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Quantitative immunohistochemical assessment of blood and lymphatic microcirculation in cutaneous lichen planus lesions

Desanka Výbohová[1], Yvetta Mellová[1], Katarína Adamicová[2], Marián Adamkov[3] and Gabriela Hešková[1]
Comenius University in Bratislava, [1]Institute of Anatomy, [2]Institute of Pathological Anatomy and [3]Institute of Histology and Embryology, Jessenius Faculty of Medicine in Martin, Slovak Republic

Summary. Latest advances have brought to light the hypothesis that angiogenesis and lymphangiogenesis are tightly connected to some chronic inflammatory diseases. The present study focuses on immunohistochemical assessment of the quantitative changes in the blood and lymphatic microcirculatory bed in common chronic dermatosis - cutaneous lichen planus. Double immunohistochemistry with CD34 and podoplanin antibodies was used to detect blood and lymphatic endothelium, while anti-human VEGF was used for the observation of a key angiogenesis and lymphangiogenesis inducer. Morphometric analysis was performed with QuickPhoto Micro image analysis software. Results confirmed statistically significant enlargement of both the blood and lymphatic microcirculatory beds. Compared to healthy skin, cutaneous lichen planus lesions revealed 1.6 times enlarged blood microcirculatory bed and 1.8 times enlarged lymphatic microcirculatory bed. Vascular endothelial growth factor (VEGF) expression in lesional skin was significantly higher in the epidermis (19.1 times increase) than in the dermis (10.3 times increase). These findings indicate a tight association of angiogenesis and lymphangiogenesis with the pathogenesis of cutaneous lichen planus.

Key words: Cutaneous blood and lymphatic microcirculation, Cutaneous lichen planus, Angiogenesis, Lymphangiogenesis, VEGF

Offprint requests to: MUDr. Desanka Výbohová, Ph.D, Institute of Anatomy, Jessenius Faculty of Medicine, Malá Hora Str. N. 4, 03601 Martin, Slovak Republic. e-mail: vybohova@lefa.sk

Introduction

Angiogenesis and lymphangiogenesis are strictly controlled processes of new blood and lymphatic vessel development from pre-existing blood and lymphatic vessels. In healthy skin, the blood and lymphatic vessels remain quiescent and neovascularization is induced only transiently in such processes as wound healing and certain phases of the growing hair follicle. Pathological angiogenesis and lymphangiogenesis are also central to the etiology and pathogenesis of some skin diseases (Velasco and Lange-Asschenfeld, 2002).

Recent advances have brought to light evidence that angiogenesis and lymphangiogenesis are tightly connected not only to tumour biology but also to chronic inflammation. Pathological angiogenesis and lymphangiogenesis were revealed in skin tumours: melanoma (Hannah and Folkman, 1996; Marcoval et al., 1996; Kashani-Sabet et al., 2002; Jonjic et al., 2003), basal cell carcinoma (Staibano et al., 1996), Kaposi's sarcoma (Kang et al., 2008), hemangioma (Boye et al., 2001) as well as in some chronic dermatoses like rosacea (Aroni et al., 2008) and psoriasis (Braverman and Sibley, 1982; Creamer et al., 1997, 2002a; Heidenreich et al., 2009).

Angiogenesis and lymphangiogenesis are tightly regulated by a balance between pro- and anti-angiogenic mediators. It is generally supposed that in quiescent cutaneous microcirculation, a balance between proangiogenic and antiangiogenic factors maintains homeostasis. Angiogenesis and lymphangiogenesis are initiated by angiogenic switch resulting from up-regulation of angiogenic factors and the downregulation

of inhibitors (Hannah and Folkman, 1996). An important feature that distinguishes physiological from pathological angiogenesis is that physiological angiogenesis is limited to a few days or weeks, although pathological angiogenesis can persist for months or years (Carmeliet and Jain, 2000; Polverini, 2002).

Cutaneous lichen ruber planus (CLP) is a relatively common subacute or chronic dermatosis with poorly defined aetiology and a not entirely understood pathogenesis (Braun-Falco et al., 2001).

Recent information has put this dermatosis into the group of T- cell mediated diseases. Lichen planus lesion formation results from an immunological response to keratinocyte antigen expression or unmasking at the lesion site induced by systemic drugs, contact allergens, mechanical trauma, bacterial or viral infection, or an unidentified agent. Both antigen-specific mechanisms and non-specific mechanisms are supposed to be involved in its pathogenesis (Sugerman et al., 2002; Lehman et al., 2009; Roopashree et al., 2010).

Clinical manifestation involves pruritic, polygonal papules, sometimes with fine scales and very often with a delicate network of white lines - Wickham striae - on the surface of the papules (Braun-Falco et al., 2001).

Histopathological manifestation of CLP (Fig. 1) includes broad epidermal hyperplasia with zonal wedge-shaped hypergranulosis and hyperkeratosis. The keratinocytes in stratum spinosum are large with pale cytoplasm. The basal cells show vacuolar alteration, squamatisation and colloid bodies like anucleated remnants of apoptotic basal keratinocytes. A band-like lymphocytic infiltrate occupies the widened papillary dermis and may be admixed with melanophages. The density of the inflammatory infiltrate reflects the evolution of the lesion. The lymphocytes often disrupt the dermo-epidermal junction and attack keratinocytes. Devolution of the lesion is accompanied by reduction of the inflammatory infiltrate and fibrotic papillary dermis with deposits of collagen bundles (Murphy, 1995; Joshi, 2013).

Excessive angioproliferation was confirmed in different types of oral lichen planus (Scardina et al., 2007a,b, 2011; Tao et al., 2007; Scardina and Mesina, 2009). However microvascular changes in cutaneous lichen planus are rarely reviewed. Therefore the objective of the present study was an immunohistochemical assessment of quantitative changes in both the blood and lymphatic microcirculatory bed within CLP lesions compared to healthy skin.

Material and methods

Tissue samples

Twenty-one skin samples of CLP lesions from untreated patients with no systemic disorder and 20 control healthy skin samples from subjects without any skin or systemic disease were excised from identical topographical regions. On the basis of our preliminary study, all samples were taken from individuals aged 31 - 57 years of both genders to eliminate age-dependent variability in skin microcirculation (Výbohová et al., 2012). Specimens were fixed in 10% neutral buffered formalin and embedded into paraffin blocks. Lesions were verified for clinical and histopathological diagnostic criteria by experienced dermatologists and pathologists. The study was approved by the local Ethics Committee at Jessenius Faculty of Medicine in Martin and registered with the Office for Human Research Protection, U.S. Department of Health and Human Services under No. IORG0004721.

Immunohistochemical analysis

Five serial perpendicular 4 μm thick sections were mounted on silane coated slides. Sections were dewaxed with xylene and rehydrated in a series of graded alcohols. Endogenous peroxidase was blocked by 0.3% H_2O_2. Heat-induced epitope retrieval was done after the slides had been immersed in the retrieval solution (Target Retrieval Solution High pH, Code No. S3308, DakoCytomation Denmark A/S). Afterwards, the specimens were incubated with diluted primary monoclonal antibodies at room temperature.

Double immunohistochemical staining was performed with CD34 (Monoclonal Mouse Anti–Human CD34 Class II Clone QBEnd-10 M 7165, DakoCytomation Denmark A/S) and D2-40 (Monoclonal Mouse Anti-Human D2-40 Clone D2-40, DakoCytomation Denmark A/S) antibodies to detect blood and lymphatic endothelium. The primary antibodies were revealed using a polymer technique - EnVision Doublestain System (Code K 1395, DakoCytomation Denmark A/S), which permitted the simultaneous demonstration of both antigens (CD34 and podoplanin) in a single specimen through double immunoenzymatic staining with two chromogens: peroxidase chromogen DAB (diaminobenzidine) and alkaline phosphatase chromogen Fast Red.

Anti-human VEGF (Monoclonal Mouse Anti –Human Vascular Endothelial Growth Factor Clone VG1 M7273, DakoCytomation Denmark A/S) was used to assess vascular endothelial growth factor expression. This primary antibody was detected by biotinylated immunoglobulins and horseradish peroxidase streptavidin – biotin complex (LSAB™ +/ HRP Kit, Code No. K0679, DakoCytomation Denmark A/S). DAB was used to complete the staining.

Finally, all sections were counterstained with Mayer's hematoxylin. All steps of the immunohistochemical staining were managed according to the manufacturer's recommendations.

Computer-assisted morphometric analysis

Sections were screened and digital images were taken at magnifications of x200 and x400 with an

Olympus Evolt E-420 installed in an Olympus BX41N. The digital images were morphometrically analysed by QuickPhoto Micro Version 2.3 (Promicra, Prague, Czech Republic). The blood and lymphatic microcirculatory beds were evaluated by blood vessel area (BVA) and lymphatic vessel area (LVA). Five serial slides from each skin sample were examined field per field by two experienced investigators to identify the areas with highest vascularization - hotspot areas (Weidner, 1995). At least ten standard fields (hotspot areas) in each serial slide from each skin sample were evaluated to depict, to a certain extent, the partial volume of the papillae. Fields with the capillary networks around hair follicles and glands were eliminated.

The blood vessel area (BVA) / lymphatic vessel area (LVA) was defined as percentage of CD34 / D2-40 – stained area (proportional area occupied by blood / lymphatic microvessels) to standard fields (0.1 mm^2) of the papillary and upper reticular dermis (Mylona et al. 2007; Deb et al., 2012; Boruah et al., 2014). Similarly, VEGF expression was evaluated by the VEGF positive area defined as the average percentage of VEGF – stained area in standard fields of epidermis and dermis (Mylona et al., 2007).

Statistical analysis

Morphometric data were expressed as the mean ± standard deviation. Statistical analysis was performed using the analysis of variance, t–test and Pearson's correlation test. A p value less than 0.05 was considered the minimum for statistical significance.

Results

The blood vessel area (BVA), lymphatic vessel area (LVA) and VEGF positive area in the cutaneous lichen planus lesions were quantified and compared with values from the control healthy skin. Individual variability of the specimens was rejected on the basis of ANOVA tests ($p>0.05$).

Table 1 shows average BVA values in the lesional and healthy skin. Results showed significantly higher

Table 1. Blood vessel area in healthy skin (HS) and cutaneous lichen planus (CLP) lesions.

topographic region	blood vessels area (BVA) in %		
	healthy skin (n=20)	CLP lesions (n=21)	HS vs CLP lesions p-value
abdominal region	0.77±0.07	1.19±0.21	4.0E-04
back region	0.75±0.15	1.15±0.13	0.006
facial region	1.07±0.16	1.41±0.21	0.040
femoral region	0.76±0.09	1.11±0.12	0.018
regional variability p-value	0.008	0.157	

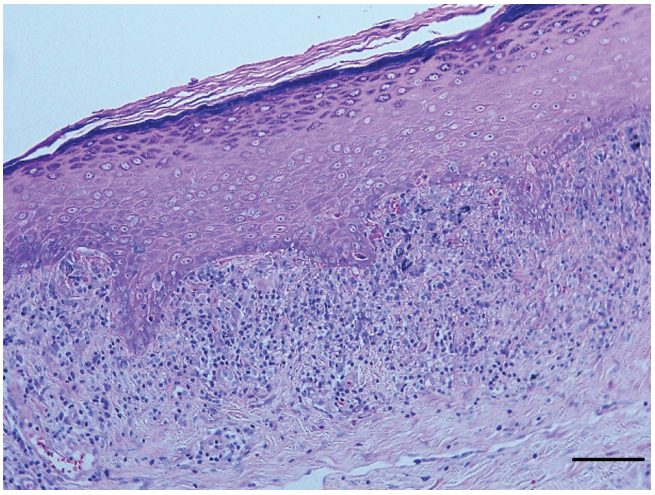

Fig. 1. Typical histopathological manifestation of cutaneous lichen planus: epidermal hyperplasia with zonal wedge-shaped hypergranulosis and hyperkeratosis, squamatization and basal cells vacuolar alteration, a band like lymphocytic infiltrate admixed with melanophages in papillary dermis. Hematoxylin-eosin staining. Scale bar: 100 μm.

Table 2. Lymphatic vessel area in healthy skin (HS) and cutaneous lichen planus (CLP) lesions.

topographic region	lymphatic vessels area (LVA) in %		
	healthy skin (n=20)	CLP lesions (n=21)	HS vs CLP lesions p-value
abdominal region	0.40±0.07	0.81±0.23	9.4E-04
back region	0.34±0.09	0.79±0.19	0.001
facial region	0.60±0.07	0.87±0.17	0.038
femoral region	0.43±0.06	0.80±0.15	0.010
regional variability p-value	0.002	0.930	

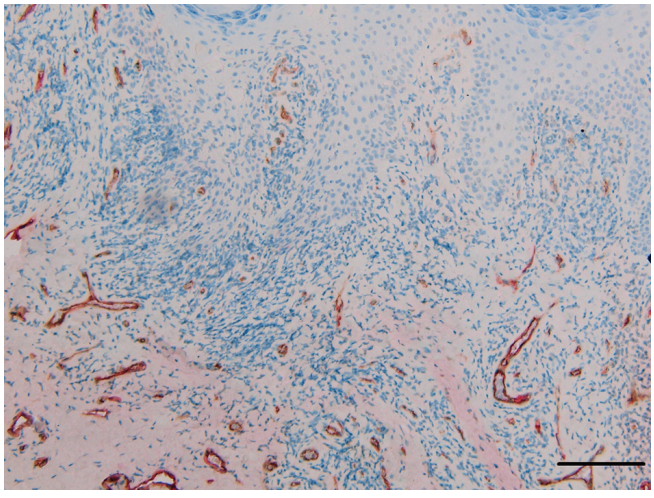

Fig. 2. Blood (brown) and lymphatic (pink - red) microvessels in cutaneous lichen planus lesion. Double immunohistochemical staining with CD34 and D2-40 antibodies. Scale bar: 100 μm.

BVA in the lichen planus lesions compared to the healthy skin in all topographical regions ($p<0.05$). The blood microcirculatory bed in the CLP lesions was approximately 1.6 times more extensive than in the control healthy skin. The total BVA average in the CLP lesions was 1.21±0.19%, while in the healthy skin it was 0.76±0.11%.

LVA in the CLP lesions was significantly higher (total average 0.81±0.18%) than in the healthy skin (total average 0.44±0.12%) in all topographic regions ($p<0.05$). Lymphatic microcirculation in the CLP lesions was approximately 1.8 times more extensive than in the control healthy skin (Table 2).

ANOVA and Student-Newman-Keuls post hoc tests revealed no significant regional differences in BVA and LVA in the CLP lesions. However, they confirmed regional variability in healthy skin samples. There was significantly higher BVA and LVA in the facial region of the healthy skin compared with the other observed regions.

Pearson's correlation test confirmed only low - statistically insignificant correlation between BVA and LVA (Table 3, Fig. 7) in both healthy skin ($r=0.403$, $p=0.078$) and CLP lesions ($r=0.233$, $p= 0.310$).

VEGF was immunohistochemically detected in the cytoplasm of keratinocytes in basal and suprabasal layers of the epidermis, in fibroblasts, and endothelial cells of the dermis. Neither ANOVA nor Student-Newman-Keuls post hoc test revealed significant regional differences in VEGF expression.

Statistical analysis confirmed significant differences between the VEGF positive area in the healthy skin and in the CLP lesions both in the epidermis and dermis (Table 4). VEGF expression in CLP lesions was 19.1 times higher in the epidermis (8.44±1.12% vs 0.44±0.37%) and 10.3 times higher in the dermis (2.79±0.44% vs 0.27±0.24%) compared to expression in the control healthy skin. The immunoreactivity intensity of VEGF expression was weak and infrequently mild in the CLP lesions and only weak in the healthy skin samples.

Discussion

Cutaneous blood vasculature is arranged in two

Table 3. Pearson's correlation between BVA and LVA in healthy skin and CLP lesions.

topographic region	Pearson's correlation	
	healthy skin r / p-value	CLP lesions r / p-value
abdominal region	0.320 / 0.536	0.341 / 0.408
back region	0.227 / 0.714	-0.323 / 0.532
facial region	0.340 / 0.660	0.288 / 0.712
femoral region	0.231 / 0.769	0.132 / 0.915
totally all regions	0.403 / 0.078	0.233 / 0.310

Table 4. VEGF positive area within the epidermis and dermis in healthy skin (HS) and CLP lesions.

localization	VEGF positive area in %		
	healthy skin (n=20)	CLP lesions (n=21)	HS vs CLP lesions p- value
epidermis	0.44±0.37	8.40±1.12	4.9E-09
dermis	0.27±0.24	2.79±0.44	5.4E-09
epidermis vs dermis t - test p-value	5.0E-04	4.1E-08	
regional variability epidermis p-value	0.988	0.620	
regional variability dermis p-value	0.906	0.551	

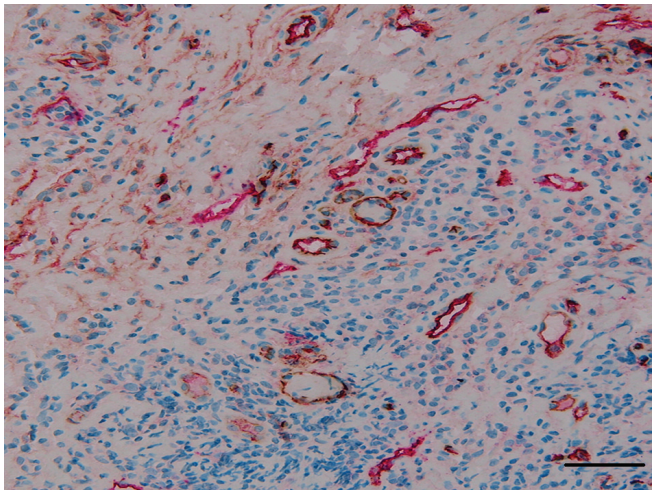

Fig. 3. Blood (brown) and lymphatic (pink - red) microvessels in cutaneous lichen planus lesion. Double immunohistochemical staining with CD34 and D2-40 antibodies. Scale bar: 50 μm.

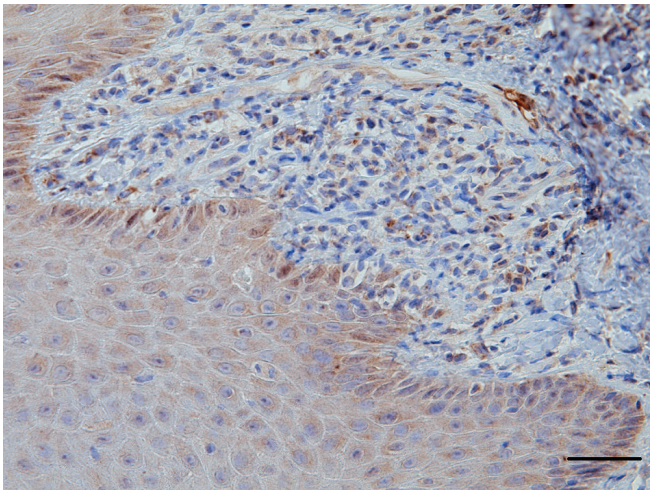

Fig. 4. Immunohistochemical positivity of VEGF - A expression in epidermis and dermis within the cutaneous lichen planus lesion. Scale bar: 50 μm.

plexuses: a deep reticular plexus and a superficial subpapillary plexus. The deep reticular plexus is formed from perforating vessels at the dermal-subcutaneous interface. Vertically ascending vessels fill the superficial subpapillary plexus situated in the papillary dermis, forming capillary loops within the dermal papillae (Braverman, 1989, 2000).

The lymphatic vasculature also forms two plexuses in the vicinity of blood vessels. Initial lymphatics (incorrectly but more often called lymphatic capillaries) of the superficial lymphatic plexus originate in the lower part of the dermal papillae near the blood subpapillary plexus but more distant from the epidermis. From the superficial lymphatic plexus, lymphatic pre-collectors drain vertically into the larger lymphatic collectors within the lower dermis to form the deep lymphatic plexus situated below the deep blood reticular plexus (Ryan et al., 1986; Skobe and Detmar, 2000).

The blood capillaries and initial lymphatics show some structural differences reflecting differences in their function. In contrast to blood capillaries, initial lymphatics are not encircled by pericytes and their basement membrane is poorly developed or absent (Skobe and Detmar, 2000). Interendothelial junctions are continuous (zipper-like) in blood capillaries but discontinuous (button-like) in initial lymphatics (Baluk et al., 2007). The lymphatics have wider and more irregular lumen than blood capillaries (Braverman, 1989; Skobe and Detmar, 2000). Lymphatic endothelial cells are attached to the collagen and elastin fibres within the dermis by anchoring filaments (Gerli et al., 2000). These fibrillin containing anchoring filaments open intercellular channels by pulling adjacent endothelial cells apart, thus permitting easy passage of the fluids and particles into the lymphatics (Skobe and Detmar, 2000).

Cutaneous microcirculation in healthy adults is predominantly quiescent, due to the dominant influence of endogenous angiogenesis inhibitors over the stimuli. However, it retains the capacity for brisk initiation of angiogenesis and lymphangiogenesis.

Cutaneous blood vessels are newly formed by the process of sprouting angiogenesis. It is initiated by vasodilatation, increased vascular permeability and enzymatic degradation of the capillary basement membrane and subsequent activation, proliferation and migration of the endothelial cells. Blood endothelial cells at the tip of the sprouts - tip cells are non-proliferating cells probing the environment. They migrate through the extracellular matrix secreting proteolytic enzymes (matrixmetalloproteinases) which digest a pathway for the developing sprout created by the proliferating endothelial cells – stalk cells. Migration of the endothelial cells is directed by a gradient of angiogenic factors and mediated by integrins, cell-adhesion molecules on the endothelial cells surface. Lumen formation is followed by the maturation and stabilisation of newly formed capillaries by pericyte recruitment and synthesis of a new basement membrane (Carmeliet, 2000, 2003; Karamysheva, 2008; Adair and Montani, 2010). Despite major advances in the field of angiogenesis, lymphangiogenesis is far less explored. Experimental studies have shown evidence for lymphatic vessel formation through tunnelling that relies on extensive matrix remodelling. Migrating lymphatic endothelial cells extend long projections into neighbouring extracellular matrix and form cord-like structures. In contrast to angiogenesis, no endothelial cell specialisation to the tip cells and stalk cells was confirmed, proliferating lymphatic endothelial cells were found both at the tips of sprouting capillaries and inside extending sprouts. Instead of adhering to the basement membrane, lymphatic endothelial cells of initial

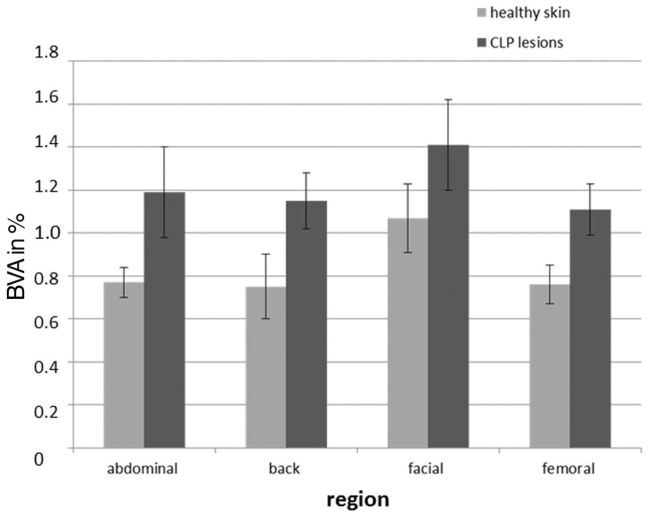

Fig. 5. Graph – Blood vessel area (BVA) in healthy skin and cutaneous lichen planus (CLP) lesions.

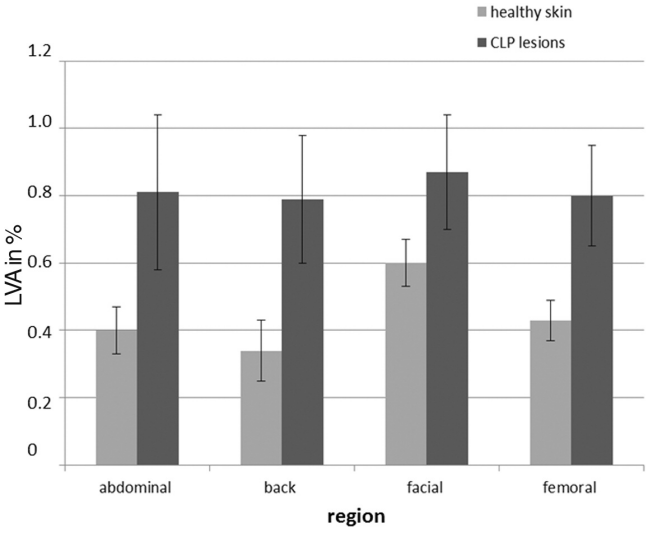

Fig. 6. Graph - Lymphatic vessel area (LVA) in healthy skin and cutaneous lichen planus (CLP) lesions.

lypmhatics are stabilised by anchoring filaments which form an intimate association with collagen fibrils in the interstitial matrix (Karpanen and Alitalo, 2008; Tammela and Alitalo, 2010; Detry et al., 2011). Both angiogenesis and lymphangiogenesis are characterised by the triad of endothelial proliferation, migration, and protease activity (Pepper, 2011).

The angiogenic activators comprise a set of some growth factors and differentiation/specification signals (VEGF family, Tie/angiopoietin system, endoglin, fibroblast growth factor, tumor necrosis factor- α, transforming growth factor -β1, platelet-derived growth factors), adhesion molecules (E-selectin, VE-cadherin, VCAM-1), chemokines (interleukin-8), integrins, and enzymes (matrix metalloproteinases, cyclooxgenase – 2, nitric oxide synthase) that participate in controlling any one of the steps that go into forming a new capillary (Carmeliet and Jain, 2000; Polverini, 2002). Lymphangiogenesis is stimulated by the VEGF family, Tie/angiopoietin system, neuropilin-2, integrin-α 9, fibroblast growth factor, platelet-derived growth factors, and endothelin-1 (Lohela et al., 2003; Christiansen and Detmar, 2011).

The VEGF family represents the main skin angiogenesis stimulators. It includes: VEGF - A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor. VEGF - A stimulates angiogenesis through interaction with VEGFR-1(vascular endothelial factor receptor 1) on blood endothelium and lymphangiogenesis through interaction with VEGFR-2 on lymphatic endothelium (Detmar, 2000; Skobe and Detmar, 2000; Ferrara, 2004; Kataru et al., 2009).

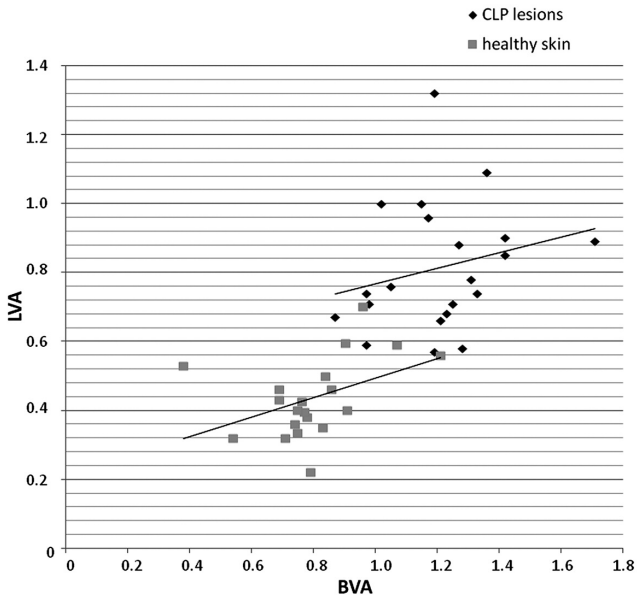

Fig. 7. Graph - Correlation between BVA and LVA in cutaneous lichen planus (CLP) lesions and healthy skin.

VEGF-B is an important proangiogenic factor which interacts through VEGFR-1 and neuropilin on blood vessel endothelium. VEGF-C and VEGF-D primarily regulate lymphangiogenesis through VEGFR-2 and VEGFR-3 on lymphatic endothelium (Detmar, 2000). The blood and lymphatic systems seem to work in a tightly regulated manner. A possible molecular link is supposed between angiogenesis and lymphangiogenesis because VEGF-C expression in vascular endothelial cells is induced by VEGF-A (Nagy et al., 2002). VEGF-A is a major multifunctional stimulator which induces lymphangiogenesis as well as angiogenesis (Nagy et al., 2002). In the skin it is produced predominantly by keratinocytes and to a lesser extent by fibroblasts when the skin is clinically involved. It also enhances microvascular permeability (Leung et al., 1989) and, through activation of specific endothelial cells, adhesion molecules contributes to recruitment of leucocytes in inflamed skin (Detmar, 2000). Animal studies of chronic skin inflammation discovered that VEGF-A induced newly-formed lymphatics that are structurally and functionally abnormal: dilatated with incompetent valves, sluggish flow and delayed lymph clearance. Whereas the angiogenic response was sustained only as long as VEGF-A was expressed, the enlarged lymphatics became VEGF-A independent and persisted long after VEGF-A expression ended (Nagy et al., 2002). Interestingly, another animal study showed that stimulation of lymphangiogenesis by VEGF-C through VEGFR-3 significantly inhibits chronic skin inflammation (Huggenberger et al., 2010).

VEGF- A expression in normal skin is low but it is over-expressed in psoriasis (Detmar et al., 1994; Creamer et al., 2002b), psoriasis-like dermatitis (Canavese et al., 2011), atopic dermatitis (Zhang et al., 2006; Genovese et al., 2012) and skin tumours (Redondo et al., 2000; Urgurel et al., 2001; Johnson and Wilgus, 2012).

VEGF expression in CLP was observed by Salem et al. (2011). In their immunohistochemical study VEGF - A expression was evaluated semiquantitatively (diffusely positive, moderately positive , weakly positive and negative) and without distinguishing of VEGF expression in epidermis and dermis. They noticed significantly higher percentage of moderately positive and diffusely positive VEGF expression in CLP lesions than in the control group. A trend of increasing VEGF positivity with a higher degree of inflammation was also supposed. In our study immunohistochemically detected VEGF-A expression was determined by precise morphometric software and separately in epidermis and dermis. Quantitative analysis revealed remarkably increased VEGF- A expression in CLP lesions both in epidermis (19.1 times higher than in the healthy skin) and dermis (10.3 times higher than in the healthy skin). In clinically involved skin VEGF - A is produced predominantly by keratinocytes and to a lesser extent by fibroblasts of the dermis (Detmar et al., 1995), similarly in our study significantly higher VEGF- A expression

was observed in the epidermis than in the dermis in both lesional and healthy skin.

Significantly increased VEGF expression was also demonstrated in oral lichen planus lesions (Scardina et al., 2009; Tao et al., 2009).

Cutaneous angiogenesis and lymphangiogenesis occur during tissue repair - wound healing (Tonnesen et al., 2000; Zampel et al., 2012) and in numerous diseases including chronic inflammatory skin diseases (Detmar, 2000; Velasco and Lange-Asschenfell, 2002) – atopic dermatitis (Velasco et al., 2009; Genovese et al., 2012), contact dermatitis (Monaco et al., 2004) and especially psoriasis (Braverman and Sibley, 1982; Creamer et al., 1997, 2002b).

In the present study, we highlighted a potent angiogenic activity in the CLP lesions. The blood microcirculatory bed in the CLP lesions was approximately 1.6 times more extensive than in the control healthy skin. Furthermore, our study provided novel morphological evidence of a potent lymphangiogenenic activity in the CLP lesions. Compared to the healthy skin the lymphatic microcirculation in the CLP lesions was approximately 1.8 times more extensive. As for angiogenesis, our results are consistent with the findings of Hussein (2007) who found 1.7 times higher microvessel density (MVD) in the CLP lesions. Salem et al. (2001) ascertained approximately 3 times higher MVD in lesionalskin than in the control healthy skin. The angioproliferation was also confirmed in oral lichen planus lesions. Immunohistochemical studies on oral lichen planus published by Tao et al. (2007), Scardina (2009) and Mittal et al. (2012) showed 1.5, 4.5 and 2.2 times increased MVD, respectively. Significantly higher MVD was observed in erosive than in reticular oral lichen planus (Tao et al., 2007; Mittal et al., 2012). Scardina and Mesina (2009) in their videocapillaroscopic study demonstrated 2 times increased vessel density per mm2, insignificantly increased loop length and significantly increased diameter. They have supposed that increased vessel density could be an indicator of the evolutionary condition of lingual lichen planus, useful for evaluating of the disease progression or regression (Scardina and Mesina, 2009). Assessment of vasculature in the papillary dermis of psoriatic lesions demonstrated a 4 times increase in endothelial surface area of lesional compared with non-lesional skin (Creamer et al., 1997)

In comparison to the mentioned papers, the present study provides novel morphological evidence of both angiogensis and lymphangiogenesis in CLP lesions.

Despite the fact that healthy skin showed regional variability in blood and lymphatic vessel area, what had been previously reviewed by Pasyk et al. (1989), CLP lesions had equal levels of blood and lymphatic vessel areas in all topographical regions, reflecting the similar inflammation stage because all biopsy specimens were taken from untreated patients who had undergone their first CLP attack with typical inflammatory activity in the histopathological picture. This level of vascularisation seems to be sufficient to fulfil the metabolic needs associated with hyperproliferation in inflamed tissue and increased displacement of the inflammatory cells in the microenvironment of the lesion.

Although the relation between angiogenesis and chronic inflammation has received much attention in recent years, at this moment it is still unclear if these processes are cause or result. While inflammation and angiogenesis are capable of potentiating each other, these processes are distinct and separable (Granger et Senchenkova, 2010).

Chronic inflammation is characterized by proliferation, migration and recruitment of inflammatory cells and tissue. Growing tissue mass with an abundance of inflammatory cells leads to relative hypoxia and increased metabolic demands in the site of inflammation (Jackson et al., 1997). Hypoxia is a common stimulus for angiogenesis and accumulation of immune cells (Costa et al., 2007). Recruitment and attraction of T-cells is activated by dendritic cells and cytokine –mediated upregulation of adhesion molecules on endothelial cells (ICAM-1, VCAM-1, E-selectin). T-cells activate, in turn, macrophages, endothelial cells and fibroblasts to produce pro-inflammatory cytokines, chemokines and matrix metalloproteinases (Costa et al., 2007; Scardina et al., 2009; Mittal et al., 2012). Furthermore, T-cells activated by some cytokines (tumour necrosis factor α, interleukin-6 and interleukin-2) stimulate fibroblasts and tissue macrophage production of VEGF at inflammatory sites, leading to angiogenesis (Monaco et al., 2004; Costa et al., 2007). Conversely, newly formed vessels maintain the chronic inflammatory state by transporting inflammatory cells to the site of inflammation and supplying nutrients and oxygen to the proliferating inflamed tissue. The increased endothelial cell area provides expanded capacity for the production of adhesion molecules and inflammatory cytokines (Jackson et al., 1997).

This feed-back mechanism and molecular cross-link between chronic inflammation and angiogenesis enhances both these processes (Monaco et al., 2004; Costa et al., 2007; Scardina et al., 2009). The newly formed vessels reserve not only tissue nutrition but also enhance the turnover of the inflammatory cells. There is strong evidence that the angiogenesis that accompanies chronic inflammation tends to prolong and intensify the inflammatory response (Jackson et al., 1997; Scardina et al., 2009).

Lymphatic vessels regulate the inflammatory response by the transport of fluid, extravasated leukocytes, and antigen-presenting cells from the inflamed tissue to the lymph nodes and to other secondary lymphoid organs (Zgraggen et al., 2013). It has been suggested that some inflammatory mediators (prostaglandins, histamin) negatively affect lymphatic contractility resulting in lymphatic insufficiency and a compensatory lymphatic vessel growth (Alitalo et al., 2005; Tammela and Alitalo, 2010). Macrophages recruited into the inflammation site can transform into

VEGF-C/D-producing cells, which can initiate lymphangiogenesis (Schoppmann et al., 2002) or they can be transdifferentiated into the lymphatic endothelial cells and incorporated into lymphatic endothelium to contribute to lymphatic vessel growth (Maruyama et al., 2005). It has been suggested that lymphangiogenesis in chronic inflammation reduces tissue edema and enhances immune responses by promoting macrophages and dendritic cell recruitment (Baluk et al., 2005; Kataru et al., 2009).

Conclusion

Finally, our results confirm the evidence that angiogenesis and lymphangiogenesis are tightly connected to another chronic inflammatory disease - cutaneous lichen planus. Although extensive evidence supports the relation between inflammation and angiogenesis/lymphangiogenesis, the pathophysiological consequences and molecular mechanisms underlying this association are largely unclear and they need further investigation.

While in acute inflammation the microcirculatory bed shows predominantly functional changes, in chronic inflammation some functional changes persist and structural changes occur (Majno, 1998). No data are available for the functional changes of the blood and lymphatic vessels in CLP yet, though experimental models of chronic inflammation have shown blood flow changes (Granger et Senchenkova, 2010), impaired arteriolar reactivity (Holzer, 1998), reduced capillary perfusion and increased vascular permeability (Nagy et al., 2008). The activation products and chemical mediators released from inflammatory cells act as inducers of the phenotypic changes in microvessel function that accompany chronic inflammation (Granger and Senchenkova, 2010).

Regulation of angiogenesis in tumour biology has been well studied and clinically implemented in antiagiogenic therapy. The interplay between angiogenesis and chronic inflammation could be of potential benefit for therapeutic approaches against both angiogenesis and chronic inflammation. Targeting angiogenesis holds the promise of decreasing turnover of inflammatory cells, reduced production of inflammatory mediators, and preventing nutrient supply to the running inflammatory process. Conversely, targeting inflammation would also negatively affect production of angiogenesis stimulators and blood vessel formation (Jackson et al., 1997; Costa et al., 2007).

Activated blood and lymphatic vasculature could be a potential therapeutic approach in chronic inflammatory diseases. The latest findings indicate that inhibition of angiogenesis and, surprisingly, activation of lymphangiogenesis might serve as a novel strategy for treating chronic inflammatory diseases. Promising results have been obtained from experimental transgenic mouse models of chronic skin inflammation, especially in psoriasis where systemic anti-VEGF treatment strongly reduces skin inflammation (Schonthaler et al., 2009).

Conflict of interest: The authors declare that they have no competing interests.

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