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ORIGINAL ARTICLE

# Comparative Analysis of the Expression of Cell Adhesion Molecules in Cutaneous T-Cell Lymphomas (Mycosis Fungoides/Sézary Syndrome) and Inflammatory Skin Diseases

**I. López-Lerma,* M.T. Estrach**

*Hospital Clinic de Barcelona, Servicio de Dermatología, Barcelona, Spain*

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**KEYWORDS**
Cutaneous T-cell lymphoma;
Mycosis fungoides;
Cell adhesion molecules;
Sézary syndrome

**Abstract**
*Introduction:* Cell adhesion molecules play a pivotal role in the establishment of T-cell populations in the skin. In this study, we quantify the expression of cell adhesion molecules in patients with cutaneous T-cell lymphoma (CTCL) and compare it with the expression found in other skin diseases.
*Material and methods:* Frozen material was obtained from 42 patients in 5 different groups: early CTCL, comprising patients with patch- and plaque-stage mycosis fungoides (n=11); advanced CTCL (n=7), comprising patients with mycosis fungoides (n=3) and Sézary syndrome (n=4); inflammatory skin disease (n=12), comprising patients with psoriasis (n=9) and atopic dermatitis (n=3); chronic skin diseases with persistent plaques that do not fulfil the histological criteria for mycosis fungoides (pre-CTCL) (n=8); and healthy volunteers (n=4). Expression of the following cell adhesion molecules was analyzed: lymphocyte function-associated antigen 1, intercellular adhesion molecule 1 (ICAM-1), ICAM-3, cutaneous lymphocyte-associated antigen, E-selectin, very late antigen 4, vascular cell adhesion molecule 1, $\alpha E\beta 7$ integrin, and E-cadherin.
*Results:* The immunohistochemical analyses used here revealed statistically significant differences between CTCL and other skin diseases but not between early and advanced CTCL. The expression of $\alpha E\beta 7$ integrin and ICAM-3 in the epidermis per high-power field (400× magnification) allowed the different groups to be distinguished from each other, except for advanced CTCL and pre-CTCL. There were statistically significant differences between advanced CTCL and pre-CTCL in terms of the expression of E-selectin at 400× magnification and the expression of ICAM-1 in a honeycomb pattern in epidermal keratinocytes.
*Conclusions:* The expression of cell adhesion molecules involved in the adhesion and migration of lymphocytes in the skin does not differ significantly between initial and advanced stages of CTCL.


*Corresponding author.
*E-mail address:* ilopez@aedv.es (I. López-Lerma).

**PALABRAS CLAVE**
Linfoma cutáneo de células T;
Micosis fungoide;
Moléculas de adhesión celular;
Síndrome de Sézary

## Análisis comparativo de la expresión de moléculas de adhesión celular en linfomas cutáneos (micosis fungoide/síndrome de Sézary) y dermatosis inflamatorias mediadas por células T

### Resumen

*Introducción y objetivos:* Las moléculas de adhesión celular (MAC) desempeñan un papel fundamental en la localización de los linfocitos T en la piel. El presente estudio cuantifica la expresión de MAC en pacientes con linfoma cutáneo de células T (LCCT) y lo compara con la observada en otras dermatosis.

*Material y métodos:* Se dispuso de material congelado de 42 pacientes distribuidos en 5 grupos: LCCT iniciales, micosis fungoide en fase de mácula y placa (11 pacientes); LCCT avanzados (n = 7): micosis fungoide y síndrome de Sézary; enfermedades cutáneas inflamatorias (n = 12): psoriasis y dermatitis atópica; dermatosis crónicas persistentes en placas que no cumplen criterios histológicos de micosis fungoide (pre-LCCT) (n = 8) y pacientes sanos (n = 4). Se ha estudiado la expresión de los antígenos LFA-1, ICAM-1, ICAM-3, CLA, selectina E, VLA-4, VCAM-1, $\alpha$E $\beta$7 y cadherina E.

*Resultados:* Los parámetros de inmunotinción valorados no han permitido demostrar diferencias estadísticamente significativas entre los grupos LCCT inicial y avanzado, pero sí con respecto a otras dermatosis. La expresión de CD103 e ICAM-3 por campo epidérmico de 400× puede emplearse como variable para distinguir entre los distintos subgrupos estudiados, salvo en el caso de la comparación entre LCCT y pre-LCCT. Estos dos últimos grupos presentan diferencias estadísticamente significativas en la observación de la expresión de selectina E a 400× y la determinación del patrón en forma de panal de ICAM-1 en los queratinocitos epidérmicos.

*Conclusión:* La expresión de MAC que participan en los fenómenos de adhesión y trasmigración de los linfocitos en la piel no difiere significativamente entre las fases inicial y avanzada de los LCCT.



## Introduction and Objectives

Recent years have seen significant advances in our understanding of cell migration towards sites of inflammation or tumors. Insights into the cellular interactions underlying this process have been made possible by the identification of the cytokines and a group of molecules that, when expressed constitutively or inducibly on the cell surface, mediate cell recognition and cell-cell or cell-extracellular matrix adhesion. These molecules are referred to as cell adhesion molecules and they play a central role in the cell migration and differentiation events associated with both physiological and pathological processes, such as embryogenesis, the immune response, and metastatic spread in cancer.[1,2]

Cell adhesion molecules are classified on the basis of sequence and structural homology into various families, including selectins, integrins, cadherins, and the immunoglobulin superfamily. In the last 20 years or so, the tissue distribution, structure, function, and ligands of these molecules have been described in detail.[1,3]

The role of cell adhesion molecules in the migration of cells from the circulation to different functional compartments of the immune system (eg, the lymph nodes) is of particular interest. This process, in which lymphocytes migrate to predetermined sites according to the expression of specific receptors is known as homing. It involves a cascade of selective binding events and can be classified into 4 phases: rolling, activation, firm adhesion, and migration.[4]

The skin functions as a barrier between the body and its environment, and it plays a role in immune surveillance. Regulated lymphocyte trafficking is essential for the control and integration of systemic immune responses. The interaction between lymphocytes and the endothelium is a key element in immune regulation that controls access of specialized subgroups of lymphocytes to certain tissues and influences the nature of the immune or local inflammatory response.[5] There is a subgroup of memory T cells that can migrate preferentially to the skin and are identified through their expression of a marker called cutaneous lymphocyte antigen (CLA). These cells are produced following stimulation of lymphocytes in skin-draining lymph nodes and they are recruited during inflammation.[6] Furthermore, in addition to their role in the immune response to external insults, these cells have been linked to the pathogenesis of cutaneous T-cell lymphoma (CTCL) and graft-versus-host disease following allogeneic bone marrow transplant, as well as many inflammatory skin diseases, such as allergic contact dermatitis, psoriasis, atopic dermatitis, and lichen planus.[7]

CLA initiates the process of T-cell extravasation from the blood to the skin. E-selectin, the endothelial ligand for CLA, is constitutively expressed at low levels in the

skin microvessels and its expression increases during inflammation. Although the interaction between E-selectin and CLA is necessary for T-cell extravasation, the T cells must also be activated by chemokines. Furthermore, firm adhesion between the T cells and the endothelium is required to complete their migration through the vessel wall, and this process requires integrins and other cell adhesion molecules. Binding of chemokines to specific receptors on the T cells modifies the structure of integrin $\alpha L\beta 2$, or lymphocyte function-associated antigen-1 (LFA-1), and integrin $\alpha 4\beta 1$, or very late antigen-4 (VLA-4), allowing them to bind intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), respectively. Once extravasation is complete, the cells can respond to chemotactic gradients. In addition, the release of cytokines by the T cells can lead to modification and expansion of the inflammatory infiltrate.[2]

The cell adhesion molecules that regulate normal lymphocyte trafficking are also present on malignant lymphocytes.[8] Thus, the mechanisms underlying the preferential migration of neoplastic cells in cutaneous lymphomas could be similar to those responsible for the selective migration of lymphocytes under normal conditions.

CTCL constitutes a heterogeneous group of diseases including mycosis fungoides, the most common form of CTCL, and Sézary syndrome.[9] The neoplastic cells usually display a $CD4^+$ T-helper phenotype, but their biological behavior is different. Whereas mycosis fungoides is considered a low-grade lymphoma, Sézary syndrome is an aggressive CTCL.

Classic mycosis fungoides develops sequentially in a number of phases over the course of years. In the early phases of the process, with lesions in the form of macules and plaques, epidermal migration of $CD4^+$ cells, either individually or in groups (Pautrier microabscesses), is observed in a process known as epidermotropism. It has an indolent course for many years in which it may affect multiple areas of the skin and even become generalized in the form of erythroderma. Progression of the disease usually involves the development of tumors and the loss of epidermotropism, coinciding with an aggressive and advanced clinical course that can include spread to the peripheral blood, lymph nodes, bone marrow, and other visceral organs. Establishing a definitive diagnosis, however, can be difficult in the initial phases of the disease because some characteristics are shared with benign inflammatory diseases and there may be insufficient evidence to support diagnosis.[10]

The inflammatory cells involved in benign T-cell–mediated inflammatory skin diseases and the neoplastic cells in CTCL employ common adhesion mechanisms in their interaction with other cells and with the extracellular matrix. However, the variability in the cytokine milieu observed in these diseases and the characteristics of the neoplastic cells themselves (capacity for spread and invasion) may result in qualitative or quantitative differences in the expression of cell adhesion molecules both in the lymphocytes and in the tissues with which they interact. In order to investigate these potential differences in the expression of cell adhesion molecules, we analyzed the expression of LFA-1, ICAM-1, ICAM-3, CLA, E-selectin, VLA-4, VCAM-1, integrin $\alpha E\beta 7$, and E-cadherin in patients with early and advanced CTCL lesions, inflammatory disease, and chronic, nonspecific skin disease.

## Material and Methods

The study included 42 patients who attended the Department of Dermatology at Hospital Clínic de Barcelona, Spain between June 1, 2002 and December 31, 2003.

Diagnosis was based on clinical and histologic parameters. Clinical assessments, skin biopsies, laboratory tests, and staging procedures were undertaken according to the diagnosis. None of the patients were receiving topical or systemic treatment at the time of sample collection and none had received prior systemic treatment for their skin disease.

The patients were divided into 4 clinical groups: 1) initial CTCL (n=11), which corresponded to macular or plaque phase, stage IA or IB mycosis fungoides (6 patients with T1 and 5 with T2); 2) advanced CTCL (n=7), corresponding to tumor phase mycosis fungoides (stage IIB) in 1 patient, erythrodermic mycosis fungoides (stage III) in 2 patients, and Sézary syndrome in 4 patients; 3) inflammatory skin disease (n=12), corresponding to 9 patients with psoriasis and 3 with atopic dermatitis; and 4) skin diseases that could develop into CTCL (pre-CTCL, n=8), corresponding to 4 patients with parapsoriasis and 4 with nonspecific chronic dermatitis.

The pre-CTCL group included patients with chronic, persistent dermatitis in the form of erythematous and often desquamative plaques that, although clinically suggestive of mycosis fungoides, did not meet the histological criteria for disease.[10] Furthermore, no disease triggers (evidence of allergic reaction or connective tissue disease) were identified. The pathology study was compatible with parapsoriasis or chronic nonspecific dermatitis.

Four skin samples taken from the borders of excised benign lesions were used as controls. Informed consent was obtained from all participants to be included in the study, which was approved by the hospital's institutional review board.

Punch biopsy samples (4 mm) were obtained. One fragment was fixed in formaldehyde for processing and conventional histology and another was placed in saline and frozen by immersion in methyl butane at –80°C in a Hurcoa Ezco freezer (Forma Scientific) prior to processing for immunohistochemistry.

The frozen tissue was embedded in optimal-cutting-temperature compound (Tissue-Tek, Sakura). Frozen sections (5 µm) were obtained using a cryostat (Reichberg-Yung 2500 Frigocut) at −26°C. The sections were stored at −80°C prior to use.

Prior to immunohistochemistry, frozen sections were fixed in acetone for 10 minutes at 4°C and dried at room temperature. The sections were then rehydrated in phosphate-buffered saline (PBS) for 10 minutes, immersed in peroxidase-blocking solution (DAKO) for 7 minutes to block endogenous peroxidase activity, and finally washed several times in PBS (5 minutes each).

Antigens were detected with the antibodies listed in Table 1 using the EnVision kit (DAKO diagnósticos, S.A, Barcelona, Spain), and the enzymatic reaction was detected with 3-amino-9-ethylcarbazole as a chromogenic substrate. The sections were counterstained with aqueous hematoxylin and mounted in Aquatex (Merck).

Antibody concentrations were optimized to obtain maximal specific staining and minimal nonspecific or background staining. The appropriate concentration was determined by testing different dilutions on frozen sections of lesions from inflammatory skin diseases.

In each skin biopsy, antibody labeling of a range of different cells was analyzed. Serial sections were evaluated by 2 independent investigators. The results were assessed on the basis of cell morphology, and staining intensity was classified semiquantitatively. When expression was observed in dermal infiltrates, the percentage of positive cells was also estimated. Intraepidermal lymphocytes were also analyzed by optical microscopy (Olympus BH-2). Microscopic fields (magnification, ×400) were quantified across the full thickness of the epidermis and the mean number of immunopositive cells in a field was recorded for each of the preparations.

The immunohistochemistry study was complemented by staining with Ulex europaeus I lectin (DAKO), a glycoprotein that binds terminal fucosyl groups on oligosaccharides. This agglutinin labels most vascular endothelial cells in all sizes of vessel; it also labels epidermal keratinocytes and skin appendages. This allowed the number of vessels present in the sample to be determined and comparisons to be made between the number and localization of vessels expressing different cell adhesion molecules in serial sections. The number of stained vascular structures was assessed per microscopic field (×400).

## Statistical Analysis

The results were stored in a database and analyzed using the Statistical Package for the Social Sciences SPSS 9.0 (SPSS Inc, Chicago, Illinois, USA). Groups of qualitative variables were compared by $\chi^2$ test using contingency tables. Ordinal and quantitative variables were analyzed using the nonparametric Kruskal-Wallis test to compare the 5 groups together, followed by the Mann-Whitney test if statistically significant results were obtained. A cutoff of $P < .05$ was set for statistical significance.

## Results

The age of the patients and the time since onset of disease are shown in Table 2. The ratio of women to men was as follows: initial CTCL, 2:9; advanced CTCL, 3:4; inflammatory skin disease, 9:3; pre-CTCL, 3:5; and healthy control subjects, 2:2. The mean (SD) body surface area affected in patients with inflammatory disease was 30.8% (19.65%), with a range of 3% to 60%. Two patients (4.3% of the total) died during follow-up (mean duration, 11 [3.6] months). Both patients were from the advanced CTCL group (accounting for 28.6% of the patients with this diagnosis).

**Table 1** Primary Antibodies Used in the Study

| Primary Antibody | | Source | | Dilution |
|---|---|---|---|---|
| Anti-human ICAM-1 | Mouse IgG 1 | Bender | CD54 | 1:50 |
| Anti-human ICAM-3 | Mouse IgG 1 | Bender | CD50 | 1:150 |
| Anti-human VCAM-1 | Mouse IgG 1 | Bender | CD106 | 1:50 |
| Anti-human E-selectin | Mouse IgG 2a | Bender | CD62E | 1:150 |
| Anti-human E-cadherin | Mouse IgG 1 | Biogenex | | Prediluted |
| Anti-human LFA-1 (α chain) | Mouse IgG 1 | Immunotech | CD11a | 1:40 |
| Anti-human CLA | Rat IgM | PharMingen | | 1:150 |
| Anti-human VLA-4 | Mouse IgG 1 | Immunotech | CD49d | 1:100 |
| Anti-human integrin αEβ7 | Mouse IgG 2a | Immunotech | CD103 | 1:150 |

Abbreviations: CLA, cutaneous lymphocyte-associated antigen; ICAM, intercellular cell adhesion molecule; Ig, immunoglobulin; LFA, lymphocyte function-associated antigen; VCAM, vascular cell adhesion molecule; VLA, very late antigen.

**Table 2**

| | No. of Patients | Age, y[a] | Time Since Diagnosis, mo[a] |
|---|---|---|---|
| Initial CTCL | 11 | 58.91 (20.16) | 111.18 (91.11) |
| Advanced CTCL | 7 | 65.86 (12.50) | 49.43 (54.84) |
| *Inflammatory skin disease* | 12 | 46.50 (20.54) | 139.00 (126.21) |
| Pre-CTCL | 8 | 63.50 (12.19) | 112.75 (109.74) |

Abbreviation: CTCL, cutaneous T-cell lymphoma.

[a]Data are shown as means (SD).

## Expression of Adhesion Molecules in Skin Biopsies

### ICAM-3 (CD50) (Figure 1)

In healthy skin, ICAM-3 immunolabeling was observed in dendritic cells (Langerhans cells) in the epidermis and in cells belonging to the monocyte lineage in the dermis. The percentage of the total number of cells varied (mean, 15.25%). Endothelial cells were completely negative and in the follicles expression was observed in dendritic cells.

In diseased skin, intense anti-ICAM-3 labeling was observed in dendritic cells and in epidermotropic lymphocytic infiltrates. Immunopositive monocyte-lineage cells accounted for a large proportion of the total number of cells in dermal infiltrates. Expression was observed in isolated dendritic cells in the dermis and in cells located in the perivascular region. The intensity of ICAM-3 expression in lymphocytes infiltrating the skin was similar in different diseases.

The number of ICAM-3-positive intraepidermal lymphocytes was independently assessed per linear epidermal field (magnification, ×400) along with the percentage of ICAM-3-positive cells in the dermal infiltrate. Table 3 shows the mean (SD) numbers of lymphocytes observed and Table 4 shows the values for significant results obtained in comparisons between the different groups.

Statistically significant differences were observed between the groups for both the numbers of intraepidermal lymphocytes ($P$=.005) and the percentage of dermal cells ($P$=.027). These differences were present between each of the different disease categories and healthy skin. In the case of ICAM-3-positive epidermal lymphocytes, differences were also observed between inflammatory diseases and both initial CTCL and pre-CTCL. The largest numbers of immunopositive epidermal lymphocytes were observed in initial CTCL, followed by advanced CTCL and pre-CTCL. The mean percentage of ICAM-3$^+$ cells in dermal infiltrates was greatest in the inflammatory skin diseases.

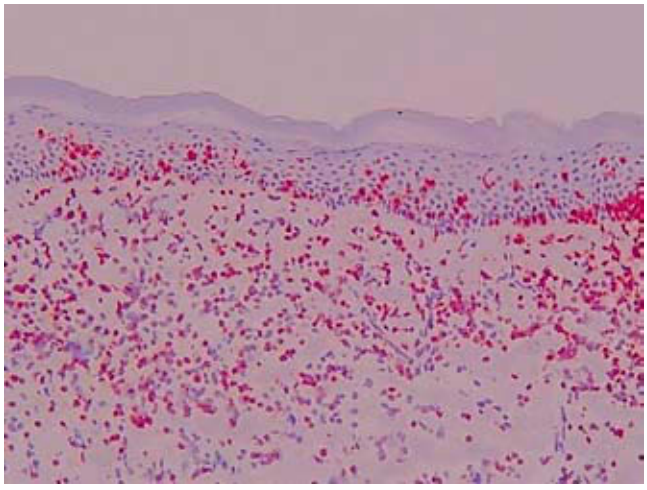

**Figure 1** Intense expression of intercellular adhesion molecule 3 in a tumor infiltrate. Skin biopsy, frozen section (original magnification, ×40).

### ICAM-1 (CD54) (Figure 2)

Analysis of ICAM-1 expression was complicated by the presence of various types of immunopositive cells. Constitutive expression was limited to the endothelium in healthy skin. In contrast, variable expression was observed in the keratinocytes in all other clinical groups. In 1 section, a microabscess positive for ICAM-1 was observed. In the dermis, labeling was observed in the blood vessels and some elongated cells, as well as in monocyte-lineage cells.

The expression of ICAM-1 was quantified separately: 1) localization of ICAM-1 immunopositivity in the epidermis (absence of staining, lower third, lower two-thirds, or entire epidermis); 2) honeycomb pattern of anti-ICAM-1 staining (absence of honeycomb pattern, +, ++, and +++); 3) percentage of ICAM-1$^+$ cells in the dermal infiltrate; and 4) expression of ICAM-1 in the blood vessels (+, ++, +++).

The epidermal labeling of the keratinocytes adopted a honeycomb pattern that could be focal, isolated, isolated multifocal, or diffuse epidermal and cover varying degrees of the epidermis. No significant differences were found between these patterns (data not shown). Nevertheless, statistically significant differences were found upon analysis of the extent of honeycomb anti-ICAM-1 staining ($P$=.01). Specifically, significant differences were found for advanced CTCL and pre-CTCL compared with healthy skin and between inflammatory skin disease and pre-CTCL (Figure 3).

Loss of expression in patients with Sézary syndrome was not observed in this study, but there was more honeycomb staining classified as +++ in the pre-CTCL and initial CTCL groups (12.5% and 18.2% of cases, respectively).

The mean percentage of lymphoid cells in the dermal infiltrate that expressed ICAM-1 was lower than the percentage observed for ICAM-3. Also, although the expression of ICAM-1 appeared stronger in inflammatory skin disease and advanced CTCL than in healthy skin, the difference was not statistically significant.

The expression of ICAM-1 in blood vessels (+, ++, +++) showed statistically significant differences between the groups ($P$=.016); these differences occurred between initial CTCL and inflammatory disease, and between inflammatory disease or pre-CTCL and healthy skin. The level of expression was highest in inflammatory diseases, with 91.7% of those patients having expression classified as +++ (Figure 4).

### LFA-1

In healthy skin, expression of LFA-1 was observed in the few lymphocytes found in the perivascular region and around skin appendages. In samples from diseased skin, variable staining of intraepidermal lymphocytes and some cells with a dendritic appearance was observed. LFA-1 expression in the dermis was observed in infiltrating tumor cells and cells in perivascular areas.

The presence of immunopositive lymphoid cells in the epidermis and in dermal infiltrates was independently assessed.

Although no differences were observed in the percentage of positive cells observed in the dermal infiltrates of the

**Table 3** Staining in Lymphocytes

| | Positive cells/epidermal field (magnification, ×400) | | Dermis, % | |
|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** |
| ICAM-3+ | | | | |
| Initial CTCL | 10.91 | 11.87 | 60.91 | 21.54 |
| Advanced CTCL | 6.57 | 5.71 | 68.57 | 27.19 |
| Inflammatory skin disease | 2.33 | 3.58 | 72.5 | 18.15 |
| Pre-CTCL | 4.38 | 3.07 | 67.5 | 24.93 |
| Healthy skin | 0 | 0 | 15.25 | 17.04 |
| Total | 5.45 | 7.64 | 62.4 | 26.38 |
| | *P*=.005 | | *P*=.027 | |
| ICAM-1+ | | | | |
| Initial CTCL | | | 30.91 | 24.17 |
| Advanced CTCL | | | 37.14 | 33.4 |
| Inflammatory skin disease | | | 38.75 | 18.36 |
| Pre-CTCL | | | 29.63 | 23.93 |
| Healthy skin | | | 5 | 0 |
| Total | | | 31.48 | 24.17 |
| | | | *P*=NS | |
| LFA-1+ | | | | |
| Initial CTCL | 15.27 | 15.54 | 59.27 | 27.89 |
| Advanced CTCL | 19.29 | 31.79 | 69.86 | 30.25 |
| Inflammatory skin disease | 3.92 | 4.12 | 69.17 | 15.64 |
| Pre-CTCL | 5.63 | 4.44 | 58.13 | 33.16 |
| Healthy skin | 0 | 0 | 35 | 28.87 |
| Total | 9.4 | 16.15 | 61.33 | 27.19 |
| | *P*=.014 | | *P*=NS | |
| CLA+ | | | | |
| Initial CTCL | 8.45 | 10.79 | 40.45 | 30.78 |
| Advanced CTCL | 7.71 | 6.9 | 29.29 | 24.23 |
| Inflammatory skin disease | 2.08 | 1.98 | 15.08 | 11.37 |
| Pre-CTCL | 2.63 | 3.02 | 23.57 | 21.16 |
| Healthy skin | 0 | 0 | 1 | 0 |
| Total | 4.71 | 7 | 24.98 | 24.17 |
| | *P*=NS | | *P*=.035 | |
| VLA4+ | | | | |
| Initial CTCL | 2.1 | 5.04 | 33.18 | 30.76 |
| Advanced CTCL | 3.2 | 3.42 | 55.83 | 32 |
| Inflammatory skin diseases | 2.25 | 3.08 | 59.58 | 29.81 |
| Pre-CTCL | 6.38 | 9.2 | 40 | 26.05 |
| Healthy skin | 0 | 0 | 5 | 4.08 |
| Total | 2.95 | 5.41 | 42.8 | 31.76 |
| | *P*=NS | | *P*=.007 | |
| CD103+ | | | | |
| Initial CTCL | 3.91 | 4.16 | 8.36 | 14.73 |
| Advanced CTCL | 8.14 | 6.36 | 12.29 | 14.17 |
| Inflammatory skin disease | 8.83 | 4.55 | 25 | 20 |
| Pre-CTCL | 3.88 | 4.52 | 19.38 | 19.54 |
| Healthy skin | 0 | 0 | 0.5 | 1 |
| Total | 5.64 | 5.27 | 15.12 | 17.93 |
| | *P*=.008 | | *P*=.012 | |

Abbreviations: CLA, cutaneous lymphocyte antigen; CTCL, cutaneous T-cell lymphoma; ICAM, intercellular adhesion molecule; LFA, lymphocyte function-associated antigen; NS, not significant; VLA, very late antigen.

**Table 4** Comparative Analysis of Cell Adhesion Molecule Expression in the Skin[a]

| | Initial CTCL/ Inflammatory Disease | Initial CTCL/ Healthy Control | Advanced CTCL/ Healthy Control | Advanced CTCL/ Pre-CTCL | Inflammatory Disease/ Pre-CTCL | Inflammatory Disease/ Healthy Control | Pre-CTCL/ Healthy Control |
|---|---|---|---|---|---|---|---|
| ICAM-3 epidermis | .008 | .008 | .039 | | .034 | .037 | .005 |
| ICAM-3 dermis | | .01 | .014 | | | .005 | .01 |
| ICAM-1 honeycomb pattern | | | .037 | .004 | | | .012 |
| ICAM-1 vascular | .007 | | | | | .001 | .047 |
| LFA-1 epidermis | .047 | .008 | .017 | | | .022 | .014 |
| E-selectin (×400) | | .004 | .007 | .024 | | .004 | .007 |
| CLA dermis | | .024 | .014 | | | .047 | .014 |
| VLA-4 dermis | .033 | .01 | .013 | | | .003 | .016 |
| E-cadherin | .014 | | | | | .005 | |
| CD103 epidermis | .024 | .015 | .017 | | .036 | .007 | .029 |
| CD103 dermis | .016 | | .034 | | | .008 | .019 |

Abbreviations: CLA, cutaneous lymphocyte antigen; ICAM, intercellular adhesion molecule; LFA, lymphocyte function-associated antigen; VLA, very late antigen.

[a]Data are shown as *P* values for statistically significant differences (Mann-Whitney test).

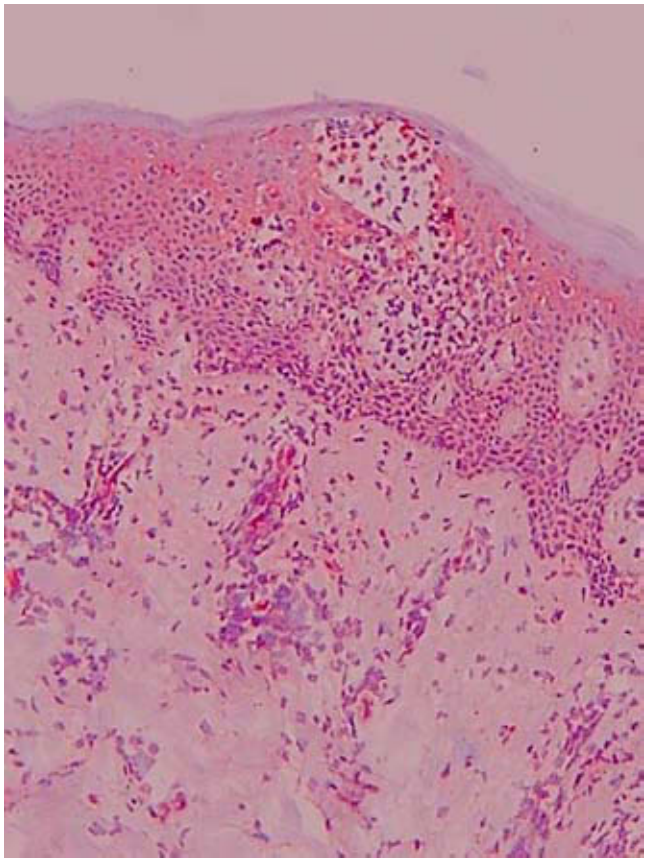

**Figure 2** Labeling of cells in dermal and epidermal infiltrates, keratinocytes, and vascular endothelial cells following immunohistochemistry for intercellular adhesion molecule 1. Skin biopsy, frozen section (original magnification, ×100).

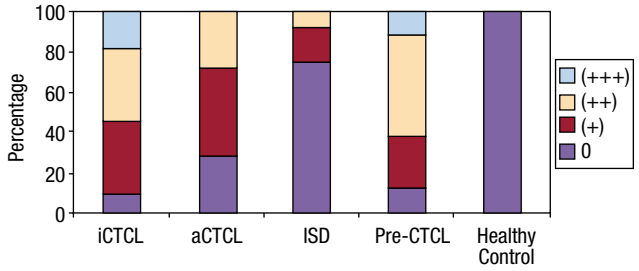


**Figure 3** Epidermal expression of intercellular adhesion molecule 1 in a honeycomb pattern in the different groups (*P*=.01). CTCL indicates cutaneous T-cell lymphoma; aCTCL, advanced CTCL; iCTCL, initial CTCL; ISD, inflammatory skin disease.

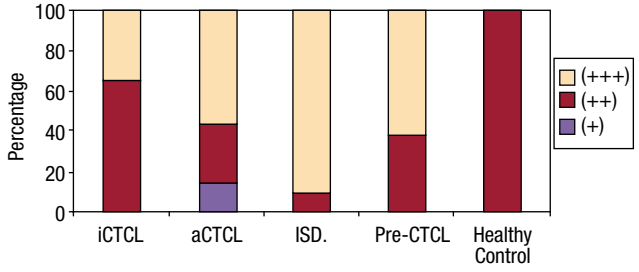


**Figure 4** Expression level of intercellular adhesion molecule 1 in the endothelium (*P*=.016). CTCL indicates cutaneous T-cell lymphoma; aCTCL, advanced CTCL; iCTCL, initial CTCL; ISD, inflammatory skin disease.

different groups, there were differences in the number of intraepidermal lymphocytes (*P*=.014). Specifically, significant differences were observed for initial and advanced CTCL, pre-CTCL, and inflammatory disease compared with healthy skin, and between initial CTCL and inflammatory skin disease. Nevertheless, no differences were observed in the percentage of cells expressing LFA-1 in early and late stages of CTCL.

## E-selectin

Only endothelial staining was observed for E-selectin in the samples analyzed. The number of E-selectin-positive blood vessels (magnification, ×400) was analyzed (*P*=.06). Statistically significant differences were observed between

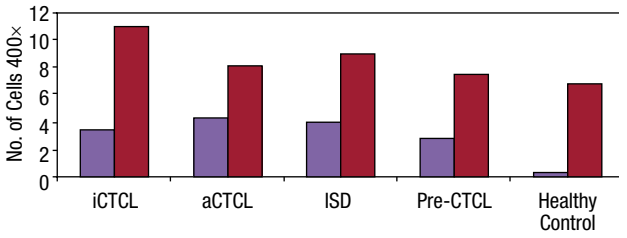


**Figure 5** Endothelial expression of E-selectin and Ulex lectin staining (number of cells per field at ×400 magnification). CTCL indicates cutaneous T-cell lymphoma; aCTCL, advanced CTCL; iCTCL, initial CTCL; ISD, inflammatory skin disease.

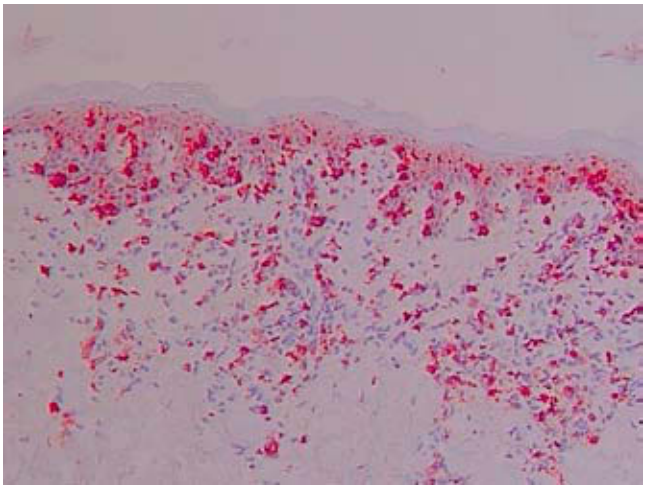

**Figure 6** Intense immunostaining of dermal and epidermal lymphoid infiltrates and of Langerhans cells with antibodies against cutaneous lymphocyte antigen. Skin biopsy, frozen section (original magnification, ×40).

all groups and healthy controls and between advanced CTCL and inflammatory skin diseases (mean of 4.29 and 3.91 cells, respectively). E-selectin was expressed in less than 50% of endothelial cells labeled with Ulex lectin, and expression was even lower in healthy skin (Figure 5).

## CLA (Figure 6)

In healthy skin, CLA expression was observed in epidermal Langerhans cells, dermal macrophages, and some cells around the skin appendages. In the other groups, expression in the epidermis was observed in abundant dendritic cells and in cells of the intraepidermal lymphocytic infiltrate. Variable expression was observed in the isolated cells and cell groups in the dermal infiltrate, although in most cases between 50% and 75% of cells expressed CLA.

The expression of CLA by intraepidermal lymphocytes, the percentage of $CLA^+$ cells in the dermal infiltrate, and the expression of CLA in large cells with a dendritic morphology (data not shown) were analyzed separately. Statistically significant differences were observed in the percentage of $CLA^+$ cells in the dermal infiltrate ($P$=.035). Individual comparisons, however, only revealed differences between the different disease groups and healthy controls, with the highest percentages observed in the initial CTCL group. The number of intraepidermal lymphocytes was greatest in initial CTCL, although statistically significant differences between the groups were not observed.

## VCAM-1

Staining for VCAM-1 was very limited in the samples analyzed. No endothelial labeling was observed in healthy skin.

In some samples from lesioned skin, anti-VCAM-1 staining was observed in cells with a dendritic appearance located among the epidermal keratinocytes and in the follicular epithelium. Given that this was only an occasional observation, it was not included in the analysis. Expression was also occasionally observed in elongated perivascular cells situated in the dermis.

VCAM-1 expression in the blood vessels was analyzed and the number of positive vessels in the whole piece assessed; no differences in vascular expression were observed between the groups. The highest level of expression was observed in inflammatory skin diseases.

## VLA-4

No VLA-4 expression was observed in the epidermis of healthy skin. Furthermore, only a very small number of cells in the dermis of healthy skin expressed VLA-4. In diseased skin, labeling was observed in epidermotropic lymphocytes and dermal infiltrates, and differed between the isolated cells in the papillary dermis and the cells located in the perivascular regions of the deeper dermis. The labeling varied substantially between different samples. The expression of VLA-4 in the general dermal infiltrate and in clusters of cells in the dermal infiltrate, and the numbers of positive cells per epidermal field (magnification, ×400) were considered independently. Data on the percentage of $VLA\text{-}4^+$ cell clusters in dermal infiltrates were only available for 3 patients and were, therefore, removed from the analysis.

Statistically significant differences were observed in the percentage of cells in the general dermal infiltrates ($P$=.007), with significant differences occurring between all disease categories and healthy controls and between initial CTCL and inflammatory skin diseases. The highest proportion of positive cells (mean, 59.58%) was observed in inflammatory diseases, although the absolute maximum value (90%) was observed in 3 disease groups.

## E-cadherin

In healthy skin, expression of E-cadherin was observed in epidermal keratinocytes and in the hair follicles and glandular epithelium. Some isolated cells with a dendritic appearance were also observed in the superficial dermis and the perivascular region.

In the other groups, expression was observed in epidermal keratinocytes, with varying staining intensity in the different layers of the epidermis of different samples. In the dermis, labeling was observed in isolated cells with a dendritic morphology and in cells around the perivascular

area. Staining was also observed in the hair follicles and the epithelium of the sweat glands.

The intensity of staining in the epidermis (+, ++, +++) was assessed along with the presence of cells with a dendritic morphology in the dermis (none, very few, moderate numbers, abundant, highly abundant) (data not shown) and the expression pattern in the different levels of the epidermis (Figure 7; $P$=.027). Differences in the expression of E-cadherin were detected between initial CTCL and inflammatory skin diseases, and between inflammatory diseases and healthy skin. The highest level of expression was observed in the control group and in initial CTCL, with the expression limited to the deep layers of the epidermis in other skin diseases.

## Integrin aEb7 (CD103) (Figure 8)

In healthy skin, staining of integrin αEβ7 was observed in isolated cells in the perfollicular area of the dermis and there was no staining in the epidermis. In the other groups, variable numbers of immunopositive cells were found in the dermis and epidermis. In some biopsies, only isolated cells were observed in the epidermis. The intraepidermal cells were distributed along the dermal-epidermal interface, although Pautrier microabscesses were negative for integrin αEβ7.

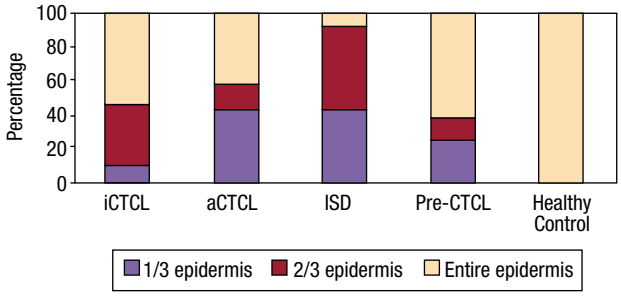


**Figure 7** Level of expression of E-cadherin ($P$=.027). CTCL indicates cutaneous T-cell lymphoma; aCTCL, advanced CTCL; iCTCL, initial CTCL; ISD, inflammatory skin disease.

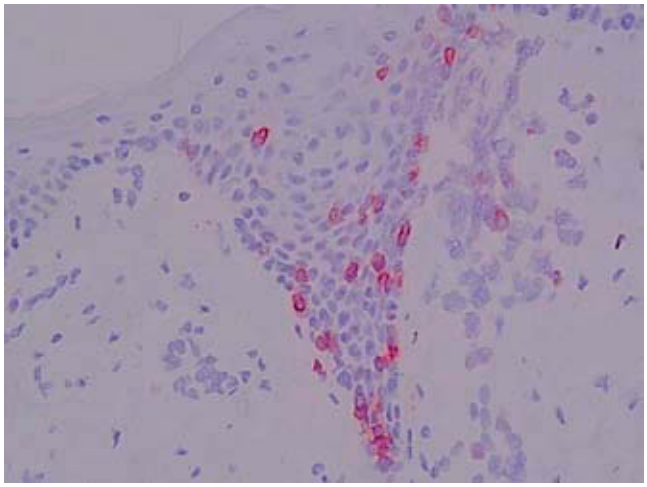

**Figure 8** Expression of CD103 by lymphocytes in the epidermis. Skin biopsy, frozen section (original magnification, ×400).

The percentage of immunopositive cells in the dermis ($P$=.012) and the number of immunopositive lymphoid cells in epidermal microscopic fields ($P$=.008) were analyzed. In both cases, there were significant differences for advanced CTCL, pre-CTCL, and inflammatory diseases compared with healthy skin and also between initial CTCL and inflammatory diseases. Differences in expression in the epidermis were also observed between initial CTCL and healthy skin and between inflammatory diseases and pre-CTCL.

Integrin αEβ7 expression was found in 83.3% of cells in dermal infiltrates, with the highest percentages in atopic dermatitis and psoriasis. Similar results were obtained for the number of immunopositive cells in the epidermis. In initial CTCL samples, patients with large numbers of immunopositive cells in the epidermis had low percentages of positive cells in dermal infiltrates, whereas patients with pre-CTCL had large percentages of immunopositive cells in dermal infiltrates.

## Discussion

The localization of T lymphocytes in the skin plays a fundamental role in immune surveillance and in the pathogenesis of inflammatory skin diseases and CTCL. The adhesion molecules that regulate lymphocyte trafficking are also expressed and functionally active in non-Hodgkin lymphomas.[5,8]

ICAM-3, also known as CD50, is a 120 kDa type I transmembrane glycoprotein. It is constitutively expressed on leukocytes (lymphocytes, monocytes, and neutrophils) and Langerhans cells.[11-13] It acts as a ligand for LFA-1 and integrin αDβ2, and it binds the lectin DC-SIGN, which is found on dendritic cells, with high affinity. Relatively uniform expression of ICAM-3 is observed in the lymphocytes that infiltrate the skin in different diseases. This observation is consistent with the in vitro finding that ICAM-3 expression is not highly regulated. The presence and distribution of ICAM-3 in the different groups included in our study appears to be related to the distribution of leukocytes in the different diseases analyzed, and the patterns observed were consistent with those reported in the literature.[12]

ICAM-3 expression has been observed in small vessels in lymphomas and other tumors, and its expression has been linked to the neoangiogenesis necessary for tumor survival.[14] In our study, which included patients with Sézary syndrome and with mycosis fungoides at different stages but without systemic spread, expression of ICAM-3 was not observed on vascular endothelial cells. The absence of staining on endothelial cells has also been observed in 15 cases of cutaneous lymphoma and pseudolymphoma[13] and in 5 patients with erythrodermic mycosis fungoides.[12] Only 1 study has reported endothelial expression in skin biopsies of cutaneous lymphomas with systemic disease.[15] Specifically, endothelial ICAM-3 staining was observed in 1 out of 10 patients with mycosis fungoides (6 with known systemic disease) and 2 out of 6 patients with Sézary syndrome. Further studies in larger groups of patients are required to determine the biological significance of these findings.

ICAM-1, or CD54, is an inducible glycoprotein that is expressed on keratinocytes, Langerhans cells, endothelial cells, and fibroblasts following stimulation with cytokines (tumor necrosis factor $\alpha$ and interferon $\gamma$).[16] It binds LFA-1 expressed on leukocytes and Mac-1 on monocytes. Nickoloff et al[17] observed that epidermal keratinocytes almost entirely lacked expression of ICAM-1 in a patient with Sézary syndrome, and it has been reported in patients with mycosis fungoides that expression of ICAM-1 by keratinocytes is reduced as the disease progresses towards more advanced stages. This would lead to a lack of LFA-1/ICAM-1 binding and a loss of skin homing.

Our observation that Sézary syndrome was not associated with loss of expression confirms the findings of previous studies.[18,19] However, we did find more extensive expression in the epidermis of patients with initial CTCL, which is consistent with the observation of more marked expression of ICAM-1 in mycosis fungoides than in other diseases.[16] Furthermore, the mean percentage of immunopositive lymphoid cells in dermal infiltrates was higher in inflammatory skin diseases and advanced lymphomas than in initial stages, but these differences were not statistically significant. Pujol et al[18] also reported that although the expression of CD54 was variable in infiltrates, the numbers of CD54$^+$ cells were greater in more advanced stages of the disease.

Integrin $\alpha L\beta 2$ or LFA-1 is an activating antigen that is expressed on lymphocytes, neutrophils, and monocytes. It binds endothelial cells and activated keratinocytes,[20] and its ligands are ICAM-1, ICAM-2, and ICAM-3. In CTCL, epidermotropic clones preferentially express this antigen and it has therefore been suggested that cells which do not express it would evade physiological immune surveillance and that this could lead to a more aggressive disease course. It has been observed that the majority of lymphomas with low levels of LFA-1 expression reduced their expression during progression of the disease,[21] and other studies have observed the loss of this antigen in up to 26% of patients with mycosis fungoides or Sézary syndrome, suggesting that it may be a common phenotype. In this study, we did not observe differences in the percentage of LFA-1$^+$ cells in early and late stages of CTCL.

E-selectin, or Endothelial Leukocyte Adhesion Molecule 1, is expressed on activated endothelial cells and binds carbohydrates. Maximal expression is observed 2 to 4 hours after cell activation and the molecule binds oligosaccharides containing sialyl Lewis X, although sialylated or fucosylated lactosaminoglycans can also act as ligands.[22] In our study, this molecule was expressed in less than 50% of endothelial cells labeled with Ulex lectin, with even lower expression in healthy controls. This expression reveals that the activation of this adhesion molecule reflects the activation of the endothelium, with no differences in expression pattern between diseases.

CLA is a carbohydrate antigen related to sialyl Lewis X that is expressed by a subgroup of memory T cells (10%-15%) that display skin-homing behavior. It corresponds to an inducible carbohydrate modification in P-selectin glycoprotein ligand-1 (PSGL-1), which is constitutively expressed on all circulating T cells, and its ligand is E-selectin.[6,7,23]

In our study, significant differences were observed in the percentage of CLA$^+$ cells in the dermis of all groups compared with healthy controls, indicating recruitment of this cell type in inflammation and skin diseases. The highest levels were observed in initial CTCL.

VCAM-1, or CD106, is an inducible endothelial glycoprotein that binds leukocytes other than neutrophils and is also present on macrophages and perivascular dendritic cells, bone marrow fibroblasts, and myoblasts.[24] It normally shows very low levels of luminal expression in unstimulated endothelial cells. Its ligands are VLA-4 and integrin $\alpha 4\beta 7$. In our study, expression was occasionally observed in intraepithelial and perivascular cells with a dendritic appearance, but the results were not included in the analysis. It has been suggested that the expression of VCAM-1 in perivascular dendritic cells is important for the maintenance of the resident population of lymphoid cells in noninflamed skin.[25]

Serial sections stained with Ulex lectin, anti-E-selectin, and anti-ICAM-1 showed that most of the vessels expressing these adhesion molecules were negative for VCAM-1, suggesting that their expression is independently regulated and that their role in the diseases studied is nonspecific.

VLA-4 is an integrin comprising a larger $\alpha 4$ subunit and a $\beta 1$ subunit. With the exception of neutrophils, most leukocytes can express VLA-4. This integrin binds to VCAM-1 via the first and fourth domains, to fibronectin, and to itself. Assessment of VLA-4 expression in dermal infiltrates allows the different diseases to be differentiated from healthy skin and initial CTCL to be distinguished from inflammatory skin diseases, in which the percentage of immunopositive cells is higher.

$\alpha E\beta 7$ integrin has been implicated in the positioning of lymphocytes within the epidermis and in their interaction with keratinocytes; reduced expression of $\alpha E\beta 7$ has been observed in advanced stages of mycosis fungoides with loss of epidermotropism.[26]

Integrin $\alpha E\beta 7$ (CD103) is an activation/proliferation antigen and is expressed on lymphoid cells, activated macrophages, and in hairy cell leukemia. It was initially described as being expressed by intraepithelial cells of intestinal lymphomas and by lymphocytes of the lamina propria, and it is expressed in less than 2% of circulating T cells. The expression of CD103 has been studied in lesioned skin from patients with psoriasis and preferential expression was observed in CD8$^+$ cells.[27,28] CD103 can bind to E-cadherin on epithelial cells and mediate the positioning of lymphocytes in the epithelium, although some authors have suggested that it acts independently of E-cadherin.[27,29]

In our study, we observed the presence of large numbers of CD103$^+$ intraepidermal cells in advanced CTCL due to the presence of numerous immunopositive cells at the junction between the dermis and the epidermis. CD103 appears to be a marker for epidermotropic T cells (including neoplastic cells) and its expression in the epidermis allowed all groups to be distinguished from each other except for pre-CTCL and advanced CTCL.

E-cadherin is a 120 kDa transmembrane adhesion molecule. Its extracellular domain mediates calcium-dependent homotypic binding while its intracellular

domain binds the actin cytoskeleton through catenins. In healthy skin, E-cadherin expression is observed in epidermal keratinocytes and in the hair follicles and glandular epithelium.[30] In this study, we observed a reduction in the thickness of the epidermal layer expressing E-cadherin from initial CTCL and healthy skin to advanced CTCL.

We did not observe statistically significant differences between initial and advanced CTCL. Such differences were observed, however, between these and other diseases. The most informative observations relate to the number of $CD103^+$ and ICAM-$3^+$ lymphocytes in the epidermis (per $\times$400 microscopic field). These allowed all of the groups except for advanced CTCL and pre-CTCL to be distinguished. These 2 groups displayed statistically significant differences in the expression of E-selectin ($\times$400 magnification) and in the honeycomb pattern of ICAM-1 expression in epidermal keratinocytes. These findings suggest that the expression of the cell adhesion molecules implicated in the adhesion and migration of lymphocytes through the vascular endothelium in the dermis does not differ significantly between initial and advanced stages of CTCL. However, these findings should be confirmed in studies including larger numbers of patients.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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