

Ioannis Maximilian Spanidis

Dr. med.

## **High Frequency Electrical Muscle Stimulation for Diabetic Polyneuropathy – Effects on Hematopoietic Stem Cells**

Fach: Innere Medizin

Doktorvater: Prof. Dr. med. Dr. h.c. Peter Nawroth

Diabetic Polyneuropathy is a common late complication of Diabetes. The underlying pathomechanism for the development of diabetic polyneuropathy remains unclear and causal treatments are still not available. Glucose control has been of little benefit in the prevention and treatment diabetic polyneuropathy in type 2 diabetes. The role of vascular disease and especially damage to vasa nervorum is of interest in the generation of nerve fiber damage that leads to diabetic polyneuropathy. Oxidative stress due to reactive oxygen species and reactive carbonyl species, like methylglyoxal, contributes to nerve damage in diabetes. Circulating stem cells of hematopoietic origin are known to be crucial in the repair of damaged endothelia and angiogenesis. Their frequency in the blood of diabetes patients and even more in patients that are diagnosed with diabetic polyneuropathy is lower than in healthy individuals. They are also less responsive to activating and migratory stimuli in this condition. Electrical Muscle Stimulation has been successfully used to alleviate symptoms especially of painful diabetic polyneuropathy. The aim of this study was to investigate possible effects of electrical muscle stimulation on glucose metabolism and hematopoietic stem cells in diabetes

28 Patients that reported symptoms of diabetic polyneuropathy underwent 4 treatment sessions of electrical muscle stimulation over a period of 2 weeks, of which 24 completed the study protocol. A control group consisting of 9 healthy individuals were treated once and 6 of these participants underwent a sham-treatment prior to the actual treatment. The effects on diabetic polyneuropathy symptoms were assessed and blood and urine samples were taken before the treatment, after the first treatment and before the last treatment. Plasma antioxidant capacity and methylglyoxal levels were measured. Peripheral blood mononuclear cells were isolated and analyzed with flow-cytometry for markers of hematopoietic stem cells, endothelial progenitor cells and activity, migration and differentiation markers of these cells.

Patients showed a significant improvement in symptoms of diabetic polyneuropathy in all scoring systems used. Markers of glucose metabolism or renal function were not affected by the treatment, neither were levels of methylglyoxal, nor the plasma antioxidative capacity. Normetanephrine and metanephrine levels in the blood significantly decreased over the study period. Electrical muscle stimulation led to an immediate decrease in the frequency of CD34 positive hematopoietic stem cells in both diabetic and non-diabetic participants. CD34 was higher expressed on the remaining hematopoietic stem cells. The treatment resulted in a higher frequency of endothelial progenitor cells and prone to migration, and differentiation subgroups of hematopoietic stem cells that express CXCR4, JAM-A or CD31. Cells that were positive for these markers also expressed more of these surface antigens after the treatment. Furthermore, the size of endothelial progenitor cells was increased after two weeks of treatment.

Findings of earlier studies that electrical muscle stimulation can attenuate symptoms of diabetic polyneuropathy were confirmed with this study. Effects were measurable after an

even shorter treatment period. Still the used tools to assess the neuropathic symptoms could be adjusted in future investigations. Glucose metabolism and levels of toxic metabolites of glucose, like methylglyoxal, were not affected by the treatment. Hematopoietic stem cells disappeared from the blood stream right after the treatment, which is hypothesized to be a result of attachment to damaged endothelia and migration of these cells. The upregulation of markers for differentiation and migration could be of benefit for the therapy of diabetic polyneuropathy. A higher fraction of hematopoietic stem cells was differentiated into endothelial progenitor cells, which are normally decreased in diabetic conditions and express angiogenic properties. These changes however were not correlated to any clinical symptom scoring and could not be found in a third sample that was taken before the last treatment. This could mean that the differentiating and migrating stem cells leave the blood stream and are therefore not found in the blood 2 days after the treatment. The initial frequency of hematopoietic stem cells was restored after this period. This study confirmed previously in vitro described effects of electrical currents on hematopoietic in an in vivo setting. Further research should be conducted on whether this treatment can provide a longer lasting effect on hematopoietic stem cells and possibly mesenchymal stem cells. Furthermore, in vitro experiments are needed to confirm the changes that were seen on these cells. Electrical muscle stimulation could be a safe and effective way to support activation of hematopoietic stem cells in vivo.