

**Epidermal innervation in type 1 diabetic patients: a 2.5-year prospective study after simultaneous pancreas/kidney transplantation**

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*Objective:* To assess the effect of normoglycemia following simultaneous pancreas/kidney transplantation (SPK) on neurological function and intraepidermal nerve fiber density (IENFD) in patients with type 1 (DM).

*Methods:* We performed vibration perception threshold (VPT) and autonomic function testing (AFT) and assessed IENFD in skin biopsies from the lower thigh and upper calf in 14 healthy controls and 18 patients with DM at the time of and at 21–40 (median 29) months post-SPK.

*Results:* At baseline, significantly increased VPTs, pathological AFT results and severe reduction in IENFD were present in SPK recipients. After SPK, an increase of IENFD in the thigh of more than 1 ENF/mm was noted in 3 patients (median 4.1; range 1.9-10.2), but changes were not significant for the group as a whole.

*Conclusions:* We conclude that irreversible nerve damage might be present in some SPK recipients, or that longer periods of normoglycemia might be needed to allow nerve regeneration.

**D**iabetic neuropathy (DN) is a common diabetic complication, which may result in grave consequences such as pain, foot ulcers and amputations. Although optimal glycemic control is considered as an effective preventive measure, intervention studies in advanced stages of DN have been almost uniformly unsuccessful (1). Only arrest of progression of DN could be achieved in patients after pancreas transplantation (PT) (2). To assess nerve regeneration following PT, Kennedy et al. (3) proposed the use of skin biopsies with quantification of intraepidermal nerve fiber density (IENFD). Previously, we documented severe IENFD reduction in lower limb skin biopsies performed at the time of PT (4). Here we present assessment of IENFD following a mean of 2.5 years of normoglycemia.

## **RESEARCH DESIGN AND METHODS**

Twenty two patients with type 1 diabetes (DM) undergoing simultaneous pancreas/kidney transplantation (SPK) and fourteen healthy controls(C) participated in the study. For details of the procedure and study subjects please see Online Appendix. The study was approved by the local Ethical Committee and informed consent was obtained from all subjects.

Skin biopsies were performed using a 3-mm punch (Stiefel Laboratories, Sligo, Ireland) from the distal thigh (2 samples at a distance of 1 cm, one assessed in Prague and the other in Würzburg) and the proximal calf (1 sample, assessed in Prague) at the time of SPK and at 30±5 (mean±SD) months post-transplant. Biopsies from controls were taken from corresponding regions. After fixation (4% paraformaldehyde for 3 hours at 4°C, then cryoprotection with 10% sucrose in 0.1 M phosphate buffered saline) and freezing (in isopentane cooled by liquid nitrogen), 40-µm sections were immunoreacted with a rabbit polyclonal antibody to the panaxonal marker

protein gene product (PGP) 9.5 (DakoCytomation, Glostrup, Denmark), followed by mouse anti-rabbit IgG conjugated with rhodamine or Cy3 (Jackson Immuno Research, West Grove, PA). Samples were imaged with an Olympus microscope BX 51(Olympus Optical, Hamburg, Germany) in Prague and with a Zeiss Axiophot 2(Carl Zeiss, Göttingen, Germany) in Würzburg. Three sections per patient were examined. The mean number of intraepidermal nerve fibers per mm epidermis was derived using the software Olympus DP-SOFT (Software Imaging Systems, Münster, Germany) and Image Pro Plus 4.0 (Media Cybernetics, Leiden, Netherlands), respectively. Established counting rules were followed (5). Changes >1 IENF/mm were considered as meaningful. In addition, the subepidermal nerve plexus was classified semiquantitatively in Würzburg as ‘normal’, ‘reduced’ or ‘absent’. Clinical neuropathy evaluation in the patients included vibration perception thresholds (VPT; Bio-Thesimeter; Bio-Medical Instrument, Newbury, USA) and autonomic function testing (AFT; VariaPulse TF3; Sima Media, Olomouc, Czech Republic)(6). The Mann-Whitney U test and Wilcoxon signed ranks test were used for inter- and intra-group comparisons, respectively.

## **RESULTS**

Normoglycemia with insulin-independence and satisfactory renal graft function was achieved in 18 patients (M/F 10/8, aged 47±10 years, with DM duration 29±9 years, P-creatinine 1.3±0.4 mg/dl at follow-up; Online Appendix Table A1). At baseline, significantly increased VPTs, reduced AFT results (Online Appendix Table A2) and severe reduction in IENFD in both regions were present in SPK recipients (Table, Online Figure A1). At follow up 21-40 (median 29) months after SPK, increases

in IENFD of the thigh samples were seen in 3 patients both in Prague and Würzburg (median: 4.1; range 1.9-10.2 IENF/mm). The subepidermal plexus was reduced or absent in all but 1 patient. A change in category from 'reduced' to 'normal' occurred in 2 patients with improvement of IENFD, but in none of the other patients. No significant changes occurred in neurological function or IENFD of the transplanted group as a whole.

## **CONCLUSIONS**

Previous reports of neuropathy follow-up in pancreas or islet transplant recipients were mostly based on clinical examination, electrophysiology, and AFT. Most recently, stabilisation of electrophysiological parameters could be shown over a 6 year period in 18 patients with islet-after-kidney transplantation (7). An innovative noninvasive approach, corneal confocal microscopy, was proposed by researchers from Manchester (8). Using this method, a significant improvement of corneal nerve fiber density and length was detected within 6 months of SPK (9).

We did not encounter a similarly significant early regenerative response of lower limb nerve fibers after SPK. While a type II error cannot be excluded and more advanced DN could have been present, other reasons may be also responsible. The length-related pattern of DN and varying regenerative capacity of nerve fibers from different body regions could play a role. Moreover, the subepidermal plexus from which epidermal reinnervation should occur was reduced in most patients. We observed

some improvement of nerve fiber counts in the biopsies from the more proximal lower thigh area in 3 patients. While this subgroup did not differ in clinical characteristics including time from SPK, a still longer period of normoglycemia might be possibly needed to achieve nerve fiber regeneration in the lower limbs in the remaining patients. Of note, in the case of diabetic nephropathy, reversal of renal lesions was seen after more than 5 years of normoglycemia following PT (10).

Irreparable damage of lower limb nerves might be also present in some advanced cases. Although generally producing an immense improvement of the recipient's clinical condition and long-term prognosis, SPK does not eliminate risks connected with DN. Matricali et al.(11) recently reported on a high rate of Charcot foot complications at a mean of 1.8 years post-transplant. Foot ulcers and gangrene, while often co-initiated by vascular disease and infection, are not uncommon throughout the post-operative period. Such complications have occurred in 62 of 200 PT recipients at our center since 1994.

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**Table**

Glycated hemoglobin (HbA<sub>1c</sub>), vibration perception thresholds (VPT) and intraepidermal nerve fiber density (IENFD; number of intraepidermal nerve fibers/mm) in healthy control subjects and in type 1 diabetic patients (DM) at baseline and at median (range) 29 (21- 40) months of normoglycemia following simultaneous pancreas/kidney transplantation (SPK)

	Controls (n=14)	DM at SPK (n=18)	DM post-SPK (n=18)
HbA <sub>1c</sub> (%)	5.6 ± 0.3*	8.2 ± 1.4 ‡	5.4 ± 0.5
VPT (V)	16 ± 5*†	34 ± 11	35 ± 11
T <sub>P</sub> (IENFD)	11.4 ± 4.2*†	0.8 ± 1.3	1.6 ± 2.5
T <sub>W</sub> (IENFD)	8.9 ± 1.8*†	0.8 ± 1.5	1.5 ± 3.2
C <sub>P</sub> (IENFD)	8.0 ± 3.0*†	0.4 ± 1.1	0.4 ± 0.8

Data are means ± SD; T<sub>P</sub> – thigh (Prague), T<sub>W</sub> – thigh (Würzburg), C<sub>P</sub> – calf (Prague)

\*p < 0.001 Controls vs. DM at SPK

†p < 0.001 Controls vs. DM post-SPK

‡ p < 0.001 DM at SPK vs. DM post-SPK