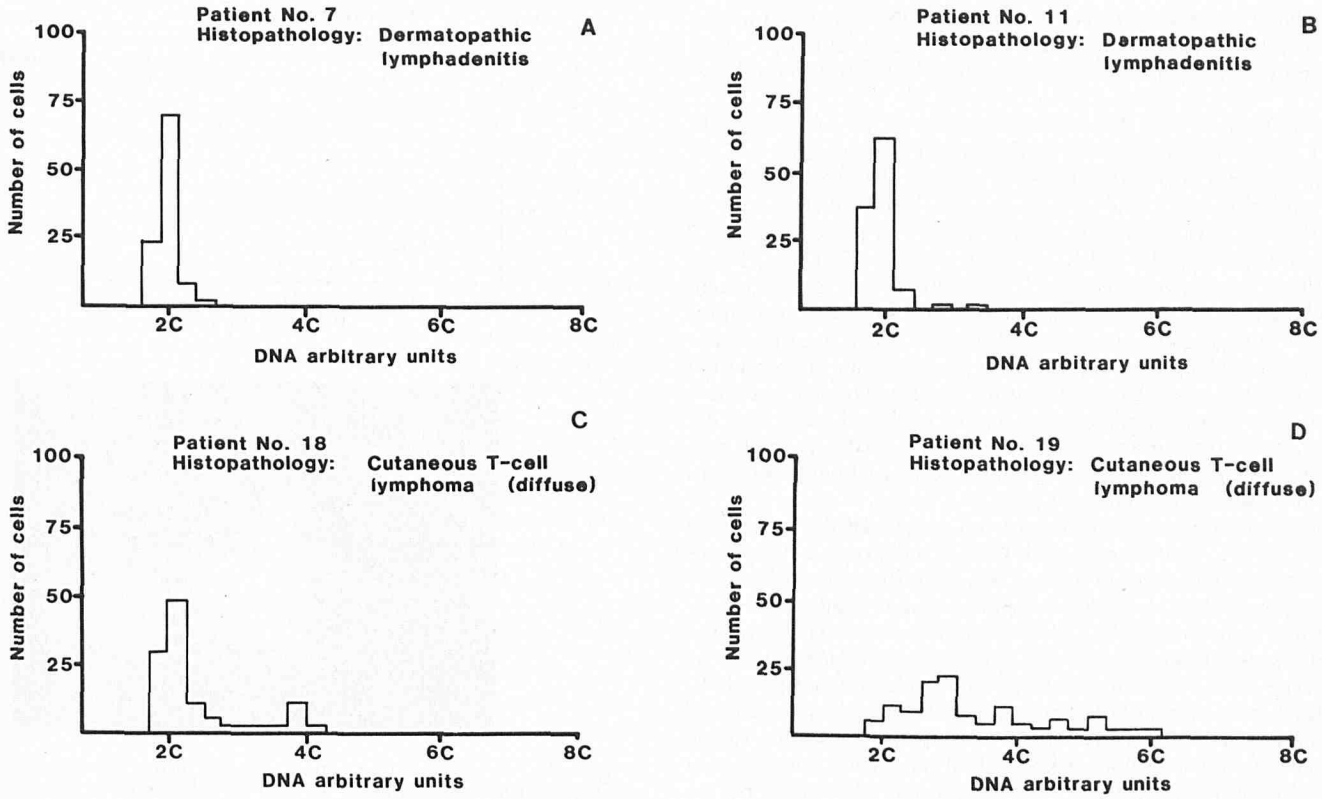

Figure 1. DNA-cytophotometry histograms: number of cells (*y* axis) versus DNA content per cell (*x* axis; 2C = diploid). *(A)* Type 1 histogram—quiescent diploid cell population. *(B)* Type 2 histogram—low degree of proliferation of a diploid cell population. *(C)* Type 3 histogram—high degree of proliferation of a diploid cell population. *(D)* Type 4 histogram—hyperproliferation and high proportion of aneuploidy (hypertetraploidy).

Table II. Results of Studies Performed on Lymph Node Specimens From Patients With Cutaneous T-Cell Lymphoma (CTCL)

Patient	Source of Lymph Node[a]	Cytophotometric Pattern[b]	Histopathologic Diagnosis[a]	Cytogenetic Findings (Source of Specimen)
1	A	1	RH	Not done
2	A	2	DL	Not done
3	I	2	RH	Not done
4	I	1	RH	Not done
5	A	2	DL	Not done
6	I	1	DL	Not done
7	I	1	DL	Not done
8	A	2	DL	Not done
9	I	1	DL	Not done
10	I	1	DL	Not done
11	A	2	DL	Random abnormalities (skin)
12	I	2	DL	Hyperdiploid clone (skin); normal study (lymph node)
13	A	3	DL	Not done
14	A	2	DL	Not done
15A	A	1	CTCL (focal)	Not done
15B	A	4	CTCL (focal)	Not done
16	A	4	CTCL (diffuse)	Pseudodiploid clone with related hypotetraploid subline (blood)
17	I	4	CTCL (diffuse)	Not done
18	I	3	CTCL (diffuse)	Pseudodiploid clone (blood)
19	I	4	CTCL (diffuse)	Polyploidy with suggestion of hypertetraploid clone (skin)
20	A	4	CTCL (focal)	Not done
21	A	2	CTCL (focal)	Pseudodiploid clone with related tetraploid subline (skin)
22	A	4	CTCL (focal)	Random abnormalities (blood)
23	I	4	CTCL (diffuse)	Not done
24	I	4	CTCL (diffuse)	Not done
25	A	4	CTCL (diffuse)	Not done

[a] A, axilla; I, inguinal region; RH, reactive hyperplasia; DL, dermatopathic lymphadenitis.
[b] See Materials and Methods for definition.

uninvolved by histopathologic criteria, DNA-cytophotometry demonstrated a type 1 histogram pattern in six specimens, a type 2 histogram in seven specimens, and a type 3 histogram in one specimen.

By contrast, of the 12 nodes diagnosed to be involved with CTCL, a type 4 histogram was demonstrated in 9 instances, indicating an excellent correlation between positive histopathologic features and this particular cytophotometric pattern ($P < 0.0001$, Fisher's exact test). Two other specimens showed a type 1 and type 2 histogram (patients 15 and 21, respectively) and had focal histopathologic involvement by CTCL with only about 5% effacement of nodal architecture. This "false-negative" result suggests that inadequate sampling may limit the usefulness of touch imprint DNA-cytophotometry in detecting early involvement, an interpretation supported by the fact that in three other focally involved specimens shown to be abnormal by cytophotometry, cells with more than 4C DNA content (hypertetraploidy) constituted a relatively small proportion of the entire lymphocyte sample compared with specimens with more diffuse involvement (Fig 2).

The remaining sample obtained from patient 18 showed diffuse histopathologic involvement with CTCL and a type 3 cytophotometric pattern indicative of marked hyperproliferation without hypertetraploidy (Fig 1*C*). This exceptional patient had overt Sézary syndrome characterized by a chromosomally abnormal clone of T lymphocytes demonstrated in skin, lymph node, and the blood. The karyotype of the abnormal cells in this clone showed pseudodiploidy, that is, a diploid (2C) amount of DNA with chromosomal rearrangements, specifically 46, XY, 2q+, abn 12. There was no evidence of a related subline of polyploid cells. Thus, it seems that the cytophotometric pattern 3 in this patient was caused by the proliferation of a large proportion of the pseudodiploid cells containing a 2C amount of DNA when quiescent. Consequently, DNA-cytophotometry did not discriminate between these abnormal lymphocytes and normal proliferating cells in the absence of associated definite aneuploidy, that is, hypertetraploidy.

Flow microfluorimetry (FMF) was performed concurrently with touch imprint DNA-cytophotometry on nine specimens (nine patients with CTCL, one normal control) with excellent correlation of the results except for one case. For the lymph node specimen from patient 2, FMF showed a fluorescence distribution characteristic of a population of cells exhibiting mild proliferation compared with the single 2C peak (type 1 histrogram) demonstrated by DNA-cytophotometry. Presumably, this discrepancy reflects the difference between sample sizes used in the two techniques. Figure 3 shows the close agreement between FMF and touch imprint DNA-cytophotometry obtained on a lymph node from a patient with Sézary syndrome. The predominant karyotype of the neoplastic clone in this patient was 50, XY, −10, −16, +2D, +4 mar with a related hypotetraploid subline with approximately 84 chromosomes constituting 10%–25% of the metaphases examined.

From a clinical perspective, a significant difference in survival patterns was observed between patients with and without histopathologic involvement ($P < 0.0005$). Of the 11 patients with involved LNs, only one patient has survived to the present time. Conversely, of the 14 patients with uninvolved lymph nodes, 2 patients have died, but in only one case was there clinical evidence of disease progression, which began 6 years after the lymph node biopsy was peformed. The probability of survival for 5 yr was 11% for the former group versus 89% for the latter group. As a type 4 histogram was the only DNA-cytophotometric pattern found to correlate strongly to the histopathologic interpretation, it is apparent that type 4 histograms also have adverse prognostic implications for patients with CTCL; however, DNA-cytophotometry did not seem to provide any additional useful information apart from the histopathologic interpretation for the 11 patients with proven lymph node involvement.

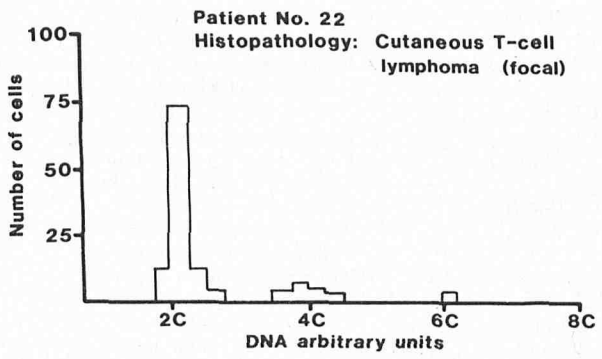

Figure 2. DNA-cytophotometry histogram: Type 4 histogram with hyperproliferation but low proportion of hypertetraploidy.

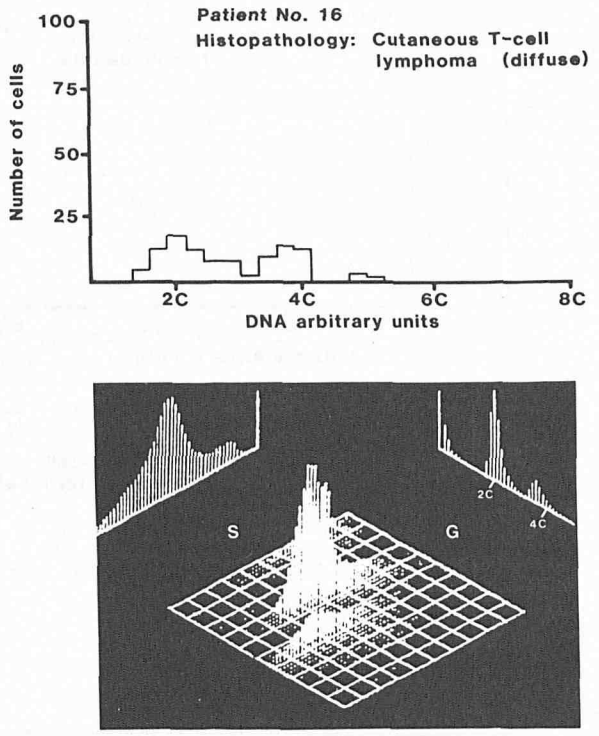

Figure 3. Top panel — DNA-cytophotometry histogram: number of cells (y axis) versus DNA content per cell (x axis). Bottom panel — dual-parameter FMF histogram from patient 16 (500,000 cells counted): number of cells (y axis) versus DNA content per cell (x axis: G = green fluorescence) versus cell size (x axis: S = forward low-angle light scatter). Note the presence of a hypotetraploid cell population detected by both techniques.

DISCUSSION

Our results confirm the previous work of van Vloten and coworkers [13,17] demonstrating an excellent correlation between DNA-cytophotometry and standard histopathology as a way of confirming LN involvement with CTCL. Of 25 LN studied by van Vloten, 10 LN were considered to be histopathologically uninvolved, of which 2 LN (10%) were abnormal by cytophotometric criteria. Of the remaining 15 LN with histologic evidence of involvement, 3 LN (20%) showed a normal DNA histogram. The 10% "false-positive" and 20% "false-negative" results in van Vloten's series compared favorably with the 0% "false-positive" and 25% "false-negative" results in our study.

To be clinically useful, however, DNA-cytophotometry must not only correlate well with the histopathologic interpretation, but also recognize early involvement prior to its demonstration by standard methods. Such a role has been shown for touch imprints of skin biopsy specimens [5], but we found no evidence that this also occurs with touch imprints of LNs because we had no instance of an abnormal DNA histogram on a lymph node interpreted as uninvolved by

histopathology. Indeed, we believe that DNA-cytophotometry actually failed to detect involvement in two specimens with focal involvement from patients with a correspondingly poor clinical course. Increasing the sample size or restricting measurements to morphologically atypical lymphocytes within a sample may improve the specificity of the test by increasing the likelihood of detecting cells with hypertetraploidy, thereby decreasing the number of "false-negatives."

FMF appears to be better suited than touch imprint DNA-cytophotometry in detection of small numbers of aneuploid cells [7,8,11]. FMF can be used to analyze, within a few minutes, 200,000 or more cells for DNA content as well as other parameters, compared with the 100 cells analyzed by scanning cytophotometry in about 1 h. Small numbers of aneuploid cells would more likely be identified by FMF not only in the hypertetraploid (> 4C) range, but also within the 2C–4C range. Caution must be used, however, when discriminating aneuploid cells within the 2C–4C range from normal proliferating cells. This is evident from studies utilizing FMF analysis of benign dermal lymphocytic infiltrates which revealed two hyperdiploid peaks, representative of proliferating and/or activated lymphocytes, present in 36% of the specimens studied [26]. These findings, plus our results indicating that not all examples of CTCL are characterized by alterations in ploidy level (for example, patient 18 in this series), suggest that abnormal T lymphocytes in such cases (with pseudodiploid cells as in the above case and perhaps chromosomally normal cells as well) possibly can be identified on large samples by multiparameter FMF sensitive to variables other than DNA content per cell, such as cell size (light scatter) or chromatin structuring [8].

In summary, the results of touch imprint cytophotometry correlate well with histopathology, especially in diffusely effaced lymph nodes, but do not seem to provide any additional prognostic information; however, on occasion, histopathologically involved lymph nodes may not manifest abnormalities in DNA-cytophotometry because of sampling error and/or the presence of abnormal cells without hypertetraploidy. Therefore, as a diagnostic tool, DNA-cytophotometry is limited, but it still maintains its heuristic value in the study of CTCL and in dermatology. Alternative modalities such as multiparameter FMF and molecular probing for T-lymphocyte clonality [20], which overcome the limitations of DNA-cytophotometry, may provide a greater capacity to detect early involvement of CTCL in LNs.

The authors gratefully acknowledge Dr. Claudio Nicolini for his valuable assistance in FMF analysis. The authors also thank Dorothy Rose and Virginia Steinmetz for preparation of the manuscript and Bill Witmer for preparation of the figures.

REFERENCES

1. Lutzner M, Edelson R, Schein P, Green I, Kirkpatrick C, Ahmed A: Cutaneous T-cell lymphomas: The Sezary syndrome, mycosis fungoides, and related disorders. Ann Intern Med 83:534–552, 1975
2. Sterry W: Mycosis fungoides. Curr Top Pathol 74:167–223, 1985
3. Crossen PE, Mellor JEL, Finley AG, Ravich RBM, Vincent PC, Gunz FW: The Sezary syndrome. Cytogenetic studies and identification of the Sezary cell as an abnormal lymphocyte. Am J Med 50:24–34, 1971
4. Erkman-Balis B, Rappaport H: Cytogenetic studies in mycosis fungoides. Cancer 34:626–633, 1974
5. van Vloten WA, Schaberg A, van der Ploeg M: Cytophotometric studies on mycosis fungoides and other cutaneous reticuloses. Bull Cancer 64:249–258, 1977
6. Hagedorn M, Kiefer G: DNA content of mycosis fungoides cells. Arch Dermatol Res 258:127, 1977
7. Bunn PA Jr, Whang-Peng J, Corney DN, Schlam ML, Knutsen T, Gazdar AF: DNA content analysis by flow cytometry and cytogenetic analysis in mycosis fungoides and Sezary syndrome: Diagnostic and prognostic implications. J Clin Invest, 65:1440–1448, 1980
8. Vonderheid EC, Fang SM, Helfrich MK, Abraham SR, Nicolini CA: Biophysical characterization of normal T-lymphocytes and Sezary syndrome. J Invest Dermatol 76:28–37, 1981
9. Nowell PC, Finan JB, Vonderheid EC: Clonal characteristics of cutaneous T-cell lymphomas: Cytogenetic evidence from blood, lymph nodes, and skin. J Invest Dermatol 78:69–75, 1982
10. Whang-Peng J, Bunn PA Jr, Knutsen T, Matthews MJ, Schechter G, Minna JD: Clinical implications of cytogenetic studies in cutaneous T-cell lymphoma (CTCL). Cancer 50:1539–1553, 1982
11. Lange Wantzin G, Larsen JK, Christenson J, Ralfkiaer E, Thomsen K: Early diagnosis of cutaneous T-cell lymphoma by DNA flow cytometry on skin biopsies. Cancer 54:1348–1352, 1984
12. Vonderheid EC, Micaily B: Treatment of cutaneous T-cell lymphoma. Dermatol Clin 3:673–687, 1985
13. Scheffer E, Meijer CJLM, von Vloten WA: Dermatopathic lymphoadenopathy and lymph node involvement in mycosis fungoides. Cancer 45:137–148, 1980
14. Colby TV, Burke JS, Hoppe RT: Lymph node biopsy in mycosis fungoides. Cancer 47:351–359, 1981
15. Sausville EA, Worsham GF, Matthews J, Makuch RW, Fischmann AB, Schechter GP, Gazdar AF, Bunn PA Jr: Histologic assessment of lymph nodes in mycosis fungoides/Sezary syndrome (cutaneous T-cell lymphoma): Clinical correlations and prognostic import of a new classification system. Hum Pathol 16:1098–1109, 1985
16. Guccion JG, Fishmann AB, Bunn PA Jr, Schechter GP, Patterson RH, Matthews MJ: Ultrastructural appearance of cutaneous T-cell lymphomas in skin, lymph nodes, and peripheral blood. Cancer Treat Rep 63:565–570, 1979
17. van Vloten WA, Scheffer E, Meijer CJLM: DNA-Cytophotometry of lymph nodes imprints from patients with mycosis fungoides. J Invest Dermatol 73:275–277, 1979
18. Weiss LM, Wood GS, Warnke RA: Immunophenotypic differences between dermatopathic lymphadenopathy and lymph node involvement in mycosis fungoides. Am J Pathol 120:179–185, 1985
19. Burke JS, Sheibani K, Rappaport H: Dermatopathic lymphadenopathy. An immunophenotypic comparison of cases associated and unassociated with mycosis fungoides. Am J Pathol 123:256–263, 1986
20. Weiss LM, Hu E, Wood GS, Moulds C, Cleary ML, Warnke R, Sklar J: Clonal rearrangements of T-cell receptor genes in mycosis fungoides and dermatopathic lymphadenopathy. N Engl J Med 313:539–544, 1985
21. Bunn PA Jr, Lamberg SL: Report of the committee on staging and classification of cutaneous T-cell lymphomas. Cancer Treat Rep 63:725–728, 1979
22. DeCosse JJ, Aiello NJ: Feulgen hydrolysis: Effect of acid and temperature. J Histochem Cytochem 14:601–606, 1966
23. Grove GL, Lavker RM, Kligman AM: Use of microspectrophotometry in dermatological investigations. J Soc Cosmet Chem 29:537–544, 1978
24. Siegel SS: Nonparametric Statistics for the Behavior Sciences. New York, McGraw–Hill, 1956
25. Breslow N. A generalized Kruskal–Wallis test for comparing K samples subject to unequal patterns of censorship. Biometrika 57:579–594, 1970
26. Lange Wantzin G, Larsen JK, Christensen IJ, Ralfkiaer E, Tjalve M, Lund Kofoed M, Thomsen K. Activated and cycling lymphocytes in benign lymphocytic infiltrates including psoriatic skin lesions. Arch Dermatol Res 278:92–96, 1985