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## Immunohistomorphometry of VEGF-Levels in Periodontitis and Systemic Sclerosis (Ssc)

**Language:** English

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**Introduction**

Vascular endothelial growth factor (VEGF) as an angiogenetic cytokine is a potent growth factor for endothelial cell proliferation (Kikuchi et al. 1998). Its level has been proven to be elevated in systemic sclerosis (SSc) as well as in periodontitis (Booth et al. 1998;Chapple et al.2000; Harada et al. 1998).

**Objectives**

It was the aim of this study to demonstrate whether VEGF-levels in immunostained gingival biopsies quantified by immunohistomorphometry may be regarded as a specific risk indicator for the progression of SSc with periodontitis in comparison to periodontitis alone.

**Material and Methods**

Within indicated surgical procedures (Fig. 1) biopsies were taken from 13 patients with SSc (Fig.2) leading to 29 preparations and from 8 patients with chronic periodontitis without SSc leading to 13 preparations.

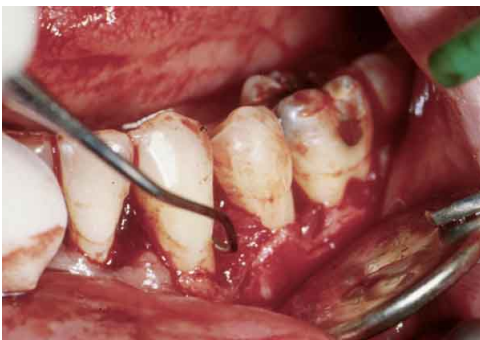


Fig.1:  
Indicated surgical procedure within a patient with advanced periodontitis.

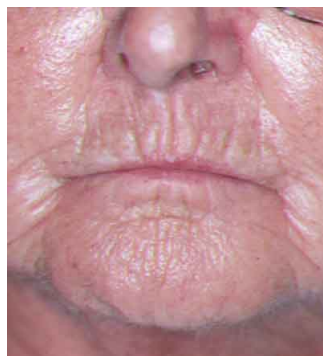


Fig. 2a:  
Patient with systemical  
sclerosis showing  
microstomia.

Fig.2b:  
Patient with systemical  
sclerosis showing perioral  
pleating.

Immunohistochemical testing was performed applying the monoclonal antibody anti-human VEGF Clone G 153-694 (Pharmingen, San Diego, CA92121,USA)(Fig. 3 and 4).

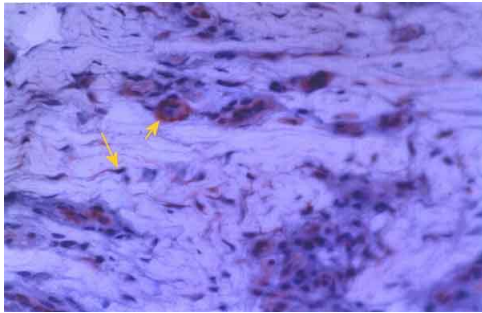


Fig.3: Positive Immunohisto-chemical staining in a patient with periodontitis. The arrows are pointing to VEGF expression within the wall of a blood vessel and around fibroblasts.

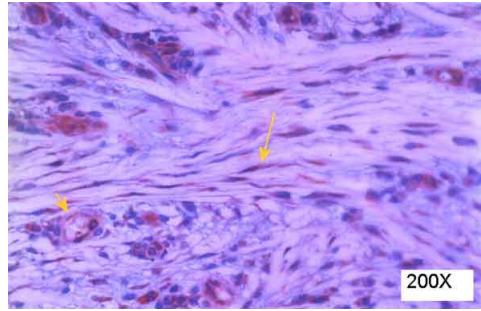


Fig.4: The slide preparation of a patient with SSc and periodontitis performs positively stained areas within blood-vessel walls and around fibroblasts too.

Transverse gingival sections from each patient sample were analyzed for VEGF expression evaluation.

The area of positive immunostaining in the gingival subepithelial connective tissue was measured using a microscope connected to a computerized video digital system (JAVA video analysis software, Jandel Scientific, Corte Madera, Ca) (Fig.5a and b) at a magnification of x100.

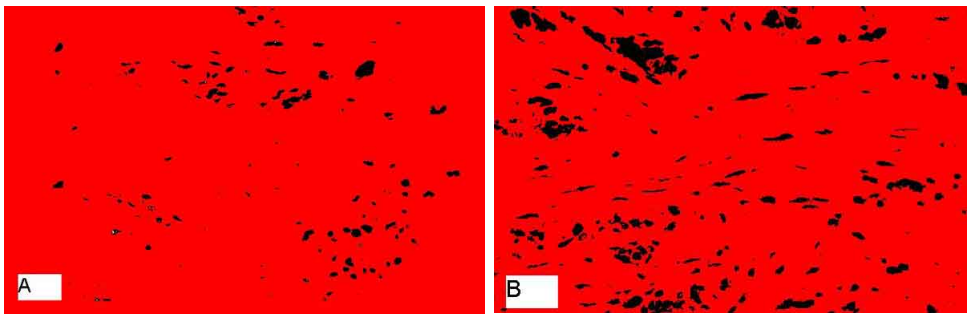


Fig.5a, b:  
As an example this figure demonstrates that the black areas in A show the positively stained areas from Fig.3 in a patient with periodontitis and Fig. 5b figuring the areas stained in the slide preparation of a patient with SSc and periodontitis from Fig.4.

For each sample five different areas were analyzed and the data were pooled to represent a mean value. The results were expressed in percentage of the positively immunostained area per total connective tissue area measuring 0.3615mm<sup>2</sup>. The data gained were combined in each group of patients to create a group mean and pooled estimate of standard error. The significance was evaluated applying the Mann-Whitney-U-test.

## Results

The mean percentage of the positively immunostained area for VEGF in SSc was 7.6%±2.2 while measuring 5.3%±1.2 for chronic periodontitis. The mean rank for SSc was 26.6 and 10.12 for periodontitis. The rank sum for SSc was 771.5 and 131.5 for periodontitis. Thus the difference was highly significant (p<0.001).

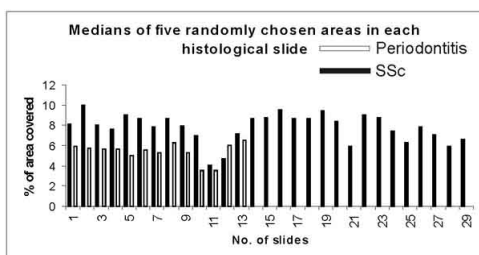


Fig.6: Periodontitis constantly provokes less staining.

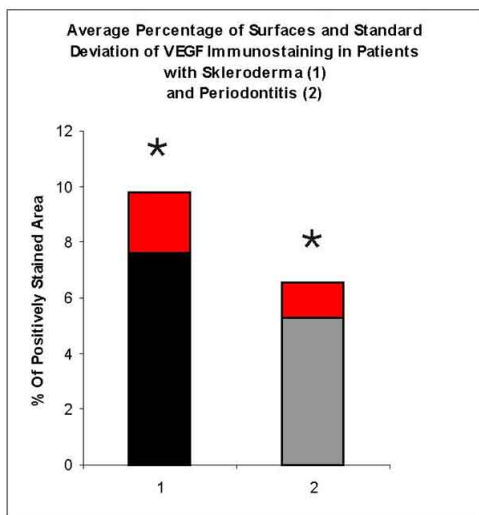


Fig.7: SSc and Periodontitis lead to a significantly higher expression of VEGF than periodontitis alone.

Tab.1: Mann-Whitney-U-Test

Disease	N	Mean Rank	Rank Sum
SSc	29	26,60	771,50
Periodontitis	13	10,12	131,50

## Conclusions

Using quantitative immuno-histomorphometry reveals VEGF expression in gingival biopsies to be elevated more in SSc than in chronic periodontitis. Our results suggest to use VEGF as a molecular marker to distinguish between SSc associated with periodontitis and periodontal diseases alone. Thus further studies have been initiated with larger groups of patients to corroborate or confute that VEGF is a sufficient parameter for risk assessment in SSc progression in comparison to progression in periodontal diseases (Johnson et al 1999) .

## Literature

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## Abbreviations

SSc = Systemic Sclerosis  
VEGF = Vascular Endothelial Growth Factor

*This Poster was submitted by Prof. Dr. med. dent. habil. Wolf-Dieter Grimm.*

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# Immunohistomorphometry of VEGF-Levels in Periodontitis and Systemic Sclerosis (Ssc)

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### Introduction and Aim:

Vascular endothelial growth factor (VEGF) as an angiogenetic cytokine is a potent growth factor for endothelial cell proliferation (Kikuchi et al. 1999). Its level has been proven to be elevated in systemic sclerosis (SSc) as well as in periodontitis (Booth et al. 1999, Chapple et al. 2000, Harada et al. 1999). It was the aim of this study to demonstrate whether VEGF-levels in immunostained gingival biopsies quantified by immunohistomorphometry may be regarded as a specific risk indicator for the progression of SSc with periodontitis in comparison to periodontitis alone.

### Materials and Methods:

Within indicated surgical procedures (Fig. 1) biopsies were taken from 13 patients with SSc (Fig. 2) leading to 29 preparations and from 8 patients with chronic periodontitis without SSc leading to 13 preparations.



Fig. 1. Indicated surgical procedure in a patient with advanced periodontitis.



Fig. 2. Patients with systemic sclerosis showing sclerotic skin and microstomia.

Immunohistochemical testing was performed applying the monoclonal antibody anti-human VEGF Clone G 153-864 (Pharmingen, San Diego, CA92121, USA) (Fig. 3 and 4).

Transverse gingival sections from each patient sample were analyzed for VEGF expression evaluation.

The area of positive immunostaining in the gingival subepithelial connective tissue was measured using a microscope connected to a computerized video digital system (JAWA video analysis software, Jandel Scientific, Corte Madera, Ca) (Fig. 5a and b) at a magnification of x100. For each sample five different areas were analyzed and the data were pooled to represent a mean value. The results were expressed in percentage of the positively immunostained area per total connective tissue area measuring 0.3615mm<sup>2</sup>. The data gained were combined in each group of patients to create a group mean and pooled estimate of standard error. The significance was evaluated applying the Mann-Whitney-U-Test.

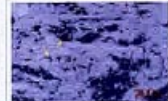


Fig. 3. Positive immunohistochemical staining in a patient with periodontitis. The arrows are pointing to VEGF expression within the wall of a blood vessel and around fibroblasts.

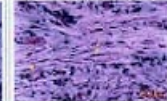


Fig. 4. The side preparation of a patient with SSc and periodontitis performs positively stained areas within blood vessel walls and around fibroblasts.



Fig. 5. As an example this figure demonstrates that the black spots in A show the positively stained areas from Fig. 3 in a patient with periodontitis and B figuring the areas stained in the side preparation of a patient with SSc and periodontitis from Fig. 4.



### Results:

The mean percentage of the positively immunostained area for VEGF in SSc was 7.6%±2.2 while measuring 5.3%±1.2 for chronic periodontitis. The mean rank for SSc was 26.6 and 10.12 for periodontitis. The rank sum for SSc was 771.5 and 131.5 for periodontitis. Thus the difference was highly significant (p<0.001).

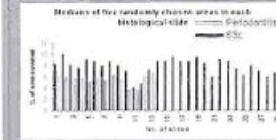


Fig. 6. Periodontitis consistently provides less staining.

Group	n	Median	Q1	Q3	Max/Min	U-Value
SSc	29	26.6	11.8	42.2	4.2/52.2	48.522
Periodontitis	13	10.12	5.3	14.9	2.2/22.2	131.5

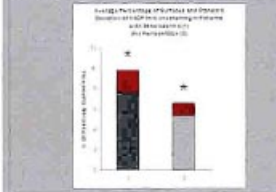


Fig. 7. SSc and Periodontitis lead to a significantly higher expression of VEGF than periodontitis alone.

### Conclusion:

Using quantitative immunohistomorphometry reveals VEGF expression in gingival biopsies to be elevated more in SSc than in chronic periodontitis. Our results suggest to use VEGF as a molecular marker to distinguish between SSc associated with periodontitis and periodontal diseases alone. Thus further studies have been initiated with larger groups of patients to corroborate or confuse that VEGF is a sufficient parameter for risk assessment in SSc progression in comparison to progression in periodontal diseases (Johnson et al. 1999).

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Booth V, Harty S, Crossley A, Zaiman M, Paveley F. Vascular endothelial growth factor in human periodontal disease. *J Periodontol Res* 1998; Nov; 33(8): 491-9.