

## SUMMARY

The diabetic foot (DF) develops based on a complex of pathophysiological processes which are presented in patients with diabetic complications. DF significantly increases a patient morbidity and mortality and markedly impairs the quality of life of these patients.

The theme of the dissertation “immunological abnormalities and infection complications in patients with the DF and therapy of the DF by off-loading” was divided into 3 parts:

- abnormalities of immune functions in connection with diabetes and its complications, especially with the DF
- infectious complications of the DF
- therapy of the DF by off-loading.

Problems concerning the impairment of immune functions in connection with diabetes and the DF were resolved in 5 studies, infectious complications of the DF in 3 studies and the therapy of the DF by off-loading similarly in 3 studies.

### **Abnormalities of immune functions in connection with diabetes and its complications, especially with the DF**

The first study deals with the effect of acute hyperglycemia and/or hyperinsulinemia on function of PMN cells in healthy subjects. The aim of this study was to assess the effect of short lasting acute metabolic changes on phagocytosis and the respiratory burst of PMN cells in healthy subjects in vivo. Acute hyperglycemia and/or hyperinsulinemia were induced by three clamp studies – HHC, HEC and HGC with endogenous blockade of insulin secretion by somatostatin. PMN phagocytosis and respiratory burst were assessed by flow cytometry under basal conditions and after E.coli stimulation. The results found during clamp studies were compared to data detected during the control clamp. Immune functions of PMN cells were not significantly influenced by short lasting hyperglycemia and/or hyperinsulinemia in healthy subjects in comparison with the control clamp.

The second study was focused on the evaluation of possible abnormalities of PMN functions caused by short lasting metabolic changes induced in vivo in patients with Type 1 diabetes. Acute hyperglycemia and hyperinsulinemia were induced by four hours lasting HHC. PMN functions were assessed similarly as in the first study. Indexes of respiratory burst detected after E.coli stimulation and all phagocytic indexes found during HHC were not significantly different in contrast to the control clamp. Short lasting hyperglycemia with hyperinsulinemia induced in vivo in patients with Type 1 diabetes did not influence significantly the immune functions of PMN cells.

The following two studies examined the potential effects of diabetes decompensation and chronic inflammation on functions of nonspecific and specific immune systems in patients with the DF. No significant differences were found in the respiratory burst of PMN cells detected under basal and the stimulated conditions between patients with the DF and healthy subjects. There was found a significantly reduced percentage of phagocytosing PMN cells after E.coli stimulation in patients with the DF compared to healthy subjects.

Significantly higher values of markers of inflammation (CRP, leukocyte and neutrophil accounts) were found in patients with the DF compared to healthy controls. However, this increase oscillated in most cases in normal values or was slightly above the norm. A higher percentage of cytotoxic (CD8+) T-lymphocytes and lower immunoregulatory index were found in patients with the DF. Immunoregulatory index correlated positively with HbA1c. Significantly higher serum levels of IgA, IgG and CIK were displayed in patients with the DF. Serum levels of IgM, C3 and C4 complement were not significantly different from the values detected in healthy subjects.

The fifth study assessed changes in the serum levels of micronutrients and their possible effect on the nonspecific immune system in patients with the DF. Significantly reduced serum levels of vitamin C were found in patients with the DF compared to patients with Type 2 diabetes without complications and healthy subjects. Significantly higher serum levels of Cu and lower levels of Mg were detected in both groups of diabetic patients compared to healthy controls. Serum levels of other micronutrients (vitamin E, its isomers, Zn and Fe) did not differ significantly among the study groups. Cu and Mg only correlated significantly with the parameters of respiratory burst of PMN cells in all study subjects.

#### **Infectious complications of the DF**

The sixth study referred to the infectious complications in different groups of patients with the DF. The aim was to assess possible differences in the occurrence of microbial agents in diabetic foot ulcers and differences in their ATB resistance among transplant, dialyzed and other patients with the DF. The occurrence of individual microbial agents did not differ significantly among study groups, but the observed groups of patients differed significantly in the ATB resistance. Transplant patients had significantly more *Staphylococcus* sp. resistant to Oxacillin, Imipenem, Co-trimoxazole and Erythromycin and *Enterococcus* sp. resistant to Ampicillin, Piperacilin and Ticarcillin/clavulanic acid compared to other groups. *Pseudomonas* sp. was more resistant to Piperacillin and Cefpirom in dialyzed patients with the DF in contrast to other two groups.

The aim of the seventh study was to evaluate whether resistance to oral ATB could form a risk factor for lower limb amputations. Consecutively included into the study were all patients

treated during one year for diabetic foot ulcers with at least one positive wound swab. According to logistic regression analysis were OM and peripheral arterial disease (PAD) together with resistance to oral ATB significant risk factors for lower limb amputations. It was shown that the association of resistance to lower limb amputations was significant especially in patients without OM.

Mycotic agents could contribute to the development of diabetic foot ulcers and could slow the healing process. The aim of the eighth study was to specify mycotic diagnosis in patients with the DF. There were compared the occurrences of mycotic agents found in hyperkeratosis and ulcers, the concordance of microscopic and culture mycotic assessments and culture mycotic findings from hyperkeratosis and ulcers. Culture and microscopic findings did not correspond in patients, the concordance of culture mycotic findings from ulcers and hyperkeratosis was only 56% in the same patient.

#### **Therapy of the DF by off-loading**

The appropriate method of lower limb off-loading is one of the factors contributing to the DF healing. The aim of the ninth study was to evaluate the effectiveness of the DF therapy by r-TCC in patients with neuropathic foot ulcers, acute ChaO and neuropathic fractures. Neuropathic fractures healed for a significantly shorter time in contrast to the other two groups as well the number of healed patients was the highest in this group. Therapy by r-TCC was also successful in patients with neuropathic foot ulcers (ulcer size reduction) and acute ChaO (decrease of disease activity).

The aim of the tenth study was to determine the incidence of complications of r-TCC therapy and to assess the effect of TCC on OM healing and risk of new OM development in patients with the DF. The most frequent complications of r-TCC therapy were technical complications and the development of a new contact ulcer. Progression of local finding was found in 17% of patients treated by r-TCC. Other complications such as mycotic infection or joint pain were rarely seen. The number of patients with healed OM was significantly higher compared to the number of patients with newly developed OM. Patients with unhealed OM

were characterized by OM location in tarsal bones and patients with newly developed OM by deeper foot ulcers infected by resistant pathogens.

The aim of the eleventh study was to compare the off-loading therapy performed by r-TCC and orthosis RCW. A significantly higher number of healed patients with OM and a lower number of patients with newly developed OM was found in patients treated by r-TCC in contrast to patients treated by RCW. A risk/benefit ratio was significantly lower in patients treated by r-TCC compared to patients treated by RCW.

**In conclusion:**

**Abnormalities of immune functions in connection with diabetes and its complications, especially with the DF**

- Acutely induced hyperglycemia and/or hyperinsulinemia did not influence significantly functions of nonspecific immune system as in healthy subjects as in patients with diabetes mellitus Type 1.

This model corresponds better with the real conditions present in patients with diabetes mellitus than to date published in vitro tests.

- *Patients with chronic infection within the DF had following changes of immune functions:*

- Slightly reduced percentage of phagocytosing PMN cells after E.coli stimulation compared to healthy controls.

- Significantly higher serum levels of IgA and IgG in contrast to healthy controls.

- Higher number of CD8+ T-lymphocytes and significantly lower immunoregulatory index in comparison with healthy controls.

- Only moderate elevation of typical markers of inflammation (CRP and leukocytes), in norm in 69% and 83% of patients.

Based on these findings, it is possible to presume that the activation of immune system and the inflammatory reaction are not adequate despite the unproven significant immunodeficiency.

- *Patients with chronic infection within the DF had the following changes of micronutrients related to immune functions:*

- Significantly lower serum levels of vitamin C in comparison with healthy controls and patients with Type 2 diabetes without diabetic complications.

- Significantly higher serum levels of Cu and lower levels of Mg in contrast to healthy subjects. The same findings were presented in patients with Type 2 diabetes mellitus without diabetic complications.

These changes could be related to immune functions, especially to the intent of impact of redox potential, differentiation of immune cells and ROI production.

**Infectious complication of the DF**

- There was found a higher occurrence of microbial ATB resistance especially in transplant patients compared to patients on hemodialysis and other patients with the DF. Based on these findings, it is possible to modify an empiric ATB treatment in risk groups of patients with the DF.

- The DF patients were compared for risk factors of lower limb amputations – not only OM and PAD but also microbial resistance to oral ATB belonged to risk factors according to logistic regression analysis. Therefore, we recommend to take this fact into consideration and perform ATB therapy adequately effectively especially in risk groups of patients.

- No concordance of microscopic and culture mycotic findings from hyperkeratosis and diabetic foot ulcers was proved. Culture findings from samples taken from hyperkeratosis and ulcers were different in the same patient.

Therefore, we recommend performing a culture evaluation to assess mycotic agents in the DF instead of microscopic assessment and to evaluate samples taken as from ulcers as from the surroundings.

### **Therapy of the DF by off-loading**

- We confirmed the beneficial effect of therapy performed by r-TCC, especially in patients with neuropathic fractures and also in patients with ChaO and neuropathic foot ulcers. We found a relatively high number primarily of technical complications; therefore, we modified the original method.
- Unlike “the traditional indications”, we verified that this method is able to apply successfully in patients with OM since the benefit of this therapy is higher than the risk of new OM development in patients with the DF, however, provided that they will be controlled carefully (!OM in tarsal bones and ulcers of W3 infected by multiresistant pathogens).
- The therapy performed by r-TCC is more suitable than orthosis RCW in the off-loading treatment of patients with OM.

The results of dissertation contributed as to the clarification of some pathogenic consequences of the DF, especially in the field of immunological abnormalities and infection, as to the improvement of therapy primarily in the field of special forms of off-loading methods. Assessment of the effect of acute hyperglycemia and hyperinsulinemia in vivo on immunological parameters, evaluation of consequences of ATB resistance with the DF complications and possible empiric therapy modification, especially in risk groups of patients and introduction of off-loading method performed by r-TCC in the Czech Republic were particularly the priorities of this work.