

# A screening of skin changes, with special emphasis on neurochemical marker antibody evaluation, in patients claiming to suffer from “screen dermatitis” as compared to normal healthy controls

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**Abstract:** In the present study, facial skin from so-called “screen dermatitis” patients were compared with corresponding material from normal healthy volunteers. The aim of the study was to evaluate possible markers to be used for future double-blind or blind provocation investigations. Differences were found for the biological markers calcitonin gene-related peptide (CGRP), somatostatin (SOM), vasoactive intestinal polypeptide (VIP), peptide histidine isoleucine amide (PHI), neuropeptide tyrosine (NPY), protein S-100 (S-100), neuron-specific enolase (NSE), protein gene product (PGP) 9.5 and phenylethanolamine N-methyltransferase (PNMT). The overall impression in the blind-coded material was such that it turned out easy to blindly separate the two groups from each other. However, no single marker was 100% able to pin-point the difference, although some were quite powerful in doing so (CGRP, SOM, S-100). However, it has to be pointed out that we cannot, based upon the present results, draw any definitive conclusions about the cause of the changes observed. Whether this is due to electric or magnetic fields, a surrounding airborne chemical, humidity, heating, stress factors, or something else, still remains an open question. Blind or double-blind provocations in a controlled environment are necessary to elucidate possible underlying causes for the changes reported in this investigation.

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Reports of skin complaints in people exposed to video display terminals (VDTs) are becoming an increasing phenomenon in several countries (1–4). Very little is known about the cause of these health complaints. The symptoms may be grouped into objective ones, including erythema, papules and pustules, as well as subjective ones including sensations of heat, itch, pain, smarting, etc. (3–8). Clinical dermatologists have regarded the symptoms to be mostly of rosacea or rosacea-like dermatitis nature (9). A large-scale epidemiological study has shown that the subjective facial skin symptoms were more common among VDT-exposed persons, but no significant differences between exposed and non-exposed groups in objective skin signs or skin disease

were reported (10). The early notion that employees with VDT-work might have specific facial histological changes could not be confirmed by Berg et al. (11) in their histopathological study. In the present investigation, the highly sensitive indirect immunofluorescence methodology (12) has been utilized. The aim of the study was to evaluate possible markers to be used for future double-blind or blind provocation investigations.

## Material and methods

### Subjects

3 groups were investigated. The material was sampled and taken by 2 professional dermatologists (see

Acknowledgements) at the Department of Dermatology, Karolinska Hospital. The groups consisted of normal healthy controls (no VDU work; no skin symptoms; n=3; males; 33, 34 and 44 years of age), "screen dermatitis" patients (VDU work; subjective skin symptoms; n=4; 1 male, 3 females; 42, 43, 44 and 52 years of age) and "screen dermatitis" patients (VDU work; subjective and objective (erythema, telangiectasia) skin symptoms; n=8; 2 males and 6 females; 26, 38, 42, 49, 49, 52, 59 and 60 years of age). Of the latter patients, 3 (females; 38, 52 and 60 years of age) were omitted from the study because of improper technical handling, such as inferior fixation. The "screen dermatitis" patients had been suffering for several years from facial skin symptoms. The patients did not have any on-going medication or any systemic or dermatological diseases, including acute infections.

### Biopsies

The subjects arrived, one at a time, to the clinic (Department of Dermatology, Karolinska Hospital). Punch biopsies (2 mm) were taken under local anaesthesia with lidocaine (0.5%) without epinephrine from the lateral part of the face, over the arcus zygomaticus. The biopsies were taken at exactly the same place regardless of the actual symptoms. The idea behind this strategy was to enable for the dermatologists to have an anatomically defined spot and, thus, to avoid variation in the material due to different localities. Finally, the biopsies were blind-coded by the dermatologists.

### Preparation of tissue

The biopsies were immersed for 2 h at 4°C in a solution of 14% saturated picric acid and 10% formalin. All the tissue samples were then rinsed for at least 24

h in 0.1 M Sörensen's buffer containing 10% sucrose, 0.01% NaN<sub>3</sub> and 0.02% Bacitracin, and 14 µm sections were cut using a cryostat (Microm, Heidelberg). The sections were thawed on to gelatine-coated slides and processed for indirect immunohistochemistry (see below).

### Antibodies

Rabbit or mouse antibodies to calcitonin gene-related peptide (CGRP), neuropeptide KG<sub>2</sub>(NPKG<sub>2</sub>), galanin (GAL), somatostatin (SOM), γ-melanocyte stimulating hormone (γ-MSH), vasoactive intestinal polypeptide (VIP), peptide histidine isoleucine amide (PHI), neuropeptide tyrosine (NPY), protein S-100 (S-100), neurofilament (NF), neuron-specific enolase (NSE), protein gene product 9.5 (PGP 9.5) and phenylethanolamine N-methyltransferase (PNMT) were used. The characteristics of the antibodies are summarized in Table 1. Two (SOM polyclonal; PGP 9.5) of the antibodies were added at a later stage of the investigation, therefore, the first biopsies were not incubated with these antibodies.

### Immunohistochemistry

The indirect immunofluorescence technique (12) was used for demonstrating the neuropeptides and neuroactive substances. The sections were kept in a humid atmosphere, incubated with the above-mentioned antibodies overnight at 4°C, rinsed in phosphate buffered saline (PBS), incubated for 30 min at 37°C in rhodamine (TRITC)-conjugated goat anti-rabbit or anti-mouse IgG (1:80 or 1:40; Boehringer Mannheim), rinsed and mounted. All antibodies were diluted in 0.3% Triton X-100. For observation and photography a Nikon Microphot-FXA or Optiphot fluorescence microscope was used. The material was evaluated by 2 independent observers using

Table 1. Description of the primary antibodies

Antibody	Abbreviation	Dilution	Species	Source
calcitonin gene-related peptide	CGRP	1:400	rabbit	Peninsula
neuropeptide KG <sub>2</sub>	NPKG <sub>2</sub>	1:400	rabbit	E. Theodorsson-Norheim, Stockholm
galanin	GAL	1:400	rabbit	Peninsula
somatostatin, monoclonal	SOM	1:200	mouse	R.P. Elde, Minneapolis
somatostatin, polyclonal	SOM	1:800	rabbit	R.P. Elde, Minneapolis
γ-melanocyte stimulating hormone	γ-MSH	1:200	rabbit	L. Terenius, Stockholm
vasoactive intestinal polypeptide	VIP	1:400	rabbit	Peninsula
peptide histidine isoleucine amide	PHI	1:400	rabbit	J. Fahrenkrug, Copenhagen
neuropeptide tyrosine	NPY	1:400	rabbit	L. Terenius, Stockholm
protein S-100	S-100	1:400	rabbit	K. Haglid, Göteborg & L. Olson, Stockholm
neurofilament	NF	1:500	rabbit	K. Haglid, Göteborg & L. Olson, Stockholm
Neuron-specific enolase	NSE	1:200	rabbit	UC
protein gene product 9.5	PGP 9.5	1:2,000	rabbit	UC
phenylethanolamine N-methyltransferase	PNMT	1:800	rabbit	M. Goldstein, New York

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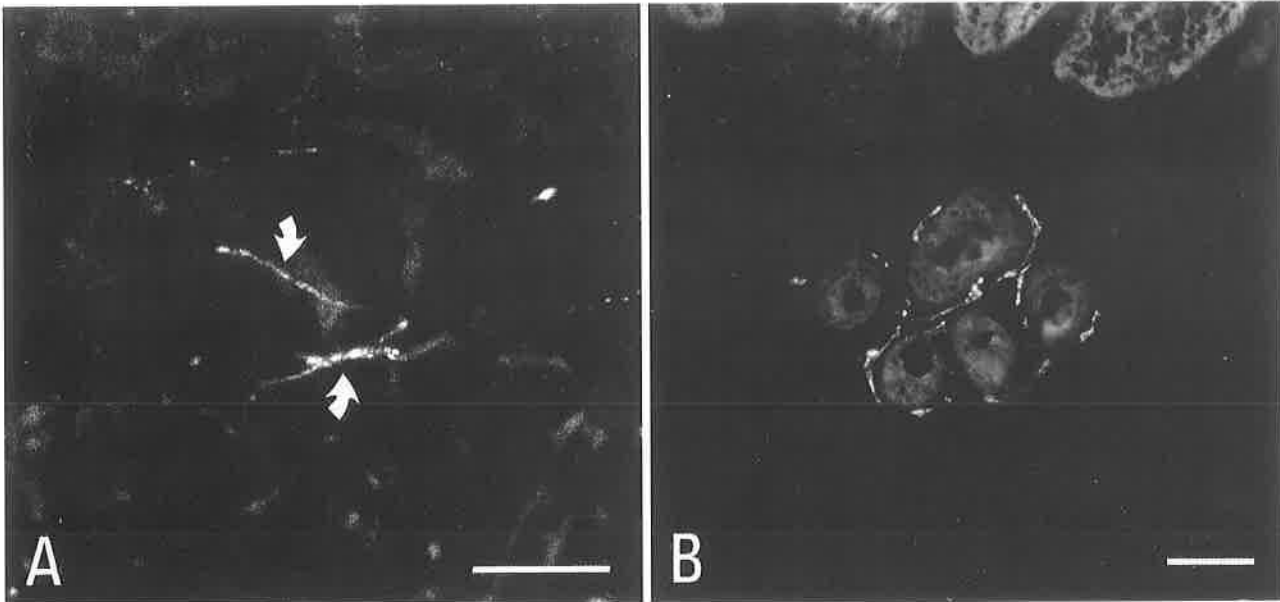


Figure 1. Immunofluorescence micrographs from "screen dermatitis" skin. (A) CGRP immunoreactive nerve fibers (arrows) in the dermis. (B) PHI immunoreactive nerve fibers surrounding eccrine sweat glands. Bars indicate 100  $\mu\text{m}$  (A) and 50  $\mu\text{m}$  (B), respectively.

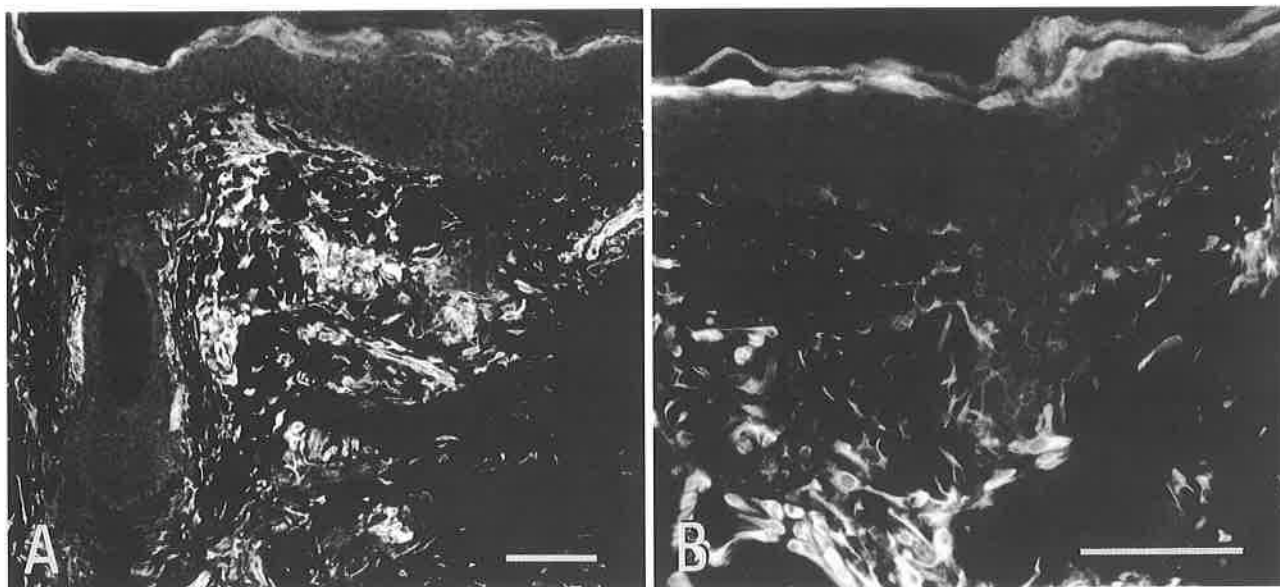


Figure 2. Immunofluorescence micrographs from "screen dermatitis" skin after incubation with a polyclonal antiserum to somatostatin. Immunoreactive cells in large numbers are seen all over the dermis. Single cells are also found in the basal part of the epidermis. Note, that the chain of cells in the upper granular layer is also seen in normal skin. Bars indicate 50  $\mu\text{m}$ .

a 5-graded semiquantitative scale. For further technical details, see Ljungberg & Johansson (13).

### Results

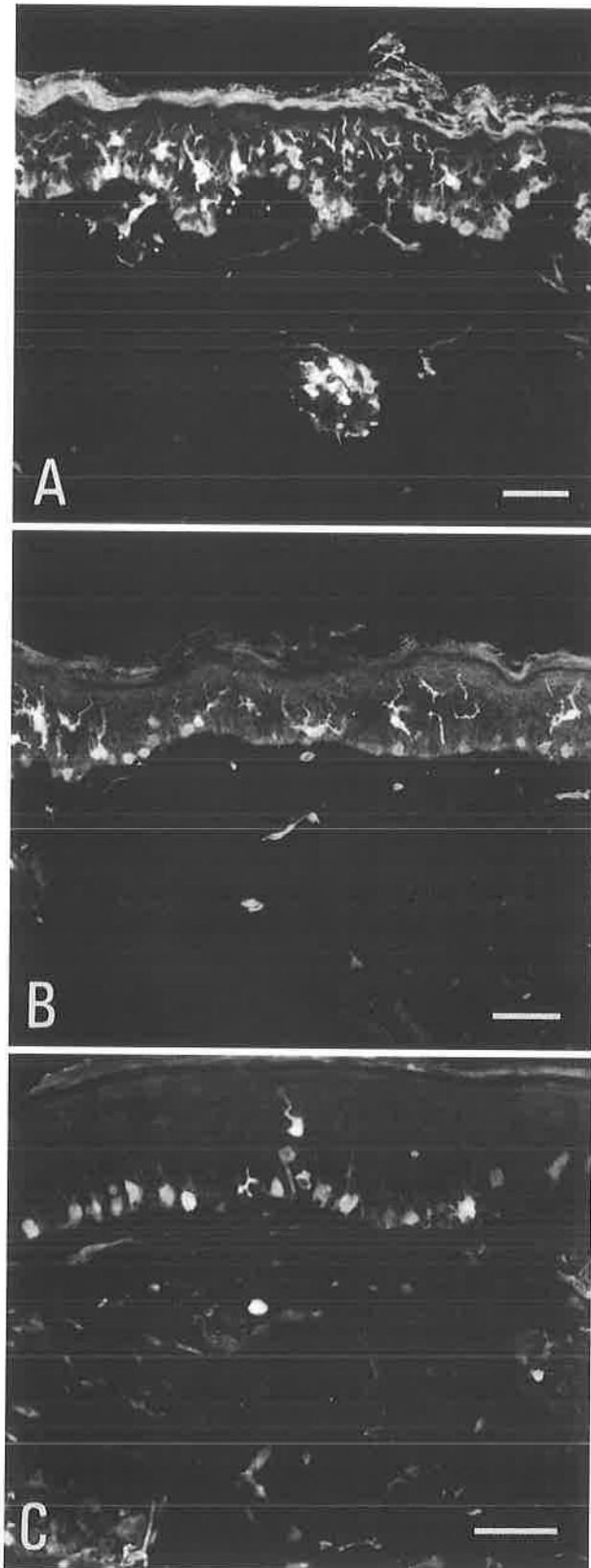
In the following, no description of the normal material is given, since this has been published in extenso in the literature. Only differences observed in the "screen dermatitis" material compared with the

normal control tissue are given below. This is against the background given in our aim, thus, to evaluate possible markers to be used for future double-blind or blind provocation investigations.

CGRP: 4 out of the 9 (4/9) "screen dermatitis" patients had few-to-very few nerve fibers in the dermis (Fig. 1A). The other patients revealed a normal-looking image concerning the nerve fiber number and distribution. However, 3 others had a very high

background in the papillary dermis. Thus, in summary, 7/9 showed an abnormal pattern.

NPKG<sub>2</sub>: no conclusive differences were observed



between the normal healthy control material and the "screen dermatitis" patients.

GAL: no conclusive differences were observed between the normal healthy control material and the "screen dermatitis" patients. It may be noted, that in 2/9 patients single GAL immunoreactive, round-to-oval dermal cells with a central, oval nucleus were observed. Such cells have not previously been observed in normal material.

SOM, monoclonal: 5/9 patients had single, very weak and very thin processes in the dermis. 6/9 had a very weak staining of the myelin.

SOM, polyclonal: 7 patients were investigated. All (7/7) showed a higher number of SOM positive dendritic cells. 1 patient had a remarkably high number as well as cells entering the epidermis (Fig. 2A). Another patient also had a remarkably high number of SOM immunoreactive dendritic cells in the dermis, but, no cells were seen in the epidermis (Fig. 2B). Four patients had cells located perivascularly. One patient instead showed very few cells.

$\gamma$ -MSH: polymorphonuclear cells immunoreactive to  $\gamma$ -MSH were found in 3/9 "screen dermatitis" patients. 8/9 had a higher background fluorescence in the stratum papillare.

VIP: 2/9 did not reveal any nerve fibers at all around blood vessels, sweat glands or other dermal appendages. Furthermore, yet another 2/9 had a decreased number of nerve fibers as compared to normal healthy skin.

PHI: 2/9 did not reveal any nerve fibers at all around blood vessels, sweat glands or other dermal appendages. Furthermore, yet another 2/9 had a decreased number of nerve fibers as compared to normal healthy skin (Fig. 1B). PHI immunoreactive cells were seen in 8/9 patients. Of these, 5/9 revealed small cells with paranuclear staining. 2/9 had somewhat larger, round cells in groups. Their nuclei were eccentrically located. 3/9 had polymorphonuclear-like cells.

NPY: 1/9 did not reveal any NPY positive nerve fibers at all. 3/9 had a decreased number as compared to normal, healthy material. Single, small dermal cells were observed in 4/9 of the patients. Such cells have not been described in normals.

S-100: in the dermis, generally the pattern looked normal. However, the S-100 immunoreactive dendritic cells of the epidermis could be grouped into several different patterns of change. First, 3/9 pa-

Figure 3. Immunofluorescence micrographs from normal (A) and "screen dermatitis" (B, C) skin after incubation with S-100 antibodies. A partial loss of epidermal dendritic cells could sometimes be seen (cf. A and B). In addition, certain cells revealed fewer and weaker dendritic processes, and even showing a complete loss of them (C). Bars indicate 50  $\mu$ m.

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tients showed partial loss of epidermal dendritic cells, i.e., along the epidermis areas of complete cel-

lular loss were seen (cf. Fig. 3A, B). In addition, the remaining cells were located to the stratum basale and revealed fewer and weaker dendritic processes, even sometimes showing complete loss of processes (cf. Fig. 3A, C). Secondly, one group (5/9) had cells located at their normal position, but their processes were fewer, weaker and sometimes even absent. A certain overlap could be observed between these two groups. Finally, only one patient had a normal-looking pattern regarding frequency, location as well as morphology.

NF: no conclusive differences were observed between the normal healthy control material and the "screen dermatitis" patients.

NSE: in the dermis, no clear-cut differences were observed between the normal healthy control material and the "screen dermatitis" patients. In the epidermis, a complete loss of nerve fibers were found in 2/9, in 2/9 nerve fibers were only seen in the stratum basale, and in yet 2/9 fewer nerve fibers were revealed, however, with a normal location within the epidermis.

PGP 9.5: 5 patients were investigated. All (5/5) showed epidermal fibers running all the way up to, and including, the stratum granulosum, however, one patient revealed a highly decreased number. This patient did not reveal nerve fibers equally high up in the epidermis. 3/5 had an increased number. In addition, it may be noted that one of these latter patients had nerve fibers running in a more straight fashion.

PNMT: 1/9 had a massive number of PNMT positive cells (with granular fluorescence) in the entire dermis (cf. Fig. 4A, B). 4/9 had a somewhat lower number of equally-looking cells (cf. Fig. 4A, C). The other "screen dermatitis" patients had a more normal appearance, i.e., single PNMT immunoreactive cells were revealed.

In summary, the two independent observers could easily, in the blind-coded fashion, distinguish, based on the above-given description, the "screen dermatitis" patients from the normal healthy volunteers. It may be noted, that two rosacea patients being processed in parallel did not differ from the normal material apart from earlier reported findings, but, were clearly not similar to the "screen dermatitis" tissue.

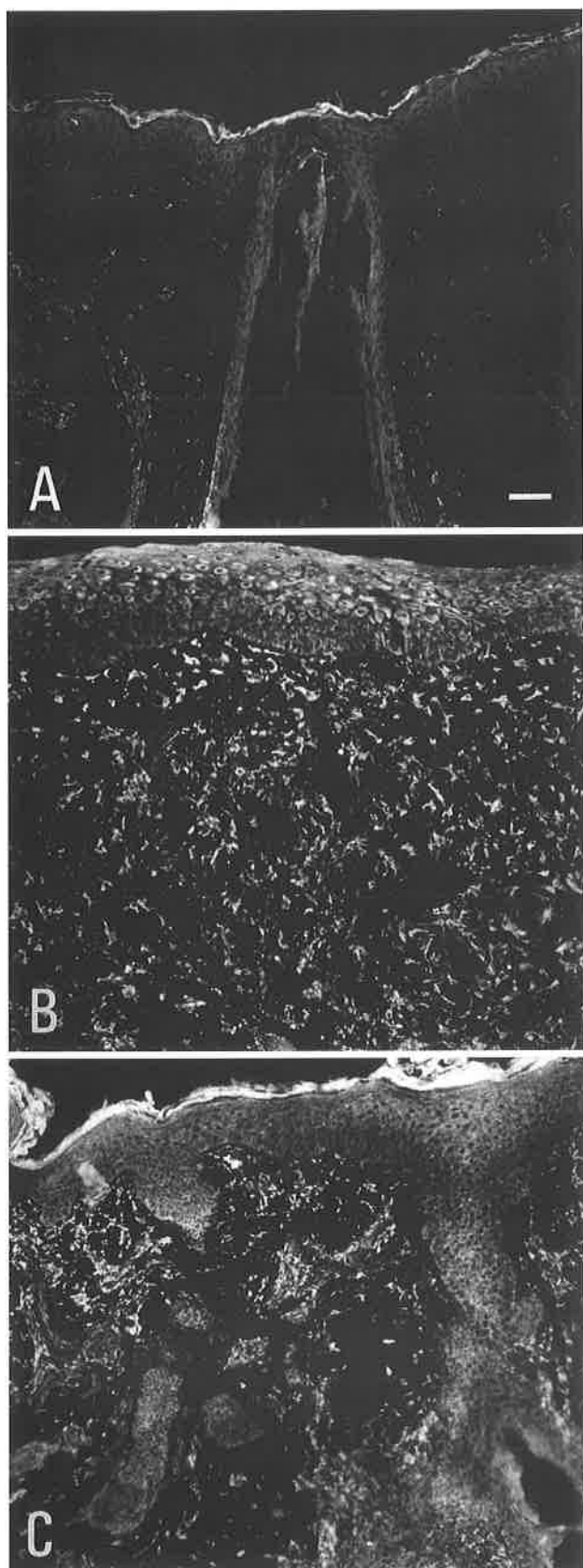


Figure 4. Immunofluorescence micrographs from normal (A) and "screen dermatitis" (B, C) skin using PNMT immunohistochemistry. One patient had a massive number of PNMT positive cells (with granular fluorescence) in the entire dermis (cf. A and B). 4 other patients had a somewhat lower number of equally-looking cells (C). In addition, the epidermis often revealed a general increase in fluorescence. Bar in A indicates 50  $\mu$ m.

## Discussion

In the following, our results will be discussed. However, it has to be pointed out that we cannot, based upon the present results, draw any definitive conclusions about the cause of the changes observed. Whether this is due to electric or magnetic fields, a surrounding airborne chemical, humidity, heating, stress factors, or something else, still remains an open question. Blind or double-blind provocations in a controlled environment are necessary to elucidate possible underlying causes for the changes reported in this investigation.

In the present study, clear differences between normal healthy skin and corresponding tissue from "screen dermatitis" patients were found for the biological markers CGRP, SOM (polyclonal), VIP, PHI, NPY, S-100, NSE, PGP 9.5 and PNMT. The overall impression in the blind-coded material was such that it turned out easy to blindly separate the two groups from each other. However, no single marker was 100% able to pin-point the difference, although some were quite powerful in doing so (CGRP, SOM (polyclonal), S-100). From a statistical point of view, it is not likely at all, that the observed differences can be explained by mass significance phenomena, but, naturally further and larger studies have to be initiated to rule out any such influences.

Certain markers could very well explain some of the claimed clinical, subjective and/or objective, symptoms. For instance, changes in the CGRP immunoreactive nerve fibers could be the basis for sensory symptoms, such as itch, pricking pain, and smarting. The autonomic markers VIP, PHI and NPY (but perhaps also (through axon-reflex actions) CGRP) may explain redness and oedema. SOM and S-100 within epidermal and dermal dendritic cells could account for the general subjective sensation of an on-going inflammation and susceptibility to skin infections as well as sensitivity to ordinary light. The morphologic markers NSE and PGP 9.5 most likely reflect structural differences between the two groups, however, it is not easily understood if they are primary or secondary in sequence. Furthermore, they are also seemingly contradictory to each other, but it should be pointed out that only 5/9 patients were incubated with PGP 9.5. It may also reflect the possibility that NSE and PGP 9.5 actually show 2 different nerve fiber populations, something never clearly investigated. Finally, PNMT is more difficult to understand, since this is regarded as a more or less negative control marker in normal healthy skin, only showing single immunoreactive cells. But, in one patient a massive number of PNMT positive cells (with granular fluorescence) was seen in the entire dermis, and 4/

9 patients had a somewhat lower number of equally-looking cells. It should be remembered that PNMT is the norepinephrine-converting enzyme, leading to the production of epinephrine. Maybe this is the chemical basis for a local stress-like reaction, not dependent on stress-mediated increased levels of adrenal medullary catecholamines, but, instead dependent on true physical factors, such as electric and/or magnetic fields, humidity, heating, etc., influencing the skin? If electric and/or magnetic fields are involved, with the on-going public debate in mind, they most probably are of high frequency nature, including both MHz and GHz ones. Of course, our data cannot exclude psychological stress as an important confounder.

The initial aim of the present study was to evaluate possible markers to be used for future double-blind or blind provocation investigations. This goal has been fulfilled. At the same time, new and highly remarkable observations were made. The fact that the two independent observers easily could, in the blind-coded fashion, distinguish the "screen dermatitis" patients from the normal healthy volunteers came as a big surprise to us. Naturally, this will lead us into further strong efforts to throw more light onto the very difficult health issue of "screen dermatitis".

It is evident from our preliminary data that biological differences are present in the patients claiming to suffer from "screen dermatitis". In view of the recent epidemiological studies pointing to a correlation between long-term exposures from magnetic fields and cancer (14, 15; Flodérus B. et al., personal communication), our data definitely ought to be further analyzed.

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