

Appendix S1

MATERIALS AND METHODS

Patients. A total of 80 adult patients on haemodialysis treated in the International Dialysis Centre, Wrocław, Poland, were included in the study. Exclusion criteria were patients applying topical agents (including emollients) within a period of 2 weeks prior to examination, unless the proper washout period of 2 weeks was achieved. Uraemic pruritus was diagnosed in 30 (37.5%) subjects (10 (33.3%) women and 20 (66.7%) men), age range 28–87 years (mean age 59.9 ± 15.5 years). These patients underwent dialysis during a period of 2–240 months (mean 56.2 ± 58.8 months). The remaining 50 (62.5%) patients on dialysis (20 (40%) women and 30 (60%) men), age range 25–90 years (mean 59.8 ± 15.8 years) who underwent dialysis for a period of 1–108 months (mean 42.1 ± 33.3 months) did not experience itch. The control group comprised 32 randomly selected healthy people (19 (59.4%) women and 13 (40.6%) men) age range 22–86 years (mean age 59.7 ± 16.0 years). The difference in age and sex distribution between controls and patients on haemodialysis did not differ significantly ($p=0.95$ and $p=0.06$, respectively); however, the patients on haemodialysis more commonly experienced arterial hypertension compared with controls (77.5% vs. 43.8%, $p=0.001$).

Evaluation of clinical parameters. Clinical evaluation of skin dryness was conducted in accordance with El Gammal's 5-point scale (grade 0=smooth skin, grade 1=patches of fine, powdery scales, grade 2=diffuse ashy appearance with many fine scales, grade 3=moderate scaling with beginning of cracks, grade 4=intense scaling, moderate cracks) (6) in 4 selected areas of the body: forearm (the upper limb without the arteriovenous fistula in haemodialysis patients; a randomly chosen upper limb in the control group), thorax, abdomen, and a randomly selected lower leg.

Non-invasive measurement of stratum corneum hydration (corneometry) was performed using the Corneometer® MPA5 (Courage+Khazaka Electronic GmbH Co., Cologne, Germany). Measurement of transepidermal water loss (TEWL) was performed using Tewameter® MPA5 instrument (Courage+Khazaka Electronic GmbH, Cologne, Germany). Measurements were taken at a stable temperature of 21–23°C and relative humidity of 45–48%. Two independent methods were used to evaluate

the intensity of itch: visual analogue scale (VAS) (at the time of examination and maximal itching within the previous 3 days) and a 4-point itch questionnaire (7).

Epidermal lipids analysis. Skin scrapings for lipid analysis were collected from a 2-cm² area of a randomly chosen lower leg, using scalpel number 15. The scrapings were placed in clean 10-ml glass tubes (Pyrex® 13 × 100 mm Tubes; Corning Inc., NY, USA) covered with Teflon cups (Corning® Reusable Phenolic GPI 13-415 Threaded Screw Cap with Teflon® Liner; Corning Inc.). Extraction of lipids from the epidermis was performed using the method developed by Bligh & Dyer (8). Extracted lipids from each patient were dissolved by adding chloroform/methanol 2:1 (v/v) solution to a concentration of 5 mg lipids in 1 ml of solution. Next, 10 µl (50 µg lipids) of this solution was placed on a 10×20-cm thin-layer chromatography (TLC) plate (Merck, Darmstadt, Germany) at a start line 1 cm from the edge of the plate. Separation of different lipid classes was performed by TLC using 3 systems of solvents: (i) methanol:chloroform:water (20:95:1), (ii) hexane:diethyl ether:acetic acid (80:20:10); and (iii) benzene. All reagents were purchased from Sigma-Aldrich (Germany) at high-performance liquid chromatography (HPLC) purity grade. Lipids were detected by charring with 20% sulphuric acid. Finally, TLC plates were scanned, images converted to greyscale and analysed by Image J software (available at: <http://rsbweb.nih.gov/ij/>). Bands were identified using lipid standards. During analysis of TLC scans, all peak areas were summed and considered as 100% of lipid content. The relative content of lipid classes was calculated by normalizing the intensities of corresponding bands to the total intensity of all bands detectable in the TLC image.

Statistical analysis. Results were analysed statistically using Statistica® 12.0 (Statsoft, Krakow, Poland). The minimum, maximum, mean values and standard deviations were calculated. For quantitative variables, differences between the analysed groups were verified by Student's *t*-test, Mann–Whitney *U* test or analysis of variance (ANOVA), along with *post-hoc* analysis of Scheffé's test, where appropriate. Numerical dependencies between the analysed parameters were verified using Pearson's correlation test. Differences in qualitative variables were analysed with a χ^2 test with Yates correction for a 4-field table, or the accurate Fisher's test, if any of the analysed subgroups were ≤ 5 . Statistical analysis was carried out with a confidence level of <0.05 .