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Circulating Sézary Cells in the Diagnosis of Sézary Syndrome (Quantitative and Morphometric Analyses)

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Plastic-embedded circulating Sézary cells were examined in semithin and thin sections (assisted by the nuclear contour index-NCI). Eight cases of Sézary syndrome were analyzed as well as 11 controls (3 cases of widespread eczemas and 8 cases of erythroderma), 7 cases of mycosis fungoides, and 3 healthy individuals. Discriminating criteria between Sézary syndrome and benign diseases were sought: in addition to Sézary cells (NCI > 6.5) intermediate lymphocytes (5.0 < NCI ≤ 6.5) proved to be helpful. Cases with Sézary syndrome were clearly differentiated when the following 3 ultrastructural criteria were fulfilled: (1) Sézary cells (SC) > 9%; (2) intermediate lymphocytes (IL) > 20%; (3) the sum of SC and IL > 37%.

A good correlation between thin and semithin sections was obtained (correlation coefficient for Sézary cells $r = 0.82$). Usually the values of SC were slightly higher on thin sections. The diagnosis of SS can be made on semithin sections when the ultrastructural criteria are fulfilled. In this way 8 of 12 samples of Sézary syndrome were correctly classified. Therefore, semithin sections (studied by light microscopy) are recommended as a routine method in the diagnosis of cases suspected of Sézary syndrome, whereas thin sections (studied by electron microscopy) appeared to be necessary in problem cases only.

In addition to erythroderma and lymphadenopathy, an increased percentage of circulating Sézary cells is a prerequisite for the diagnosis of Sézary syndrome (SS). Sézary cells (SC) are atypical lymphocytes usually with T-cell membrane properties [1–8], ultrastructurally characterized by cerebriform nuclei [9,10]. They are found not only in the blood of patients with SS, but also occasionally in mycosis fungoides [6,11–15], in a variety of benign diseases, mainly erythrodermas of atopic dermatitis, contact dermatitis and psoriasis [15,16], and even in healthy individuals [15,17–19].

However, the results of quantitative analyses of these cells are not always consistent with each other. This may be due to 4 different methods used for the determination of these cells: (1) blood smears (Pappenheim or Wright-Giemsa stain); (2) semithin sections of plastic-embedded mononuclear blood cells (analyzed by light microscopy (LM); (3) thin sections of plastic-embedded mononuclear blood cells (analyzed by electron microscopy (EM); (4) thin sections assisted by morphometric analyses ("nuclear contour index"—NCI) [17,20].

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Abbreviations:
EM: electron microscopy
IL: intermediate lymphocyte(s)
LM: light microscopy
NCI: nuclear contour index
NL: normal lymphocyte(s)
SC: Sézary cell(s)
SS: Sézary syndrome

Myrie, Zucker-Franklin, and Ramsey [21] found blood smears to be unreliable, whereas semithin and thin sections of plastic-embedded lymphoid cells were more conclusive. Even better results appear possible through application of morphometry and particularly through the determination of the NCI [15,17,20].

The clinical significance of quantification of circulating SC consists in the possible early diagnosis of SS and its differentiation from other benign erythrodermas. In addition "Sézary-cytemia" appears to be a prognostically unfavorable sign in mycosis fungoides [15,21].

However, little is known as to the reliability of SC as a discriminating factor between SS and benign diseases and which methods are necessary for routine purposes. We will describe our own investigations using the most sensitive method (thin sections assisted by morphometric analyses) to determine circulating SC in SS and benign diseases and to examine their discriminating power. Furthermore, we studied the question of whether thin sections are always necessary to the diagnosis of SS. It will be shown that semithin sections are usually, but not always, sufficient.

MATERIALS AND METHODS

The examinations were carried out in 8 cases of SS in 12 samples [4 samples, each 3 months apart, in 1 patient (FGI–FG-IV), and 2 samples in another (LK-I, LK-II)], 7 cases of mycosis fungoides (in different stages), 10 cases of benign skin diseases (7 cases of erythroderma, 3 cases with widespread eczema), 1 case of angioimmunoblastic lymphadenopathy (with erythroderma), and 3 healthy individuals. The classification of patients with SS is found in Table I.

Cell Preparation

Circulating mononuclear cells were isolated from some heparinized blood by Ficoll-Isopaque (d = 1.007 g/ml) centrifugation for 20 min and 500 *g*. After fixation in 1.5% glutaraldehyde and 1% osmium tetroxide they were embedded in Epon 812. Semithin sections were stained with methylene blue azur II and thin sections poststained with uranyl acetate and lead citrate.

Evaluation of Thin Sections

In each sample at least 100, usually 150, lymphoid cells were photographed (magnification × 4025) in a Zeiss EM 9 electron microscope. The nuclear area and circumference were determined semiautomatically (Kontron MOP AM 03); from these data the NCI of each cell was calculated. The NCI is the ratio of nuclear circumference to the square root of the nuclear area [17,20]. The minimum value for the NCI will be reached by a circle: area = $r^2\ \pi$; circumference = $2\ r\ \pi$; NCI = $\frac{2r\pi}{\sqrt{r^2\ \pi}} = \frac{2\ \pi}{\sqrt{\pi}} = 2\ \sqrt{\pi} \approx 3.54$.

Lymphoid cells with a NCI > 6.5 were defined as SC (Fig 1) [15]. The remaining lymphoid cells were further differentiated into 2 groups: first, normal lymphocytes (NL) (Fig 2) with a NCI ≤ 5.0 (normally found in healthy individuals—see Table II) and, second, intermediate lymphocytes (IL) (Fig 3) with a 5.0 < NCI ≤ 6.5. From each sample a histogram of the NCI values was established (in Fig 4 typical histograms of a case of SS and of a benign erythroderma are shown) and the percentages of the 3 types of lymphoid cells were determined. These results, along with the absolute counts of peripheral lymphocytes, allowed us to calculate the absolute SC counts per mm^3. Furthermore, in each sample the highest NCI was recorded and the 25th and 70th percentiles of the NCI distribution were determined.

TABLE I. *Clinical data and staging of the patients with Sézary syndrome and benign skin diseases at the time of blood cell investigation*

Patients (initials)	Sex	age	staging[a] / Diagnosis
Sézary syndrome			
GS	F	69	IV
GH	M	79	III
GK	M	66	IV
LK-I[b]	F	60	III
LK-II	—	—	III
FG-I[b]	M	73	III
FG-II	—	—	III
FG-III	—	—	III
FG-IV	—	—	III
BM	F	73	II
JS	F	77	III
LL	F	78	IV
Benign eczemas and erythrodermas			
FB	M	62	Parapsoriasis en plaques
WJ	M	75	Parapsoriasis en plaques
CB	F	23	Nummular eczema
OR	F	57	Contact dermatitis (erythroderma)
GG	M	37	Sebostatic eczema (erythroderma)
EO	M	39	Atopic dermatitis (erythroderma)
HD	M	64	Contact dermatitis (erythroderma)
EB	M	75	Contact dermatitis (erythroderma)
RW	M	36	Nummular eczema (erythroderma)
FH	M	49	Pseudo "Sézary syndrome" (erythroderma)
ME	F	50	Angioimmunoblastic lymphadenopathy (erythroderma)

[a] Stage I = Sézary syndrome (SS) limited to the skin; stage II = SS with lymphadenopathy (clinically enlarged lymph nodes); stage III = SS with specific lymph node involvement; stage IV = SS with visceral involvement.

[b] Sequential analyses in intervals of 3 months.

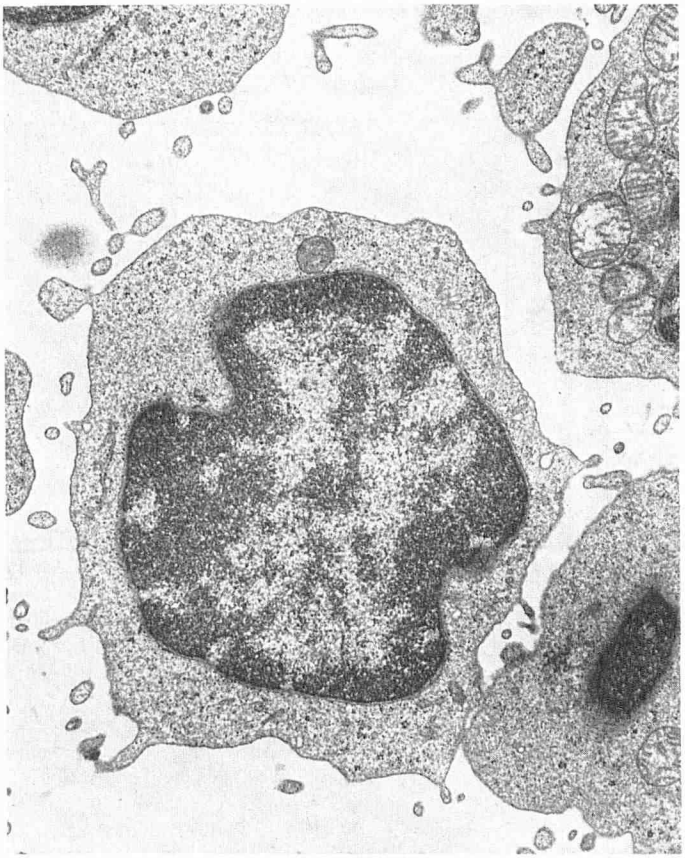

FIG 2. Normal lymphocyte characterized by a nucleus with a smooth outline (NCI = 4.0). Thin section, × 12,600.

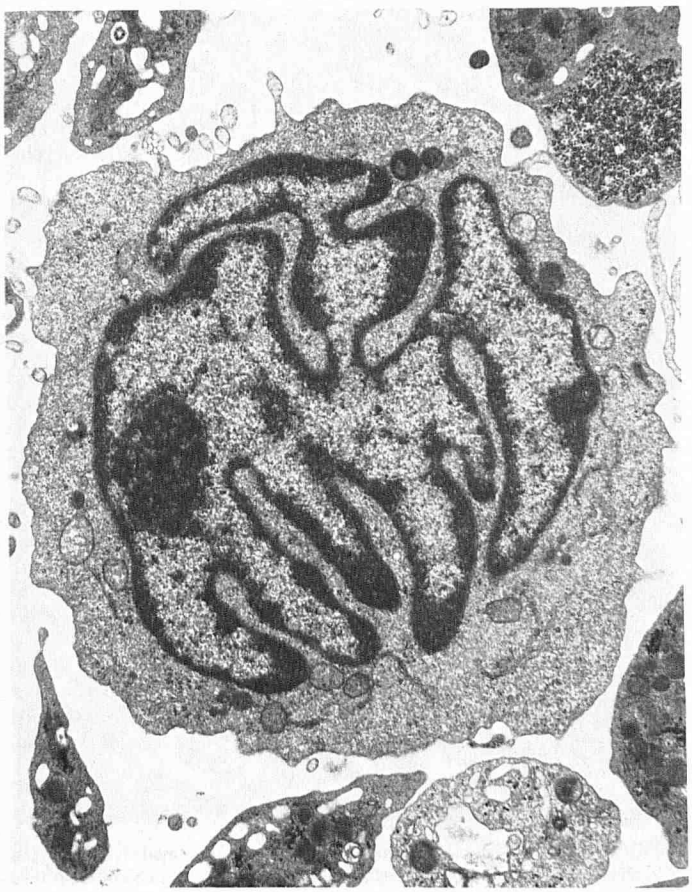

FIG 1. Sézary cell characterized by its cerebriform nucleus (NCI = 11.2) Thin section, × 12,600.

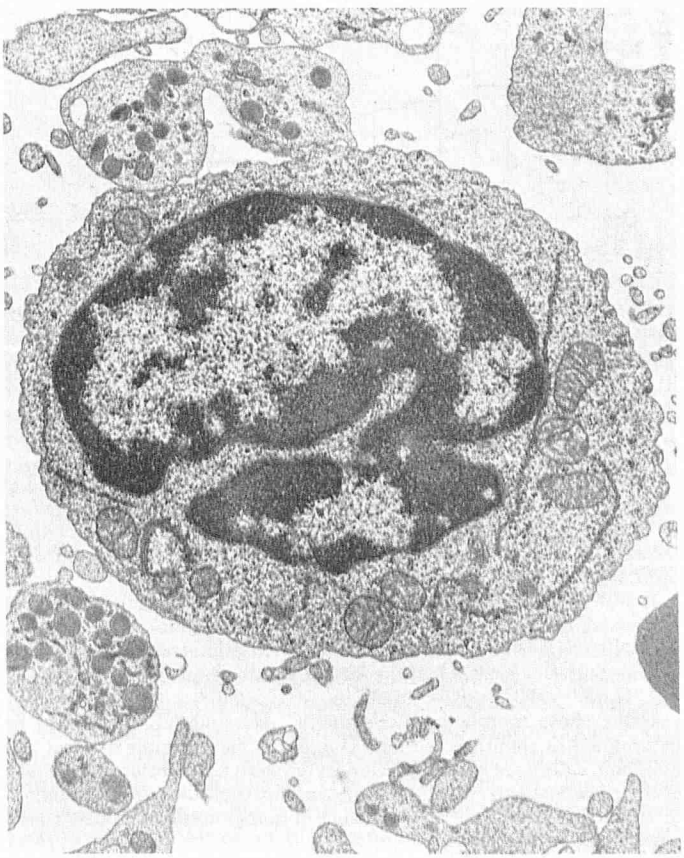

FIG 3. Intermediate lymphocyte, characterized by a moderately indented nucleus (NCI = 5.9). Thin section, × 12,600.

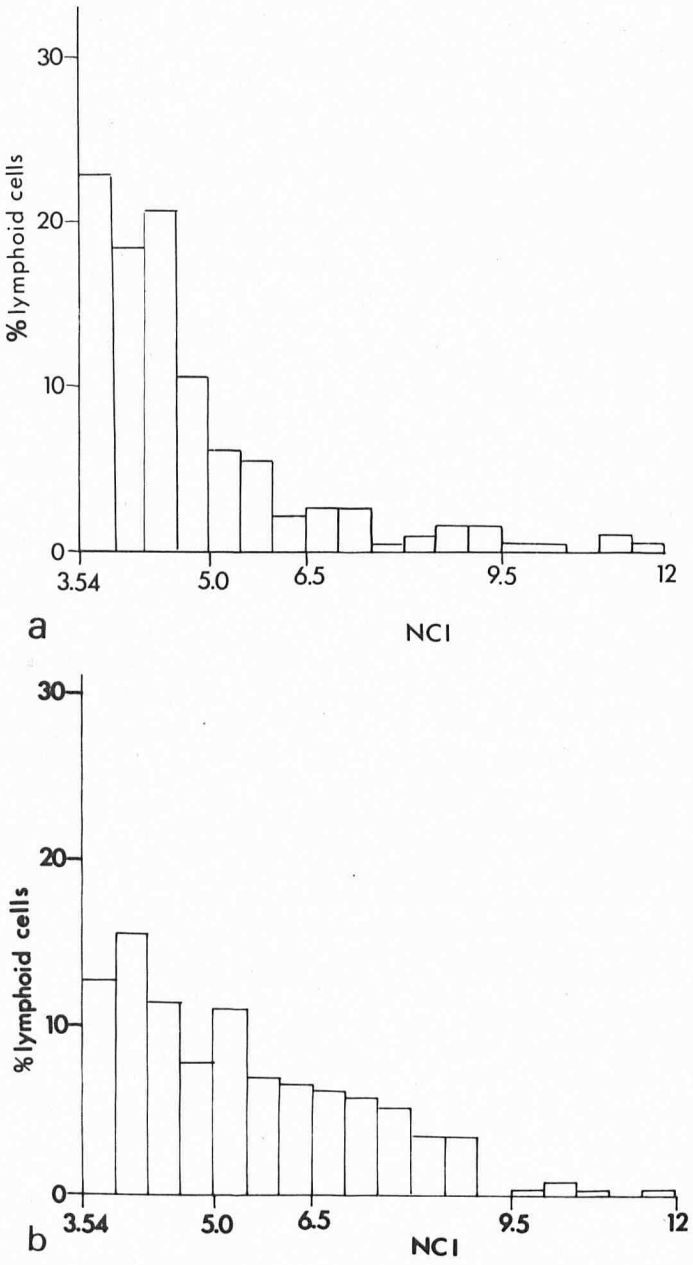

FIG 4. Histograms of 2 typical cases: contact dermatitis with erythroderma (*a*) and Sézary syndrome (*b*). A higher number of IL and SC is noted in *b*, whereas the highest values of the NCI do not appear to be a discriminating criterion.

Evaluation of Semithin Sections

Since the NCI is not applicable to semithin sections, criteria had to be determined allowing LM classification of lymphoid cells with regard to their degree of nuclear indentations. The following correlations between the NCI and nuclear indentations on EM were found on a pilot study:

1. SC (NCI > 6.5): nuclei with several indentations, the sum of which measures at least 1.5 of the largest diameter.
2. NL (NCI ≤ 5.0): nuclei with few indentations, the sum of which measures at most 0.5 of the largest diameter.
3. IL (5.0 < NCI ≤ 6.5): all others.

Using these morphologic criteria SC, IL, and NL could also be determined in semithin sections (Fig 5). In each sample at least 300 lymphoid cells were analyzed. Monocytes were differentiated by means of their low nuclear/cytoplasma ratio and by their blunt nuclear indentations. Ultrastructurally the presence of numerous dense bodies allows a more secure recognition.

RESULTS

The percentages of the IL and SC obtained through the analyses of semithin and thin sections are shown in Table II. The absolute SC counts per mm^3 and the highest NCI of each sample are also given. The percentages of SC among all lymphoid cells (absolute SC counts in parentheses) obtained through NCI-assisted thin section observations ranged from 12.1–57.8% (500–3900/mm^3) in cases of SS, 0.8–28.5% (10–1200/mm^3) in cases of mycosis fungoides, 1–13.4% (30–300/mm^3) in benign skin diseases, 3.2% (1000/mm^3) in a case of angioimmunoblastic lymphadenopathy, and only ≤ 1.2% in healthy individuals. Taking into account the variations of SC counts, and the lack of a clear-cut interval between benign and malignant cases, an additional criterion, the percentages of IL, was evaluated. Fig 6 depicts a scatter diagram showing the percentages of SC and IL: a differentiation of SS from benign cases including the angioimmunoblastic lymphadenopathy was possible when the following 3 criteria were fulfilled:

1. SC > 9%;
2. IL > 20%
3. The sum of SC and IL > 37%

Benign eczemas and erythrodermas could also be differentiated from the cases of SS by means of absolute SC counts. None of the benign cases (with the exception of the angioimmunoblastic lymphadenopathy) reached more than 300 SC/mm^3, whereas all cases with SS had SC counts higher than 500 SC/mm^3. The highest NCI was not found to be a good discriminating factor, because a NCI ≥ 11.5 was found in only 7 out of 12 samples of SS but also in 3 of 10 benign cases.

The 70th percentile (25th percentile in parentheses) ranged

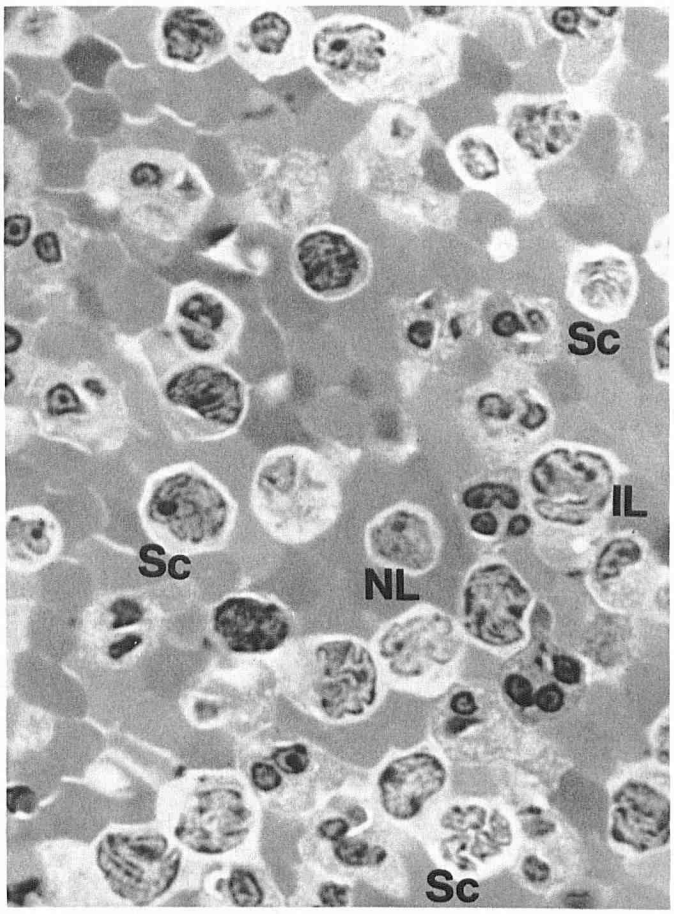

FIG 5. Semithin section showing normal peripheral lymphocytes (*NL*), intermediate lymphocytes (*IL*), and Sézary cells (*Sc*). × 1700.

TABLE II. *Percentages of Sézary cells (SC) and intermediate lymphocytes (IL) among the lymphoid cells obtained in semithin and thin sections. The percentage of normal lymphocytes = 100% − IL − SC*

Patients (initials)	Percentage IL		Percentage SC		SC/mm³	Highest NCI value
	EM[a]	LM[b]	EM	LM		
Sézary syndrome						
GS	36	34	12.1	10	2100	9.8
GH	42.5	37	21.9	13	800	9.9
GK	27.5	22	13	11	600	9
LK-I[c]	33.2	26	18.8	8	3900	12
LK-II	28.1	25	19.8	9	3000	9.4
FG-I[c]	25	22	26.8	21	700	11.7
FG-II	24.2	21	23.3	25	500	12.1
FG-III	26	27	38.6	12	600	12.9
FG-IV	23.2	28	19.6	10	600	12.2
BM	34.2	32	31.7	12.5	2900	11
JS	25	34	57.8	27	2500	12.5
LL	36.8	26	27.7	10	1700	12.6
Range	23.2–42.5		12.1–57.8		500–3900	9–12.9
Mycosis fungoides						
HW	7.6	17	0.8	4	20	6.6
JZ	10.6	11	3.8	1	70	7.5
GP	15.2	10	8.9	3	200	8.7
FU	12.9	21	28.5	6	1200	13
JE	19.7	25	5.7	5	n.t.[d]	8
AG	13.7	20	1.6	4	10	7.8
FP	29.8	26	17.7	11	400	13.3
Range	7.6–29.8		10.8–28.5		10–1200	6.6–13.3
Benign eczemas and erythrodermas						
FB	22	16	11.5	2	300	12.9
WJ	6	15	1	2	30	7.5
CB	11.5	12	4.2	2.5	n.t.	9
RW	8.7	6	1.7	1	50	6.9
OR	14	13	13.4	7	200	13
HD	24	24	1.9	3	60	9.3
EB	20.5	9	7.4	3	100	10.5
GG	14.7	11	4.7	2	200	10.8
FH	21.7	19	8.8	5	300	9
EO	26	21	8.3	10	300	12.4
ME	43.9	42	3.2	6	1000	8.8
Range	6–43.9		1–13.4		30–1000	6.9–13
Healthy individuals						
FR	2.7	8	0.9	1	n.t.	7.5
CS	8.5	6	1.2	1	n.t.	7.5
WS	12.7	8	1.2	1	n.t.	7.5
Range	2.7–12.7		0.9–1.2			

[a] EM: results obtained in thin sections by electron microscopy.
[b] LM: results obtained in semithin sections by light microscopy.
[c] Sequential analyses in intervals of 3 months.
[d] n.t. = not tested.

from 5.5–8.2 (3.9–5.4) in cases of SS, 4.2–5.2 (3.7–4.1) in benign skin diseases, 5.5 (4.1) in the angioimmunoblastic lymphadenopathy, and 4.0–4.4 (3.7–3.8) in healthy individuals. With the exception of the case of angioimmunoblastic lymphadenopathy all benign eczemas and erythrodermas could also be differentiated from the cases of SS by the means of the 70th percentile.

The correlation coefficients between thin and semithin sections were found to be: for normal lymphocytes $r = 0.89$, for intermediate lymphocytes $r = 0.86$, and for Sézary cells $r = 0.82$ (Fig 7); this indicates a relatively high correlation between LM and EM findings. Even though good correlations between semithin and thin sections were obtained, the values of SC counts were usually equal to or lower on semithin sections compared with thin sections (in only 8 samples higher values with a maximum difference of 3.2% were seen). By and large, the same holds true for the IL.

DISCUSSION

Elevated circulating SC are, in addition to erythroderma, the dominant criterion in the diagnosis of SS. The results of studies of SC in cases of SS and benign diseases published in the literature are given in Table III (only studies using semithin and thin sections were considered). The lowermost percentage of SC still compatible with the diagnosis of SS is not well known. This constituted the first object in the present study. For this reason the 8 cases of SS seen in our institution during the past 10 years were analyzed. The second aim of this study regards the distinction between SS and other benign erythrodermas. Since in benign diseases higher values of SC can be seen than in some cases of SS (as shown in Table III), we were interested in whether or not a distinction is possible through the analyses of the degree of nuclear indentations in circulating lymphoid cells. The third purpose of this study was a comparison of semithin and thin sections to determine whether LM alone might be sufficient in the diagnosis of SS. Semithin sections were described earlier as a simple and useful diagnostic method for cutaneous lesions [24] and blood cells [21] in T-cell lymphomas. Usually lower counts of circulating SC were seen in our cases compared to findings in the literature (Table III). This may be due to a generally earlier stage of the disease. Duncan and Winkelmann [16] found 1000 SC/mm³ to be necessary for establishing the diagnosis of SS; however, these results were obtained in blood smears. Other authors [25,26]

indicated 10% peripheral SC to be the lower limit in SS. In contrast, we observed cases with only 500 SC/mm^3 and with less than 10% SC among the white blood cells.

A good distinction between SS and benign diseases was possible when, in addition to SC, levels of IL were considered. The following 3 ultrastructural criteria were fulfilled in all 12 samples of SS, but in none of the benign cases: (1) SC > 9%; (2) IL > 20%; (3) the sum of SC and IL > 37%.

As to the comparison of semithin and thin sections, the former were found to be sufficient in this study. However, this needs to be differentiated: usually the percentages of SC were lower in semithin sections compared to thin sections. This may be due to narrow nuclear indentations not showing in semithin sections and to difficulties in distinguishing monocytes and SC. Since the SC values can be higher on thin sections, SS can be excluded only through EM. In contrast, this diagnosis can be established through LM when the ultrastructural criteria are fulfilled; an ultrastructural investigation then becomes unnecessary, because the following ultrastructural investigation would usually yield equal or higher values. Applying this procedure in our study, 8 out of 12 samples were correctly classified on semithin sections. In the remaining 4 samples, EM would have been necessary.

Determining the absolute SC counts proved to be not necessary in this study, since the results using percentages of SC and IL among the lymphoid cells were equally sufficient.

Myrie et al [21], comparing levels of SC in semithin and thin sections, found an even better correlation ($r = 0.98$ in 34 cases). The prior elimination of monocytes may have been a cause. However, they did not study cases with benign erythroderma nor did they use morphometry (NCI).

Van der Loo et al [15] found a NCI ≥ 11.5 to be specific for circulating SC in cases of mycosis fungoides. We found higher values even in 3 of 10 benign cases, but these were rare findings. In another study Van der Loo et al [27] also used the 25th and 70th percentiles as discriminating factors between cutaneous lesions of benign eczemas and T-cell lymphoma and proved their ability in undiagnosed cases of suspected cutaneous T-cell lymphoma. However in this study the use of the 25th and 70th percentiles did not improve the results. Furthermore, the

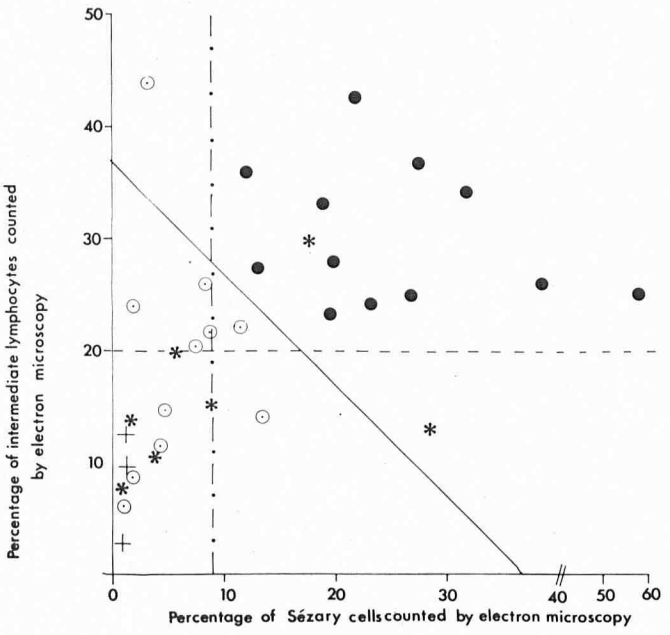

FIG 6. Scatter diagram showing percentages of Sézary cells and intermediate lymphocytes determined by electron microscopy (● = Sézary syndrome, ⊙ = benign skin disease, * = mycosis fungoides, + = healthy individual). A distinction can be made between cases of Sézary syndrome and benign skin diseases by means of 3 criteria: (1) SC >9% (— · —), (2) IL >20% (— — —), (3) the sum of SC and IL >37% (——).

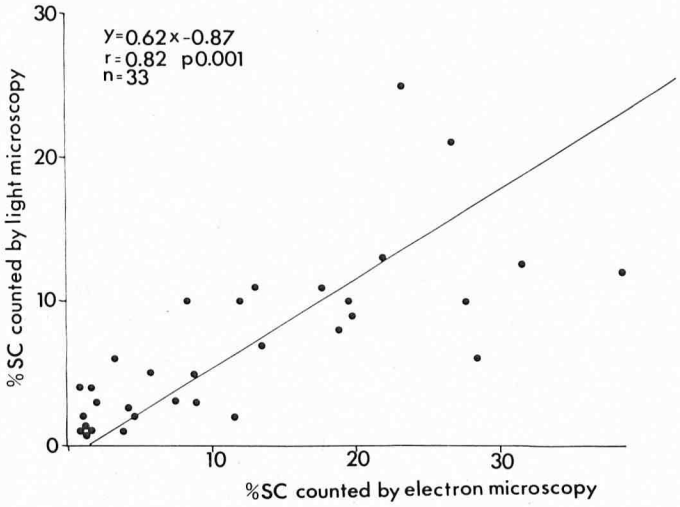

FIG 7. Correlation between semithin and thin sections of Sézary cells. The correlation coefficient is 0.82; this indicates a good correlation. In most of the cases the values were lower in semithin sections.

TABLE III. *Circulating Sézary cells (SC) in Sézary syndrome (SS) and benign diseases reported in the literature (only studies using semithin or thin sections are listed)*

Reference	Method[a]	Diagnosis	No. of cases	SC/mm^3	%SC/L[b]
[6] (1974)	EM	SS	8	60–23,000	2–72
[22] (1975)	EM	SS	5	7,500–118,000	78–90
[21] (1980)	EM	SS	8	n.t.[c]	9–95
[17] (1974)	NCI	SS	2	n.t.	~40–70
[23] (1979)	SD	Actinic reticuloid	1	860	30
[15] (1981)	NCI	Atopic dermatitis (erythroderma)	1	225	15
[15] (1981)	NCI	Psoriasis (erythroderma)	2	90–100	10–16
[19] (1981)	SD	Healthy donors	4	n.t.	4
[19] (1981)	NCI	Healthy donors	4	n.t.	6–7
[18] (1977)	EM	Healthy donors	6	n.t.	3.2–13.3
[18] (1977)	EM	Human cord blood	5	n.t.	3.8–9.6
[17] (1974)	NCI	Healthy donors	1	n.t.	~2
[15] (1981)	NCI	Healthy donors	5	n.t.	2–8

[a] Method: SD, investigations with light microscopy on semithin sections; EM, investigations with electron microscopy on thin sections; NCI, nuclear contour index-assisted analyses of thin sections.

[b] %SC/L = percentage of Sézary cells among all lymphoid blood cells.

[c] n.t. = not tested.

NCI cannot be determined on semithin sections and therefore percentiles cannot be calculated.

Summarizing the results, we recommend the use of semithin sections in the determination of circulating SC in cases suspected to have SS. Furthermore, this technique constitutes a staging procedure in mycosis fungoides as more than 20% SC were found to be an adverse prognostic sign. Thin sections are necessary for borderline cases only, when higher values of SC cannot be excluded by LM.

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