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Evaluation of Angiogenesis in Early Mycosis Fungoides Patients: Dermoscopic and Immunohistochemical Study

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Key Words
Angiogenesis · Dermoscopy · CD34 · Mycosis fungoides

Abstract
Background: Angiogenesis is the production of new blood vessels from an existing vascular network; it plays a critical role in solid tumor development and metastasis. ***Objectives:*** To assess angiogenesis in early cases of mycosis fungoides (MF) and to determine vascular patterns in MF dermoscopically. ***Methods:*** 25 patients with MF and 20 healthy controls were included. The MF lesions were assessed dermoscopically. CD34 immunohistochemistry was performed to count dermal microvessel density (MVD). ***Results:*** The total dermal MVD was significantly higher in MF patients (19.77 ± 5.81) than in controls (4.44 ± 3.16; p = 0.013). Among them, there were 10.8 ± 4.1 sprouts of endothelial buds (clusters of cells per field) in patients and 2.4 ± 2 in controls (p = 0.000). The dotted pattern of blood vessels was the most frequently encountered pattern in the MF lesions by dermoscopy. ***Conclusions:*** Our findings support that neoangiogenesis is significantly increased in early MF lesions and that the main dermoscopic feature of MF is dotted blood vessels.

Background

Tumor angiogenesis is the process by which new blood vessels form in neoplasms; it starts in the early stages of the disease and is a crucial step in the growth and spread of tumors [1]. Without forming new blood vessels, tumors cannot grow beyond a certain size due to the lack of oxygen and other essential nutrients [2]. Angiogenesis has become the focus of intense study in recent years, for example, in the development of antiangiogenesis pharmacological agents as attractive antitumor targets [3, 4].

Some studies have indicated that tumor microvessel density (MVD), measured by CD34, CD31 or von Willebrand factor expression, is increased in lymphoproliferative disorders [5]. Mycosis fungoides (MF), a low-grade lymphoproliferative disorder, is the most common form of cutaneous T-cell lymphomas (CTCL) and accounts for around 60% of new cases [6]. In a retrospective study, angiogenesis was found to play a role in advanced cases of MF [7].

Dermoscopy has proven its diagnostic capabilities in pigmented skin tumors [8] as well as in inflammatory skin disorders [9]. It may also be of value for the assessment of vascular structures and color variations that are not clinically visible; thus, dermoscopy may be regarded as an intermediate step between clinical examination and dermatopathology [10].

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Materials and Methods

Aim

The aim of the current work is to study the vascular pattern of MF by hand-held dermoscopy and evaluate neoangiogenesis in early MF cases using CD34 immunohistochemistry and correlate it with the clinical picture of the disease.

Patients

Following approval by the Dermatology Research Ethics Committee, Cairo University, this prospective investigational study was carried out. Patients and controls were recruited from the Dermatology Outpatient Clinic, Cairo University Hospitals, and written informed consents for participation were signed by all participants.

The study included 25 patients with confirmed MF. Their disease staging was carried out following the TNM staging system [11]. Twenty age- and sex-matched healthy volunteers served as controls, who were subjected to full history taking and examination to exclude any associated cutaneous or systemic disease. Exclusion criteria were patients with autoimmune diseases, collagen, vascular diseases, psoriasis, and history of any previous or associated cancer.

Methods

Clinical Examination of MF Patients

The clinically most affected skin lesion was determined as patch or plaque, and the following scoring system for its severity at the time of inclusion was adopted:

grade I = faintly erythematous or skin-colored lesion with no or mild scaling lesion and/ or mild pruritus and no infiltration;

grade II = erythematous and moderately scaling lesion with mild pruritus and infiltration;

grade III = deeply erythematous and severe scaling lesion with pruritus and moderate-to-severe infiltration.

Dermoscopic Examination of MF Lesions

The selected skin lesions from patients were subjected to dermoscopic examination using a Dermlite II Hybrid with a magnification of ×10 (3Gen, USA). An attachment piece was used to connect the dermoscope to a Nikon 1 J1 digital camera (10.1 megapixels).

Dermal MVD Evaluation by Immunohistochemical CD34 Detection

Five-millimeter punch biopsies were obtained from the selected skin lesions of patients with MF as well as from the skin of control subjects. Skin specimens were paraffin embedded, and immunohistochemistry was performed with primary antibody against CD34 as an endothelial cell marker to count the total dermal MVD according to Restucci et al. [12]. CD34 primary antibody is a mouse monoclonal antibody (catalogue No. MS-363-R7) that requires no special pretreatment. Immunostaining is completed by the use of an ultravision detection system, horseradish peroxidase polymer and diaminobenzidine plus chromogen (catalogue No. TL-015-HDJ). Counterstaining was done using Mayer's hematoxylin (catalogue No. TA-060-MH). CD34 primary monoclonal mouse antibody, ultravision detection system and Mayer's hematoxylin were purchased from Lab Vision Corporation, Vermont, Calif., USA, and Thermo Fischer Scientific, Runcorn, UK. Sections were examined by light microscopy for CD34 staining.

Morphometric Study

In the CD34 immunohistochemically stained sections of patients and controls, dermal MVD was counted collectively as mean number. Among it there were single endothelial cells clearly separated from one another or clusters of endothelial cells termed vascular sprouts.

This was done in 5 nonoverlapping fields at a magnification of ×200 in a field area of 20,286.63 μm^2 (for blood vessels and immunostained cells positive for CD34) for every subject using a Leica Qwin 500C image analyzer computer system (UK). Images were captured live on the screen from sections under a light microscope (Olympus BX-40, Olympus Optical Co. Ltd., Japan) with a fixed video camera (Panasonic Color CCTV camera, Matsushita Communication Industrial Co. Ltd., Japan). The video images were digitalized using Leica Qwin, which is Leica's Windows-based image analysis tool kit fitted to an IBM-compatible personal computer with a color monitor. The positive immunoreactivity for CD34 appeared as brown deposits. Interactive counting was done on the captured images on the monitor.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, Ill., USA) version 15 for the Microsoft Windows program. The χ^2 test was used to compare differences between the frequencies. The Mann-Whitney U and Student's t tests were used to compare mean values between groups. The Spearman rank correlation test was used for the assessment of correlation. The statistical significance was accepted as a p value ≤ 0.05.

Results

Twenty-five MF patients were enrolled in the study, 8 (32%) males and 17 (68%) females; their ages ranged between 16 and 70 years with a mean of 39.84 ± 18.32 years. Clinical variants included 16 (64%) classic, 7 (28%) hypopigmented and 2 (8%) poikilodermatous MF. Their disease duration ranged between 8 and 120 months with a mean of 53.52 ± 40.23 months (online suppl. table 1; see www.karger.com/doi/10.1159/000382124 for all online suppl. material). Twenty healthy individuals served as controls, including 8 males (42.1%) and 11 females (57.9%). Their ages ranged between 25 and 55 years with a mean of 37.47 ± 9.3 years. No statistically significant difference was found between the two groups as regards age and sex (p = 0.713 and 0.54, respectively).

Results of Dermoscopic Examination of MF Lesions

The dotted pattern of blood vessels was the most frequently encountered pattern in the MF lesions (fig. 1) followed by the linear pattern (online suppl. table 2). Light brown focal hyperpigmentation was detected in poikilodermatous MF lesions (online suppl. fig. 1).

Angiogenesis Evaluation in Patients and Controls

In the dermis, the total MVD was determined in patients and controls (fig. 2; online suppl. fig. 2, 3). The mean total dermal MVD was significantly higher in patients (range = 8.6–31.4, mean ± SD = 19.77 ± 5.81) than controls (range = 0.20–9.40, mean ± SD = 4.44 ± 3.16; p = 0.013). The mean number of microvessels with lumen per field was significantly higher in patients (range = 3.4–14, mean ± SD = 9.1 ± 2.7) than controls (range = 0.0–4, mean ± SD = 1.8 ± 1.28; p = 0.000). Patients had significantly higher numbers of vascular sprouts (endothelial buds; range = 1.6–17.4, mean ± SD = 10.8 ± 4.1) than controls (range = 0.2–5.6, mean ± SD = 2.4 ± 2; p = 0.000).

Correlations of Angiogenesis with Clinical Setting of MF

No statistically significant difference was found between the different clinical variants of MF as regards the mean number of microvessels positive for CD34 (p = 0.672) and angiogenic vascular sprouts (p = 0.499; online suppl. table 3). When correlating the total dermal MVD with possible prognostic factors, no relation was detected. There were no significant statistical correlations between microvessels and the vascular sprouts with disease duration and the grade of severity of the skin lesion. No statistically significant difference was found when evaluating the MVD with respect to the lesion type clinically, whether patch or plaque, as regards the mean number of microvessels positive for CD34 (p = 0.567) and vascular sprouts (p = 0.454).

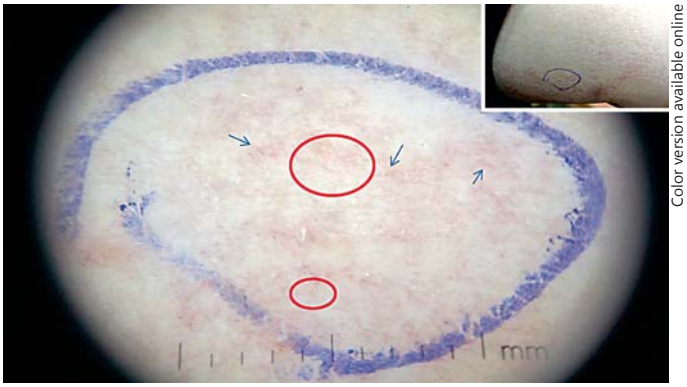

Fig. 1. Dermoscopic picture of classic plaque-stage MF. Blue arrows (colors refer to the online version only) indicate comma-shaped blood vessels, red circles show dotted blood vessels. ×10.

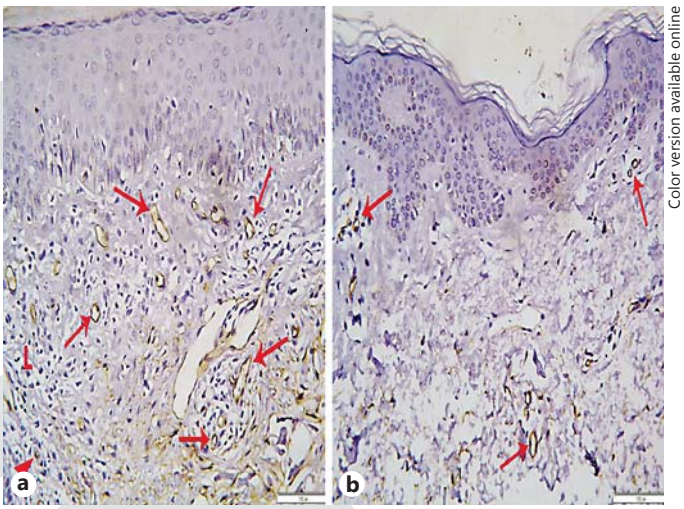

Fig. 2. CD34 immunostaining of blood vessels. **a** MF case No. 2. Blood vessels (arrows) and angiogenic bud (arrowhead); note the superficial lymphoid infiltrate and epidermotropism. **b** Control No. 9. Note the minimal staining of the blood vessels (arrows). Avidin-biotin complex, diaminobenzidine. ×200.

Discussion

Neoangiogenesis, the formation of new blood vessels from existing ones, plays an important role in the growth and progression of tumors, beginning with vessel dilation and the detachment of pericytes from preexisting vessels followed by angiogenic sprouting and the proliferation of endothelial cells with new vessel formation [13]. The use of MVD as a marker for tumor angiogenesis has been reported [14].

The current study revealed that total dermal MVD is statistically significantly higher in patients with early MF than controls. In a previous study [7], angiogenesis was evaluated in advanced MF, stages III and IV, by measuring the expression of CD34 in skin specimens of 25 patients and 8 controls. Vascular structures, whether microvessels or endothelial buds, were counted collectively as MVD, which was found to be statistically significantly higher in late stages of MF than in the control population.

In the current study angiogenesis in MF was not only evaluated by estimating the total number of blood vessels, but by further counting tubes with lumen as well as vascular sprouts in tumor specimens. The mean count of vascular sprouts was found to be slightly higher than vessels with lumen, denoting an active process of neoangiogenesis in cases of early MF. Both counts proved to be higher in tumor specimens in comparison to normal skin specimens in a highly statistically significant manner. These results provide evidence that active angiogenesis

occurs even in early cases of MF, with low-grade malignant potential and a disease which is known to have an indolent course.

In an earlier study [15] using factor VIII to detect vascular structures, it was found that disease progression and MF upstaging were paralleled by an increase in angiogenesis as measured by microvessel area. Values of MF patch stage overlapped that of control skin, while values were significantly higher in the plaque stage. This is in contrast to the current study, where no difference was found when evaluating the MVD with respect to the lesion type clinically, whether patch or plaque. In the same context, it was expected to find a relation between increased angiogenesis in MF patients and grade of severity of skin lesions or disease duration; however, none was detected. This may be related to the selection of the most affected skin lesion to be examined not the most chronic lesion.

The exact mechanisms of angiogenesis in CTCL remains unclear; however, lymphoma growth and progression are potentiated by at least 2 distinct angiogenic mechanisms: autocrine stimulation of tumor cells via expression of VEGF and VEGF receptors by lymphoma cells, as well as paracrine influences of proangiogenic tumor microenvironment on both local neovascular transformation and recruitment of circulating bone marrow-derived progenitors [16]. Indeed, neovessels promote growth, which seems to explain why the S-phase fraction and morphologically the number of blasts rise in the transition from the patch to the nodular stage [17]. Neovessels favor invasion and metastasis [18], which could explain why loss of epidermotropism and invasion of lymph nodes and parenchymal organs are frequently observed in plaque and, even more so, in nodular stage patients [17].

Although the mean number of the total blood vessels and the new angiogenic buds was higher in the poikilodermatous variant of MF than the classic and hypopigmented variants, it does not reach a statistical significance. This is related to the small number of patients in each group.

In the current study, MF lesions were examined with hand-held dermoscopy and exhibited a characteristic vascular pattern consisting mainly of dotted and linear blood vessels while orange-yellowish patchy areas were seen in the background in only few cases. Arborizing blood vessels were detected in the poikilodermatous variant of MF. Only 2 studies in the published literature could be retrieved evaluating MF lesions dermoscopically. Lallas et al. [19] revealed that MF shows a characteristic dermoscopic pattern consisting of fine short linear vessels in 93% and dotted vessels in 55% of its cases, along with orange-yellowish patchy areas. Furthermore, they described characteristic vascular structures resembling spermatozoa in half of their cases. This vascular pattern could not be identified in our study, as with a magnification of ×10 by the dermoscope, it was not readily possible to differentiate a spermatozoa-like pattern from a comma-shaped vascular pattern. In a recent study [20], short linear vessels in 19% and spermatozoa-like vessels in 14% of cases were observed on a pink homogenous background. It is noteworthy that dark globules and light brown multifocal pigmentation were observed in almost half of their MF cases. Similar light brown multifocal pigmentation was observed only in poikilodermatous lesions among our patients. Thus, further studying of vascular patterns of MF lesions is necessary to reach a consensus on its dermoscopic criteria.

Recommendations

It is recommended to perform further studies on patients with tumor lesions of MF to compare the process of angiogenesis in early and advanced stages of the disease.

Further studies on the dermoscopic picture of MF need to be performed.

Limitations of the Study

All cases of MF were early stages, which did not allow for comparison between different stages of MF. Furthermore, the dermoscopic magnification power is ×10, which is not enough for obtaining clear images for precise blood vessel pattern evaluation.

Conclusion

A significantly higher number of new microvessels is found in early MF compared to normal skin indicating that angiogenesis plays a role in the growth of early stages of CTCL, raising the possibility of using angiogenesis inhibitors in CTCL therapy.

Disclosure Statement

No conflicts of interest declared.

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