**lek. Magdalena Katarzyna Maciejewska**

# Nowe biomarkery zaburzeń mikrokrążenia w twardzinie układowej.

**Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne**

Promotor: prof. dr hab. Lidia Rudnicka

Katedra i Klinika Dermatologiczna Warszawski Uniwersytet Medyczny

Kierownik Kliniki: prof. dr hab. n. med. Lidia Rudnicka


Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych Warszawskiego Uniwersytetu Medycznego

Warszawa 2023

# SUMMARY

## NEW BIOMARKERS OF MICROVASCULAR IMPAIRMENT IN SYSTEMIC SCLEROSIS.


Introduction

Systemic sclerosis is an autoimmune connective tissue disease associated with progressive fibrosis of the skin and internal organs. According to the current concept of disease pathogenesis, endothelial cell damage, microcirculation disorders, and inflammation develop initially. The process of fibrosis itself seems to be secondary to stimulation by cytokines released from the endothelium. Clinical manifestations of circulatory disorders include renal crisis, pulmonary hypertension, or Raynaud's phenomenon, which often precedes the appearance of other features of systemic sclerosis by several years and is found in over 95% of patients. Critical ischemia together with ulceration of the distal parts of the fingers is the most severe form of microcirculation disorder, leading to pain, disability, and reduced quality of life.

Currently, it is challenging to identify patients at risk of rapid disease progression and development of severe organ complications at an early stage. For these reasons, the aim of the study is to search for new biomarkers associated with vascular damage in the course of systemic sclerosis, to assess their relationship with disease activity and response to vascular therapy.

Objective

The aim of the study was to analyze microcirculatory disorders leading to Raynaud's phenomenon and renal pathology in patients with systemic sclerosis, as well as to search for new biomarkers associated with vascular damage in systemic sclerosis, including in particular:

1. Evaluation of the potential use of copeptin and hypoxia-inducible factor-1α (HIF-1α) as biomarkers for microcirculatory disorders in systemic sclerosis.
2. Assessment of the potential correlation between the concentration of copeptin and hypoxia-inducible factor-1α and the activity of systemic sclerosis.
3. Analysis of the impact of rheological treatment with alprostadil on the concentration of selected biomarkers of microcirculatory disorders in patients with systemic sclerosis.

## Material and methods

50 patients with systemic sclerosis and qualified for rheological treatment with alprostadil have been enrolled in the study according ACR/EULAR classification criteria. The control group included 30 healthy volunteers who were matched for age and sex. Organ involvement was assessed according to the diagnostic and therapeutic recommendations of the Polish Society of Dermatology. Vascular disorders have been assessed on skin of the face and limbs (number and location of digital ulcers, telangiectasia), nailfold capillaroscopy image classified according to Cutolo scale.

Hypoxia-inducible factor-1α (HIF-1α) and copeptin were selected as potential biomarkers for analysis. HIF-1 and copeptin concentrations were assessed by ELISA. Statistical significance level was assumed for $p<0.05$.

## Results

We found significantly higher copeptin concentration in patients with systemic sclerosis (4.21 pmol/L [3.04-5.42]) in comparison to control group (3.40 pmol/L [2.38-3.76], $p<0.01$). Copeptin significantly correlated with Raynaud's condition score ($r=0.801$, $p<0.05$). Patients with "late" capillaroscopic patterns had higher copeptin concentrations (5.37 pmol/L [4.29-8.06]) than patients with "early" (2.43 pmol/L [2.25-3.20], $p<0.05$) and "active" patterns (3.93 pmol/L [2.92-5.16], $p<0.05$]). Copeptin was found to be significantly higher in systemic sclerosis patients with DUs (5.71 pmol/L [IQR 4.85–8.06]) when compared to systemic sclerosis patients without DUs (3.31 pmol/L, [2.28-4.30], $p<0.05$). Additionally, copeptin concentration had good diagnostic accuracy in discriminating between patients with and without digital ulcers (AUC=0.863). Alprostadil decreased copeptin concentration from 4.96 [4.02-6.01] to 3.86 pmol/L [3.17-4.63] ($p<0.01$) after 4-6 cycles of administration.

The results show a marked increase in hypoxia-inducible factor-1α levels in patients with systemic sclerosis (3.042 ng/ml [2.295-7.749]) when compared to the control group (1.969 ng/ml [1.531-2.903] $p<0.01$). Patients with both, diffuse cutaneous SSc (2.803 ng/ml, IQR 2.221-8.799) and limited cutaneous SSc (3.231 ng/ml, IQR 2.566-5.502) displayed an increased serum hypoxia-inducible factor-1α levels compared to the control group ($p<0.01$). We found a notable increase in hypoxia-inducible factor-1α plasma concentration in patients with an "active" pattern (6.625 ng/ml, IQR 2.488-11.480) compared to those with either an "early" pattern (2.739, IQR 2.165-3.282, $p< 0.05$) or a "late" pattern (2.983 ng/ml, IQR 2.229-3.386, $p<0.05$). Patients with no history of digital ulcers had significantly higher levels of hypoxia-

inducible factor-1α (4.367 ng/ml, IQR 2.488-9.462) compared to patients with either active digital ulcers (2.832 ng/ml, IQR 2.630-3.094, $p<0.05$) or healed digital ulcers (2.668 ng/ml, IQR - 2.074-2.983, $p<0.05$).

Conclusion

1. Copeptin and hypoxia-inducible factor-1α (HIF-1α) may serve as potential biomarkers for peripheral microcirculatory disorders in patients with systemic sclerosis
2. The serum concentration of copeptin shows a positive correlation with the severity of Raynaud's phenomenon and the occurrence of peripheral ulcers in systemic sclerosis. It is also a sensitive parameter for response to rheological treatment. The serum concentration of copeptin is a sensitive parameter for monitoring the response to rheological treatment
3. Serum hypoxia-inducible factor-1 alpha concentration is statistically higher in patients with an "active" capillaroscopic pattern of systemic sclerosis according to Cutolo's classification, compared to patients with an "early" and "late" pattern
4. Monitoring the concentrations of copeptin and hypoxia-inducible factor-1 alpha in patients with systemic sclerosis may allow the identification of individuals at high risk of severe microcirculation disorders and, consequently, the early initiation of intensive rheological treatment