ECTP 2013

Copenhagen, Denmark
13-14 November 2013

European Congress on Tattoo and Pigment Research

We are pleased to announce the 1st European Congress on Tattoo and Pigment Research (ECTP) which will take place in Copenhagen, Denmark. November 13-14 2013.

This 1st ECTP congress is organized to present cutting edge research on tattoos and industrial pigments ranging from basic research to clinical research including health aspects and social sciences.

The congress invites chemists, biologists, physicists, toxicologists, formulation scientists, medical doctors including dermatologists and plastic surgeons, epidemiologists, sociologists and experts working with regulatory affairs to attend and to contribute with their presentations.

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Venue:
Bispebjerg University Hospital,
Copenhagen, Denmark

Deadline for abstracts:
August 14, 2013
Online submission www.ectp2013.org

Deadline early registration:
September 1, 2013

Congress Chairman
Jørgen Serup, Professor, DMSc, Bispebjerg University Hospital, Copenhagen, Denmark

Vice Chairman
Nicolas Kluger, MD, Montpellier University Hospital, France & University of Helsinki, Finland

Allergies and Tattoing

Jørgen Serup, MD
Bispebjerg University Hospital, Copenhagen, Denmark
Dept. of Dermatology, ”Tattoo Clinic”*

* The ”Tattoo Clinic” specialised in tatoos and tattoo reactions in skin was established in 2008

German Federal Institute for Risk Assessment BfR

The Tattoo-problem range, two risk levels

TATTOO COMPLAINT (mild) *– common and neglected!*
Any unusual condition, sensation or visible reaction in the tattooed skin that *differs from normal skin* of the same person. Accepted or treated ”at home”.

TATTOO COMPLICATION (advanced) *– uncommon, non infectious and infectious*
More serious adverse reactions in tattoos associated with *objective clinical pathologies of the tattoo in combination with major subjective symptoms* and significant discomfort, i.e. events that would typically make the patient consult a Doctor.

Prevalence of mild Tattoo symptoms or COMPLAINTS

Denmark (BBH data from two recent studies)* **

Tatooed Individuals, overall:

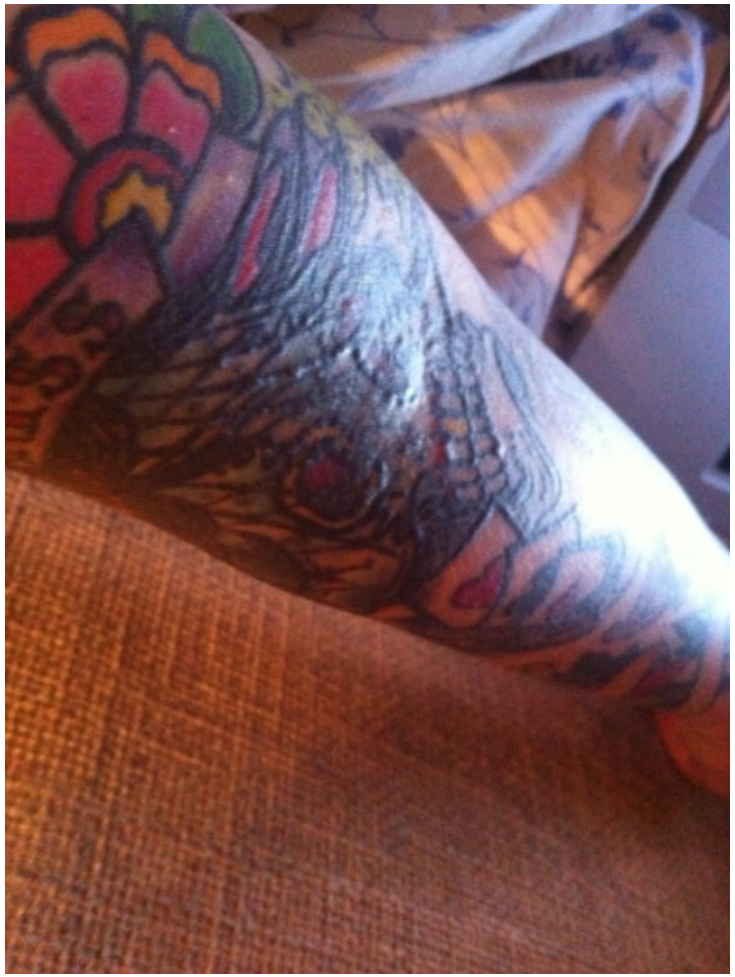
Of all tattoed nearly half have compliants, and 1/5 have abnormal sensitivity to light in their tattoo!

	STD Clinic Study*	"Beach" Study**	Pooled/overall
Total number tottoed	154	144	298
Complaining (vs total),	65/154, 42%	60/144, 42%	125/298, **42%**
Photosensisitive (vs complaints)	24/65, 37%	31/60, 52%	55/125, **44%**

Høgsberg T, Carlsen KH, Serup J. High prevalence of tattoo complaints among a young population Tattooed with recent organic pigments, J Eur Acad Dermatol, in press*

Carlsen KH, Serup J. Photosensitivity and photodynamic events in black, red and blue tattooes are Common. A "beach study", J Eur Acad Dermatol, in press**

Complaints exemplified - iPhone photo taken by patient JJ



Photosensitivity reaction, sun provoked, reproducible, mainly in dark colours

Allergy by clinical definition and circumstance

General principles applicable to tattoos

- Aquiered reaction (against non-self) though exposure to an ALLERGEN
- Initial sensitisation phase (some 2-12 weeks), and rechallenge with short reaction time
- Highly specific to the culprit albeit cross-reactivity may occur
- Hyperreactive to very low dose tolerated by others
- In the clinic often typical reaction patterns independent of the allergen
- Mediated by the immune system, affects the entire skin and body
- Two (or four) mechanisms, immediate and delayed
  - humoral mediated by circulating antibodies, immunoglobulins, urticarial
  - cellular mediated by T-lymphocytes, inflammatory
- Once aquired, allergy is life-long, code remembered
- Treatable but not curable, elimination of the allergen is essential

Allergy testing: Prick test/specific IgE, patch test by occluded chamber

Clinical diagnosis of tattoo complications beyond 3 months after tattooing, new classification system*

1. ***Elevated and inflamed "plateau" reactions*, possibly scaly or furrowed; affets every part of a tattoo holding a culprit colour; if a line or writing the entire line is swolen**

2. ***Elevated papular, nodular or globoid reactions***

3. ***Exophytic reactions* with excessive hyperkeratosis, possibly with well-like intrusions going deep, possibly with crypts undermining the epithelium**

4. ***Ulcerting and invasive reactions predominated by* necrosis**

5. ***Pigment leakage and lymfopathy patterns***

6. ***Neuro-sensory pattern or other neuropathy***

7. ***Systemic disease initiated by or triggered by tattoo (infections excluded)***

8. ***Miscellaneous patterns***

Blue: Allergic reaction patterns Classification to become presented at ECTP2013*

Tattoo Reactions of the ”plateau” type

Dominated by inflammation of outer and mid dermis,
with only minor reaction of the epidermis

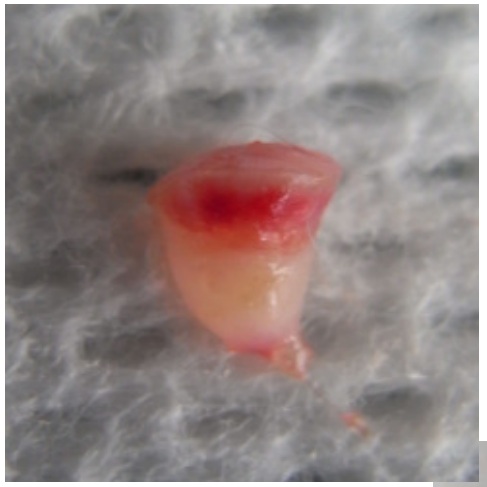
- Every piece of skin tattoed with culprit colour must react uniformly
- Amble delay (months/years) between tattooing and development of reaction
- Once full blown, persistent over time

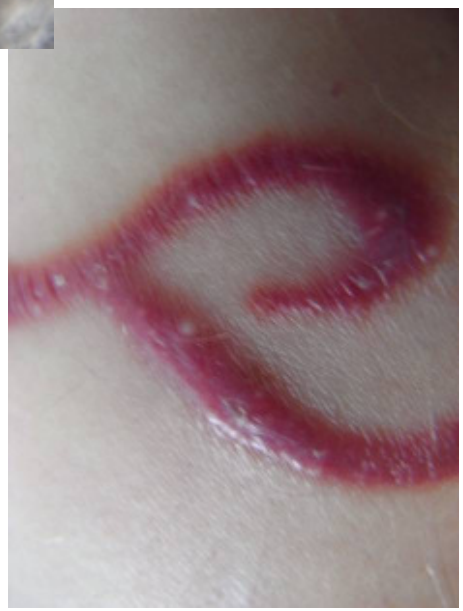
Possibly

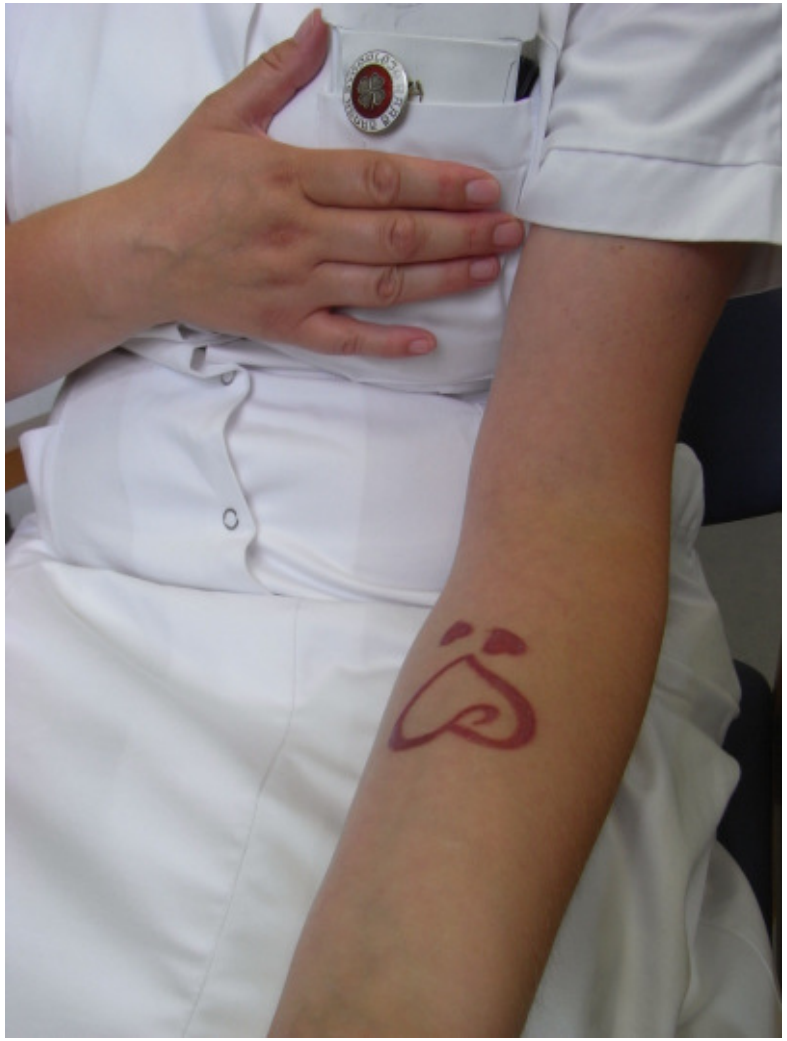
- Confirmed by patch test (if allergen or epitope is available in test material!)
- Cross-reactivity manifestations vs group allergens (such as textile azo dyes)

Pattern Elevated ”plateau” reaction in red, non-scaly

1.1 patient AMT

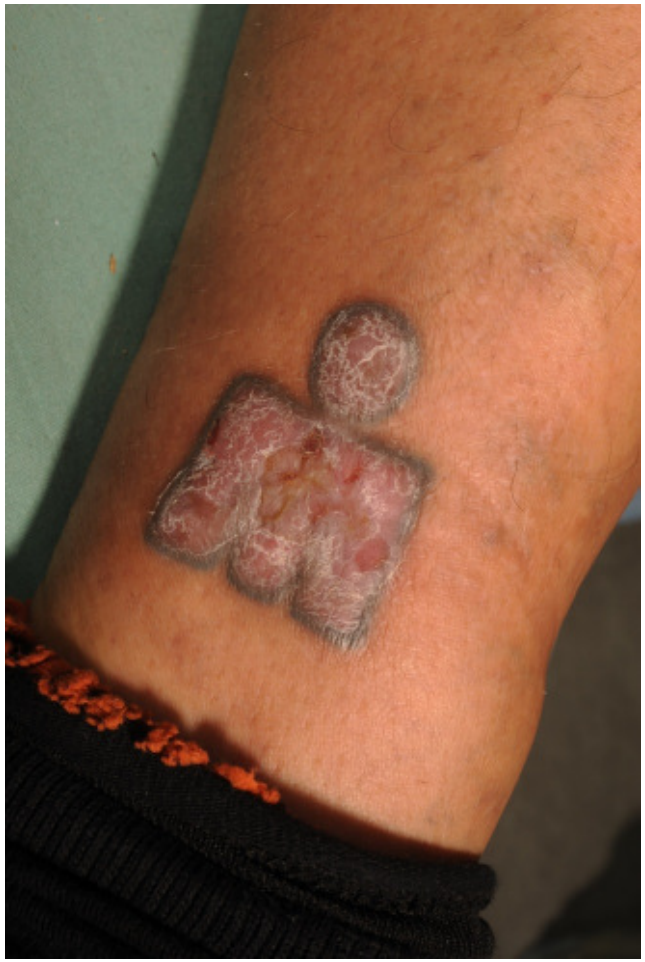




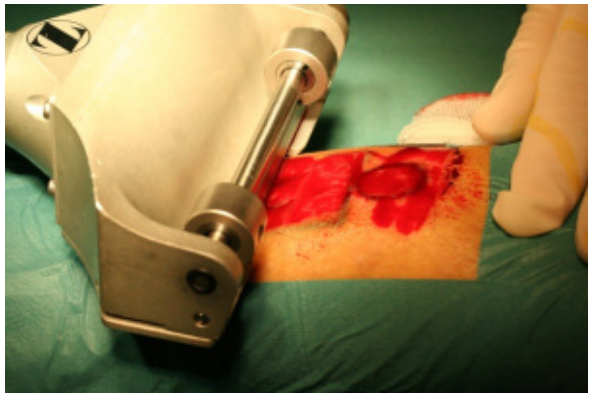


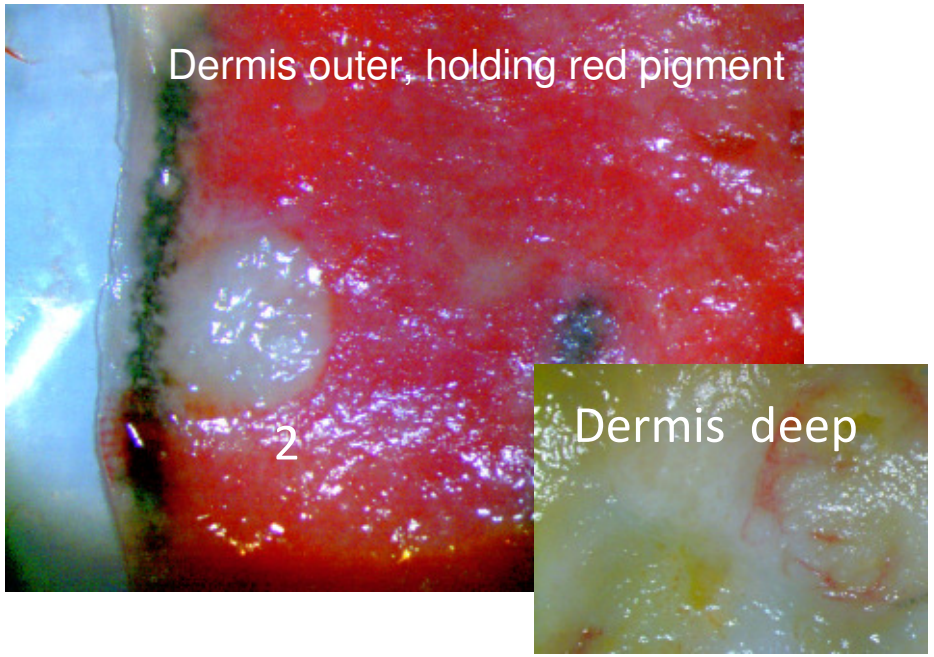
Pattern Elevated ”plateau” reactions, scaly and lichenoid

1.6 patient BC



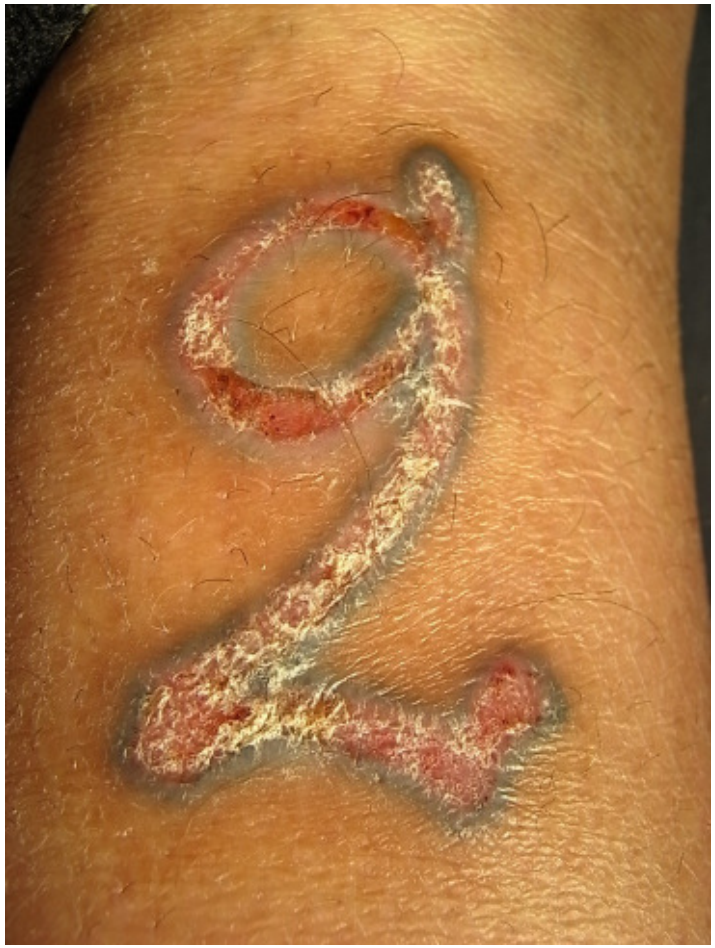
Dermatome Shaving

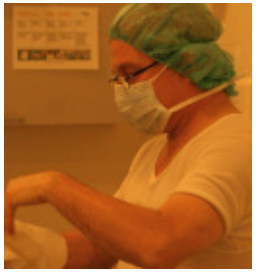




Pattern 1. Elevated ”plateau” reactions in red, scaly and ”lichenoid”

1.4 patient GH

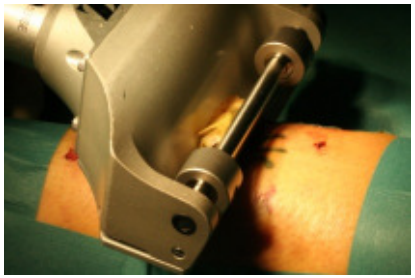


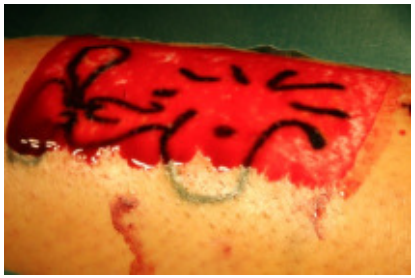


Bo Jørgensen MD

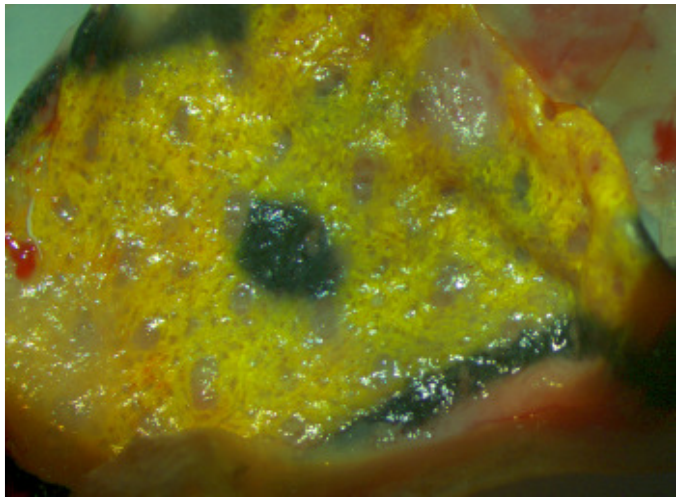
Elevated ”plateau” reactions in yellow, lichenoid

1.7 patient TPH – the pigment layer in the dermis on shaving

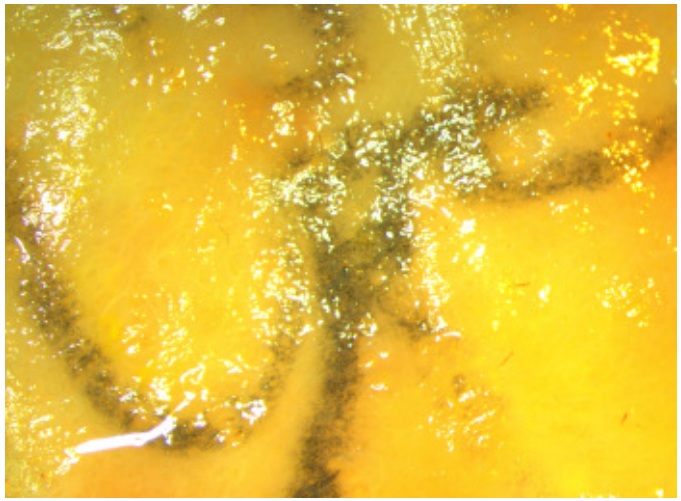




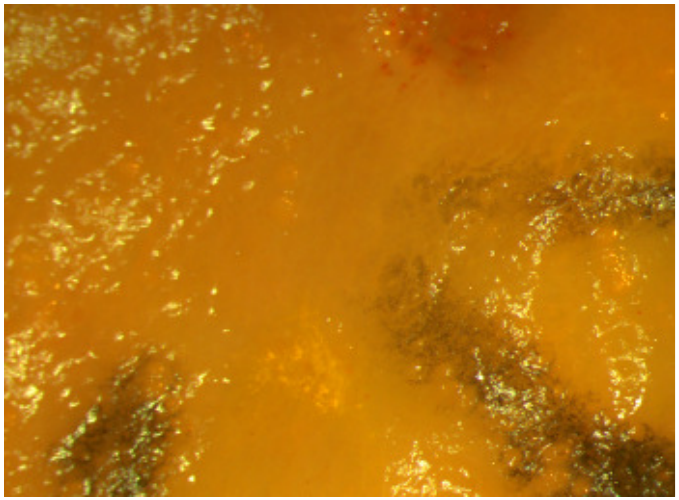
Dermis, outer



Dermis, mid



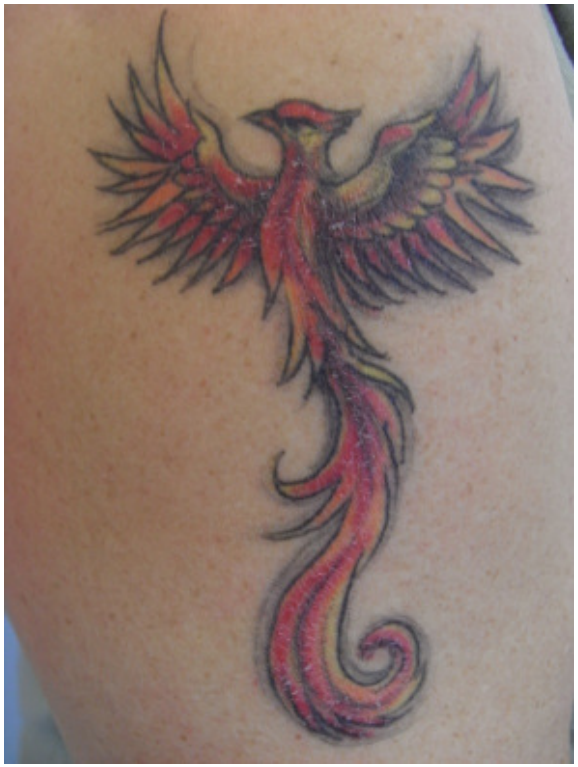
Dermis deep

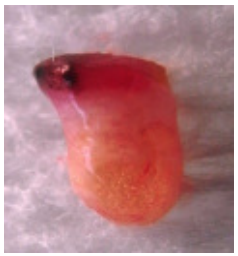


Elevated ”plateau” reaction in red, scaly lesion, clue case allergy

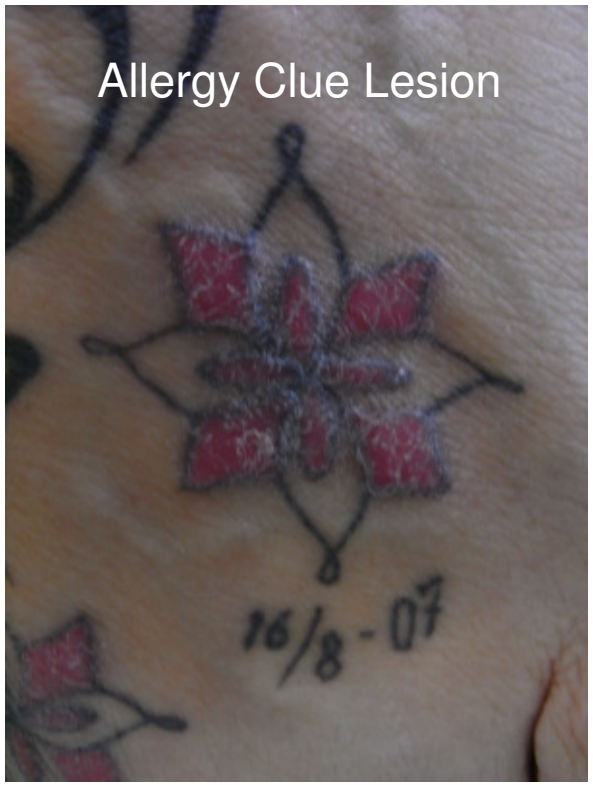
1.5 patient PH, Cross reaction in red (leg) in tattoo hitherto tolerated, provoked by new tattoo on the shoulder

Trigger tattoo on shoulder, new





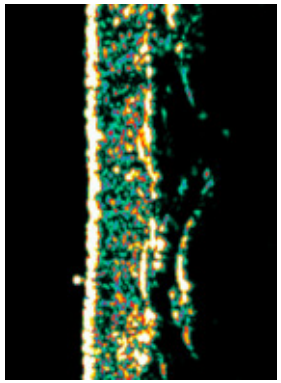
Cross reacting tattoos on leg, old and hitherto tolerated well



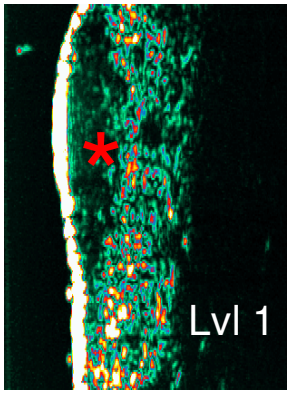
Level of inflammation in the dermis by 20 MHz ultrasound correlates with histology. N=58*

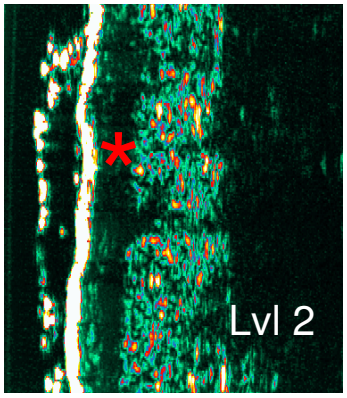
Ultrasound thickness, reactions 2.07 mm (0.67) vs. normal skin 1.18 (0.27)

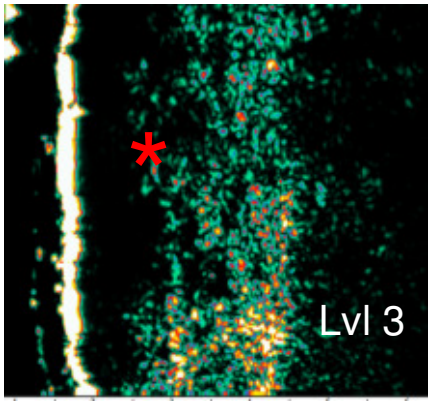
Depth of dermal Infiltrate, histology	**Number of Samples** n	**Echolucent band** mm, mean (SD)
Level 1, outer	12, 21%	0.45 (0.33)
Level 2, middle	35, 60%	0.89 (0.56)
Level 3, deeper	11, 19%	1.48 (1.18)



Normal



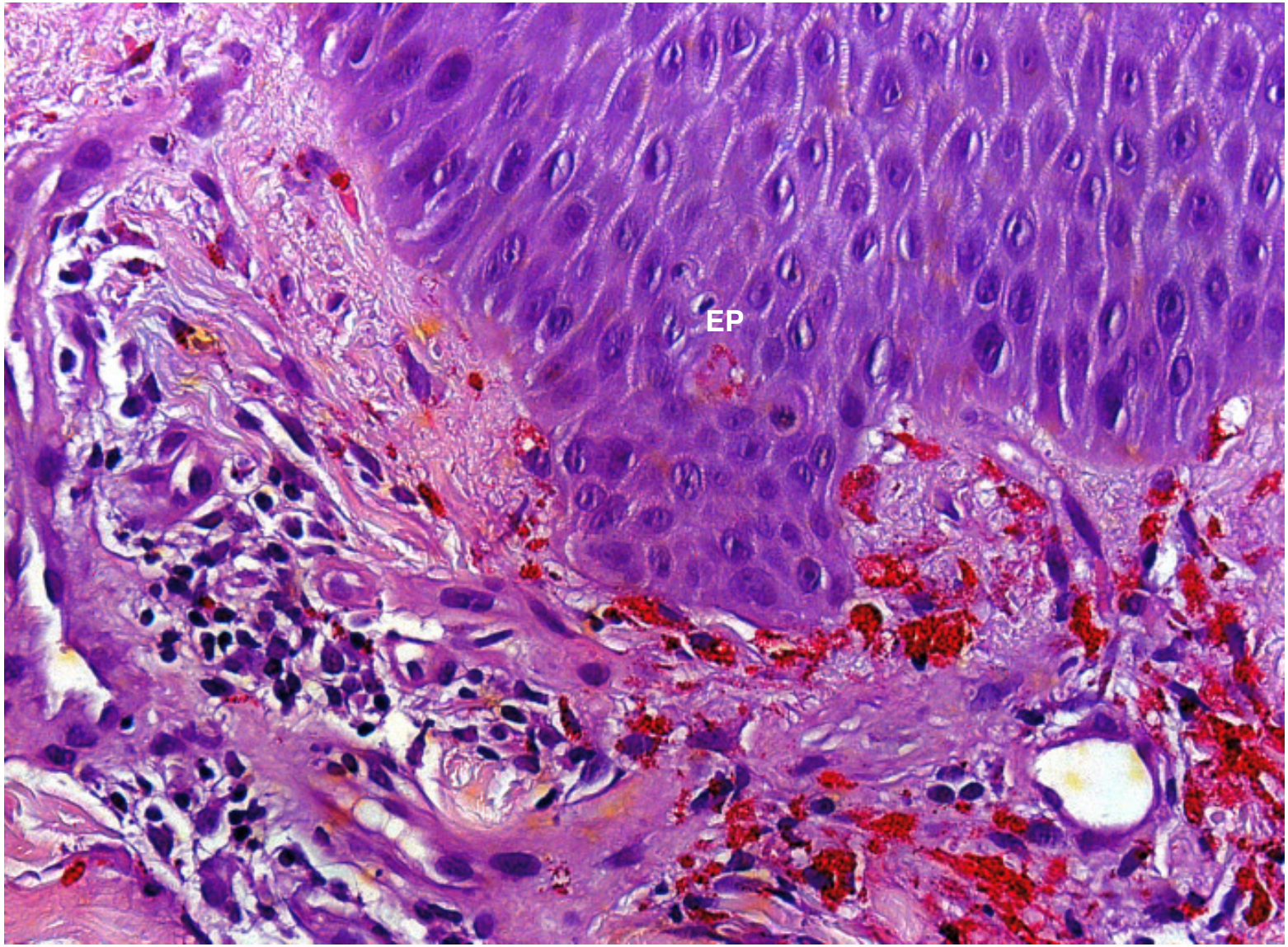




* Carlsen KH, Tolstrup J, Serup J, Skin Res Technol in press. * Echolucent band, outer dermis

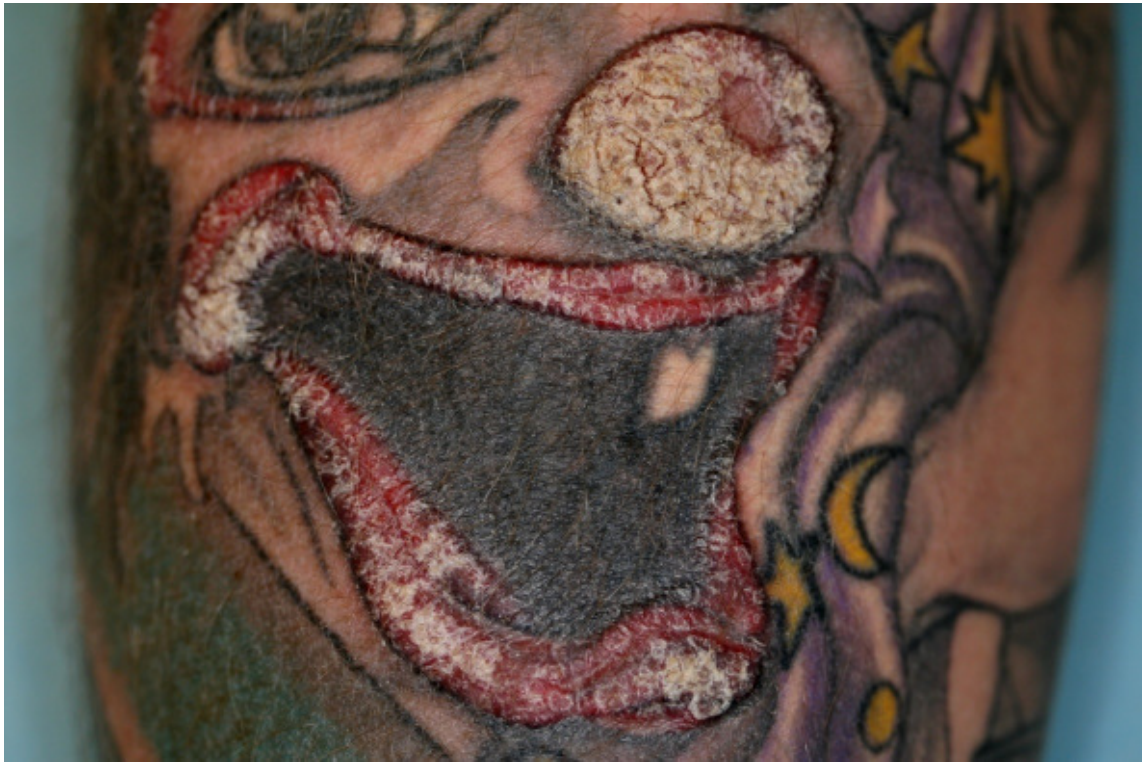
Allergic tattoo reactions with
advanced hyperplastic reaction of epidermis
(additional til inflammation of the dermis)

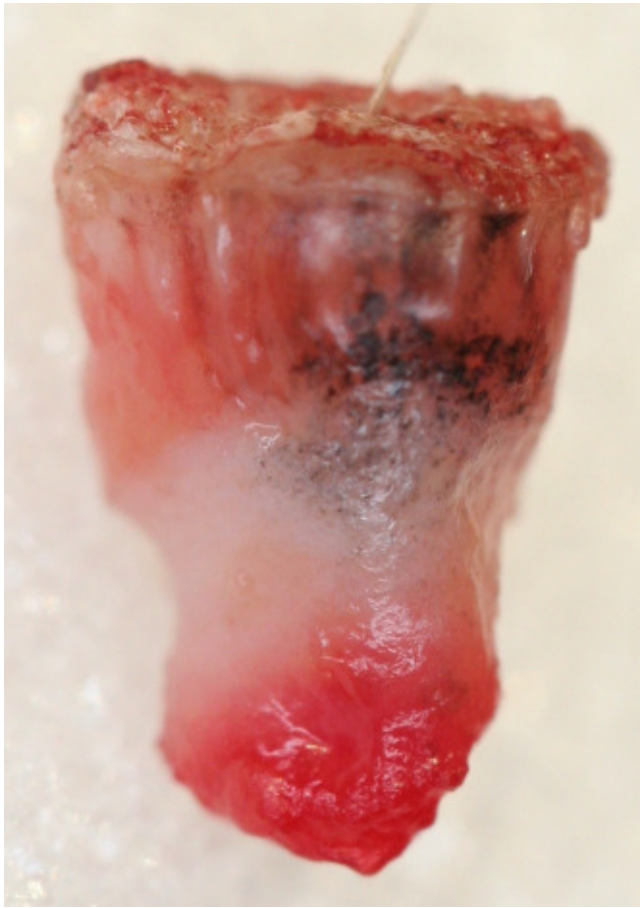
Interphase dermatitis with lymphocytic infiltration surrouding clustered red pigment in the outer dermis. Note pigment leaked to epidermis (EP)



Exophytic reactions with excessive hyperkeratosis and pigment leakage

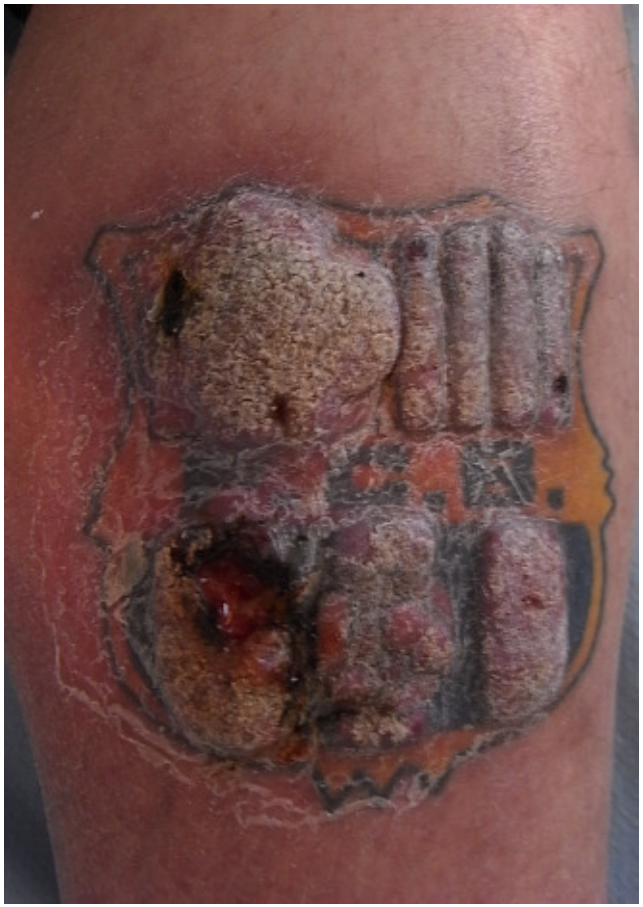
3.1 patient TH

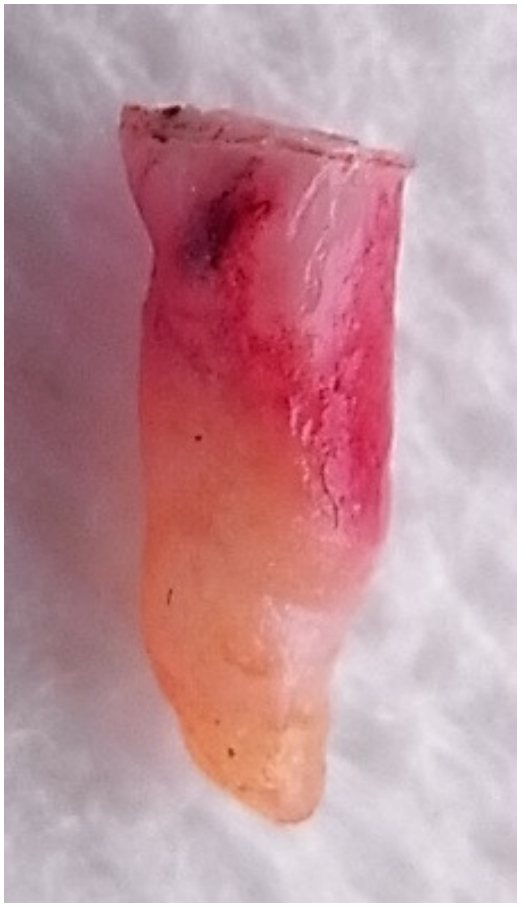


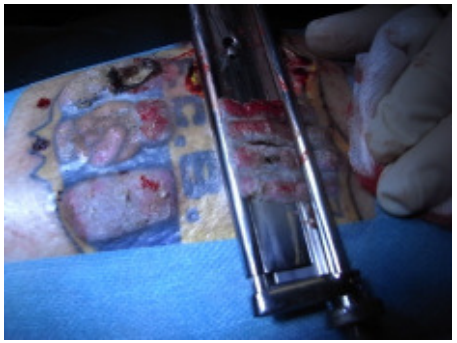


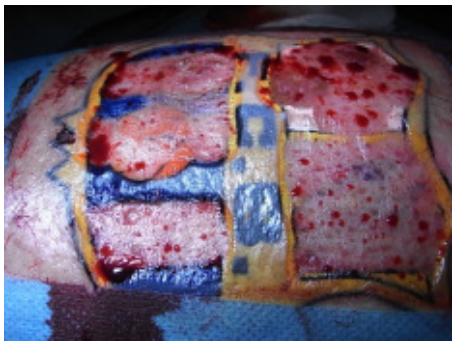
Exophytic reactions with excessive hyperkeratosis

3.2 patient BL



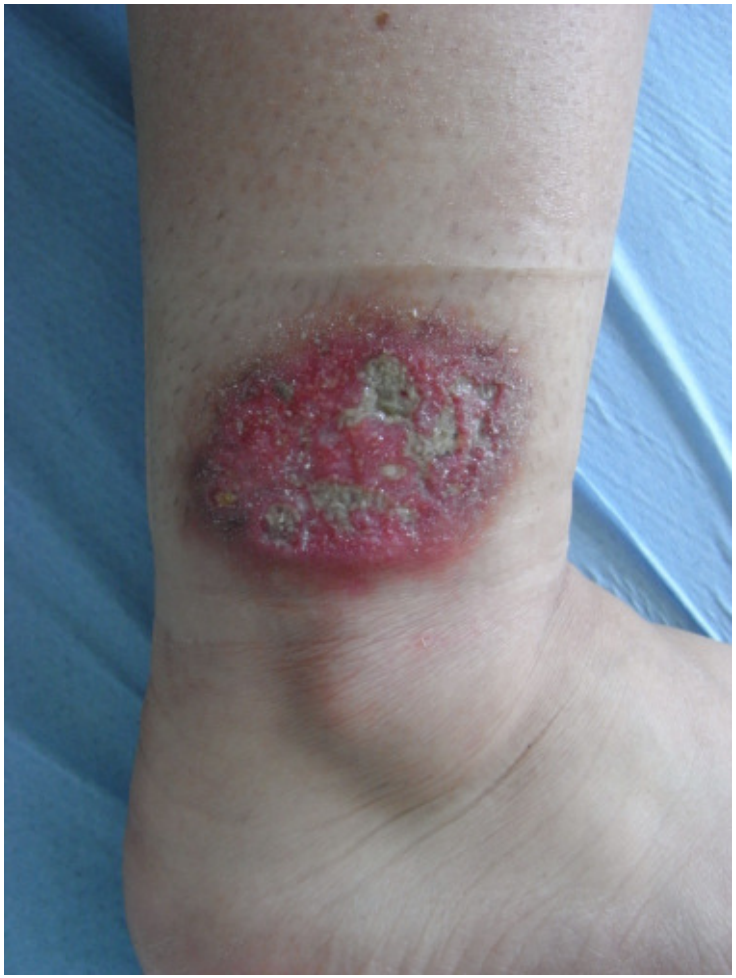


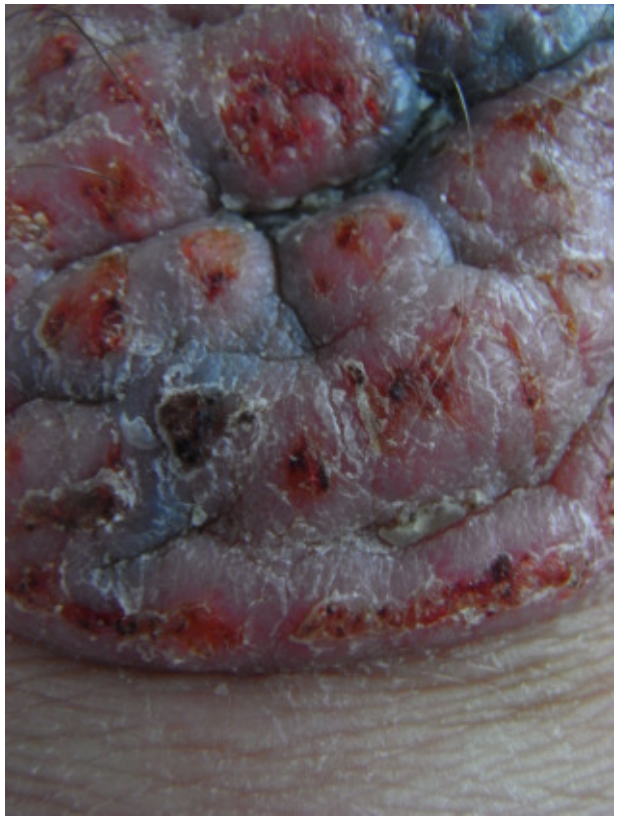


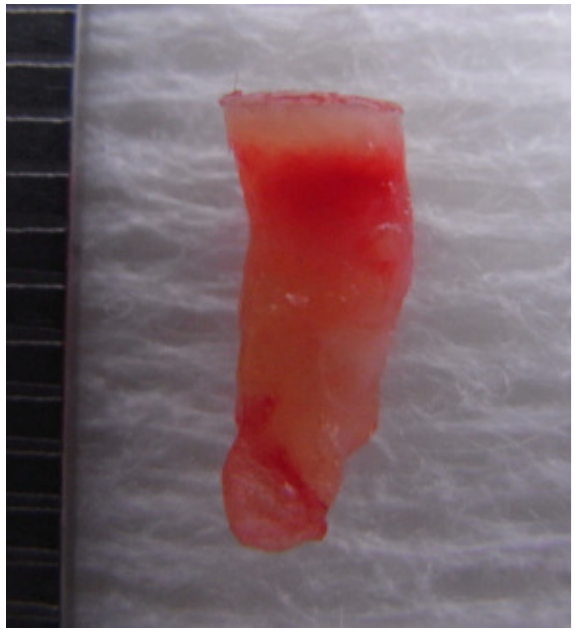


Exophytic reactions with excessive hyperkeratosis

3.3 patient SS

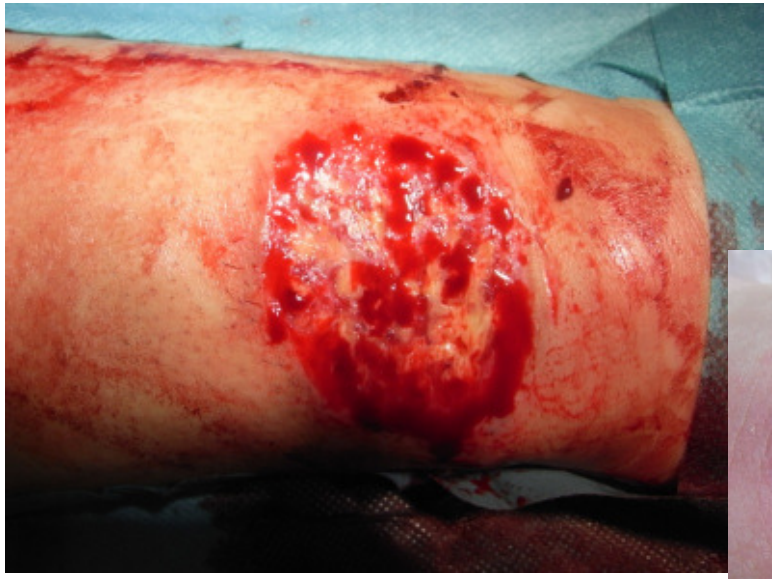


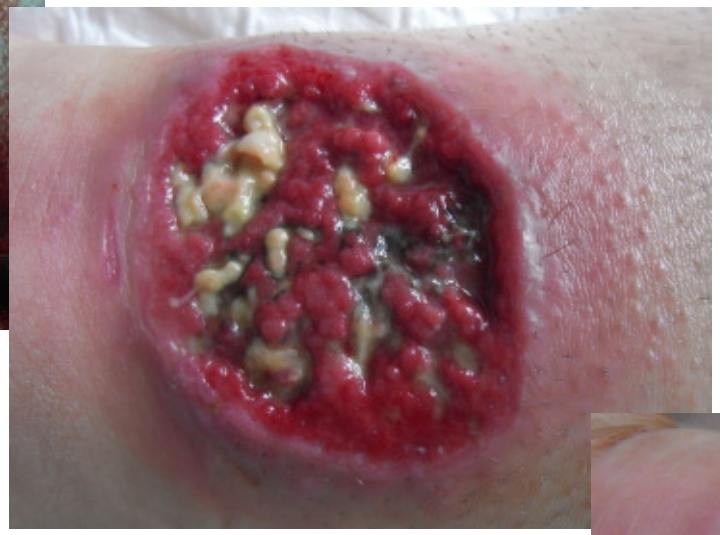


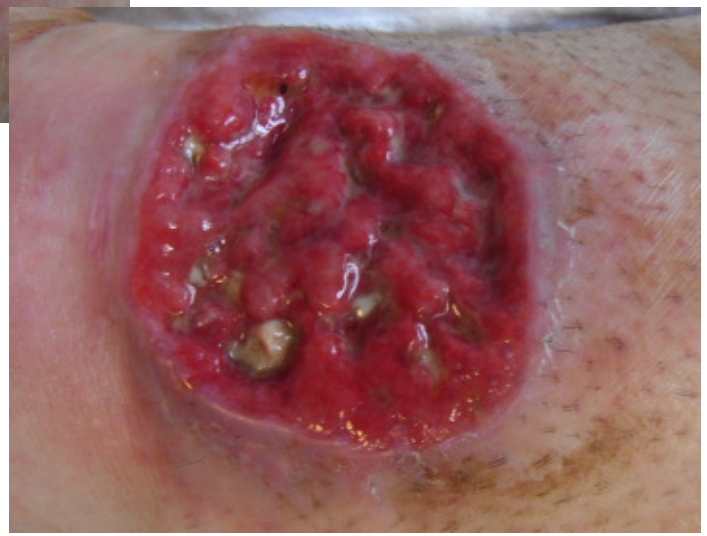


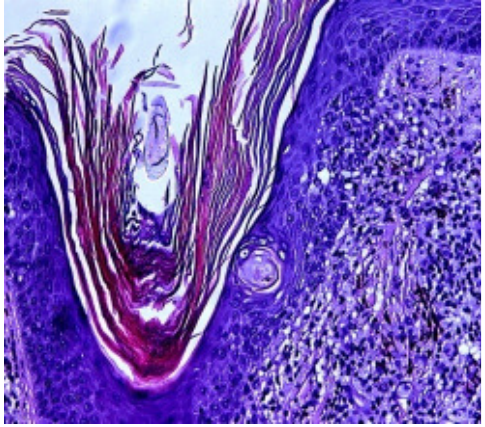
Patient 3.3 SS continued

During surgery (dermatome shaving)









”Notches” from hyperplastic epidermis may intrude into dermis, remain hyperplastic and delay healing.
IS BENIGN.

HD: Pseoepitheliomatous epidermal hyperplasia

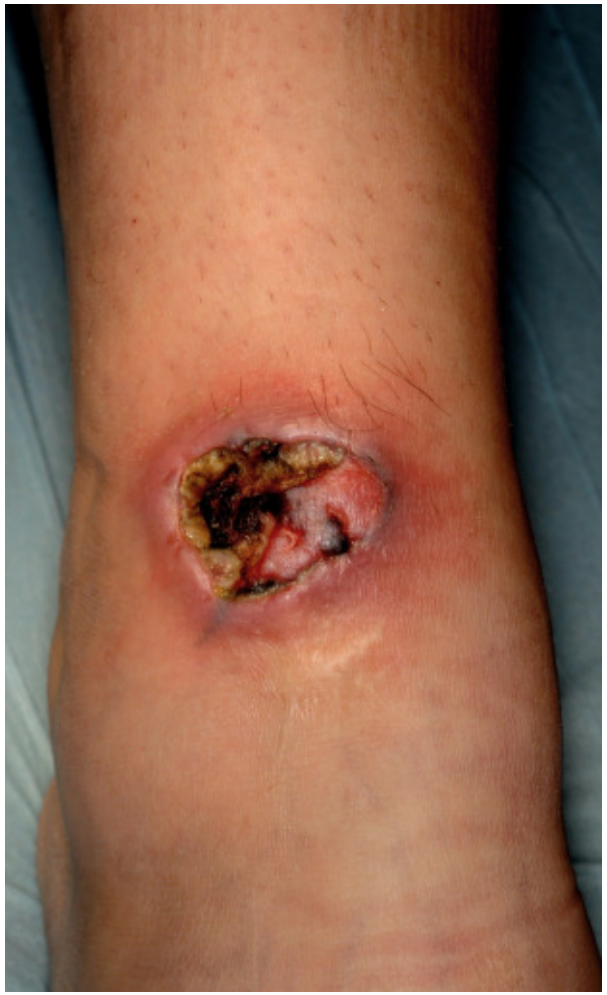
Pseudocanceroses in the literature

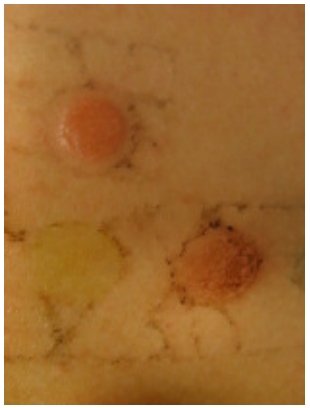
Epithelioma cuniculatum
Buschke-Löwenstein tumour
Verrucous carcinoma

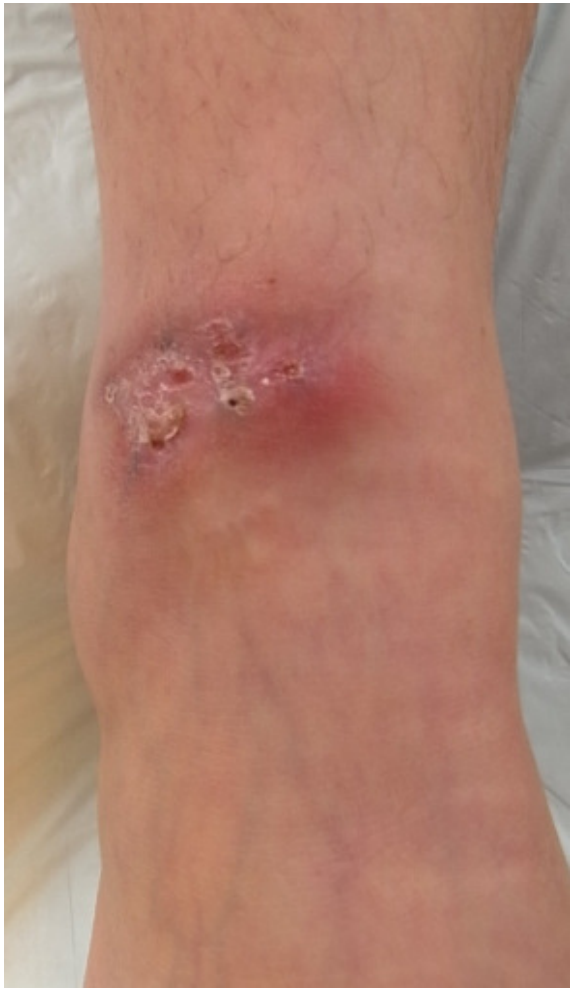
Allergic reactions going deep with ulceration, gangrena and, possibly, attack of the general skin

(the immunologic ”self”)

Ulcerating and invasive reaction predominated by necrosis, red tattoo
4.1 patient BJ

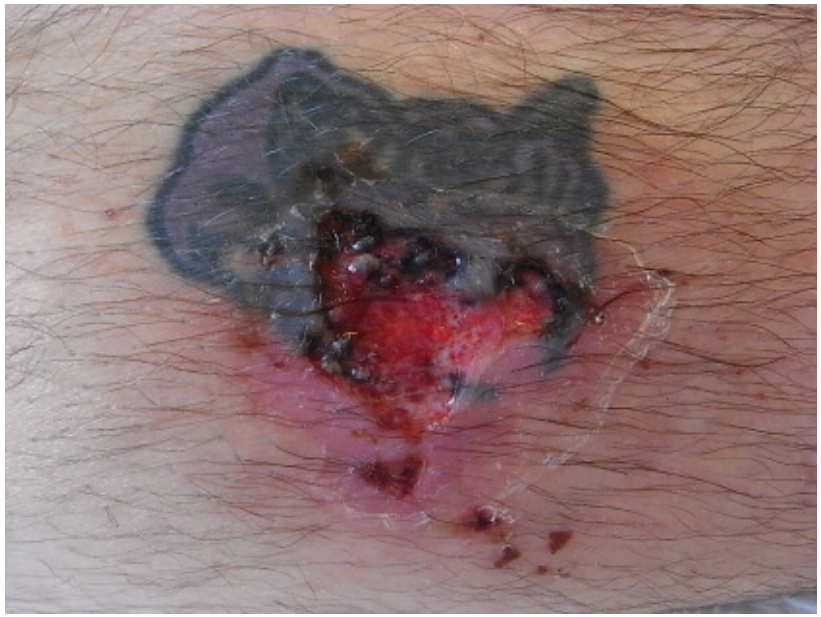


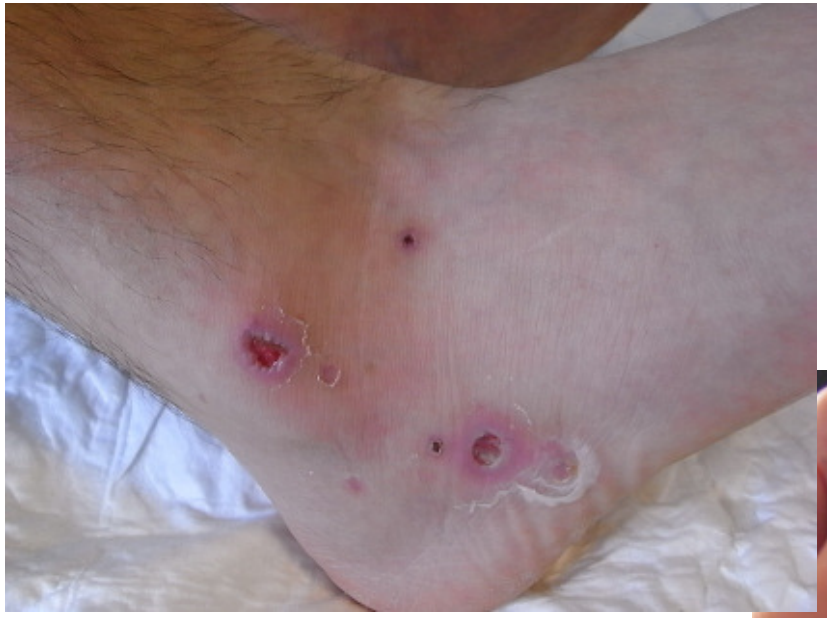


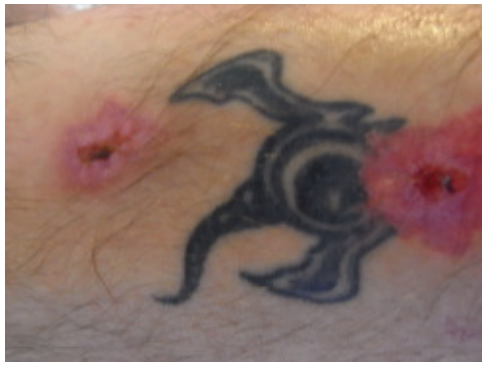


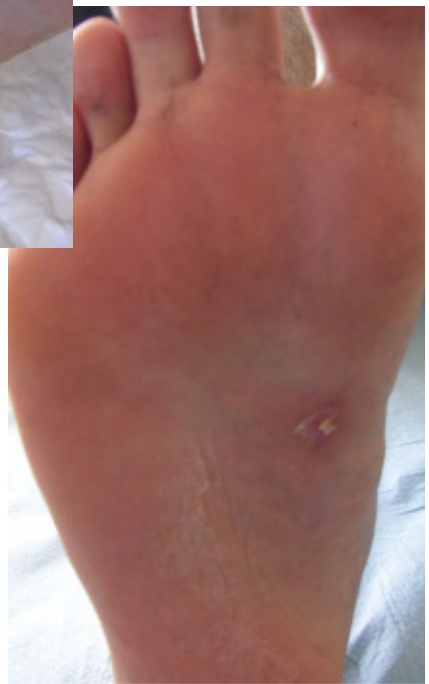
Systemic or general skin/other organ disease initiated or triggered by tattoo

7.1 patient MH





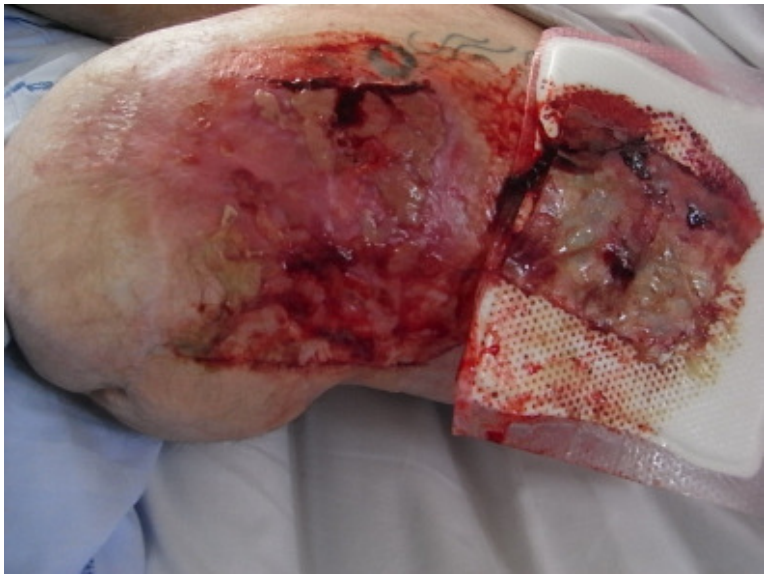




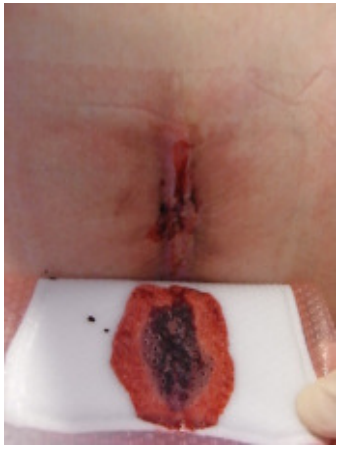
Histology: Perivasculitis rather than vasculitis

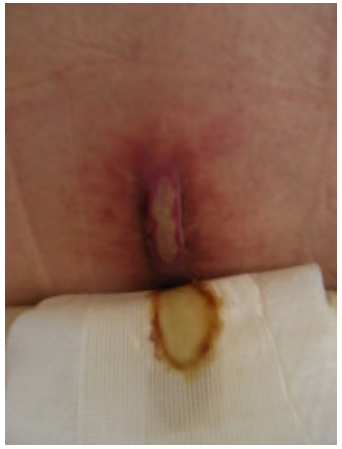
Extensive tattoo on leg, ulcer, gangrena,
several operations, finally amputation.
Entire skin now with delayed wound healing,
Herniated disc not healed two years after surgery.

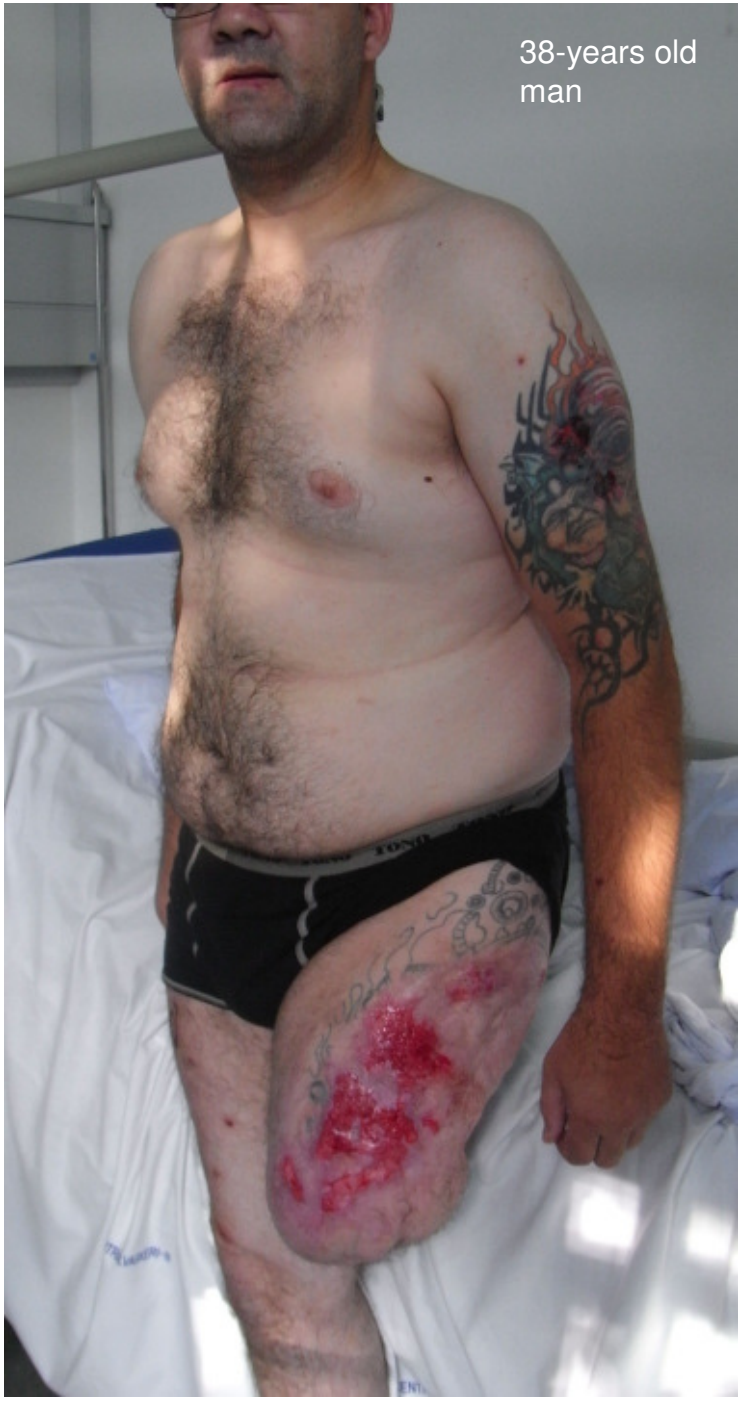
Thigh, non healing, fragile vasculature



Non healing, surgery of herniated disc





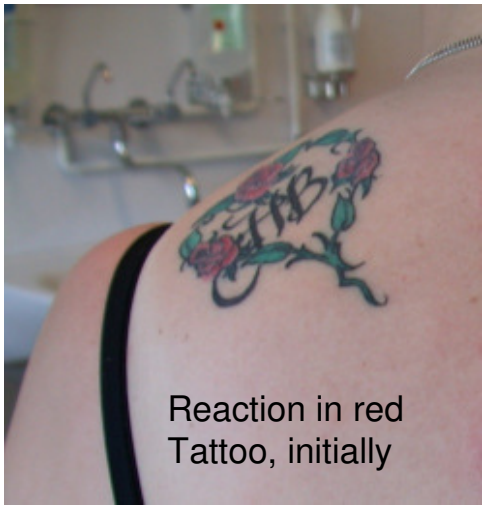


Allergic reaction in red tattoo with lymphadenopathy with necrosis and very advanced inflammation in skin andnode

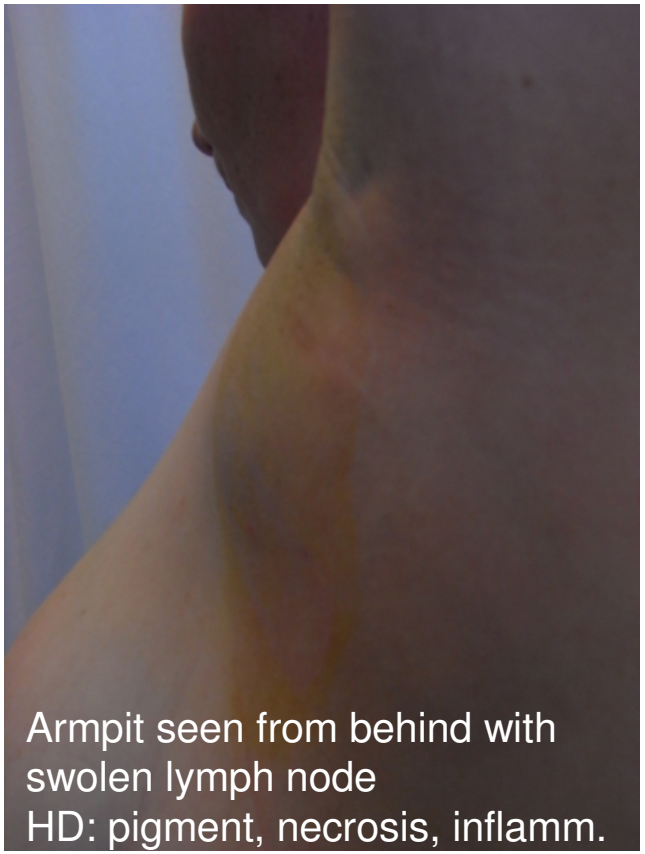
patient HB

Patient was extensively examined for malignant lymphoma (not found), TB, sarcoidosis and other malignancy, excluded.

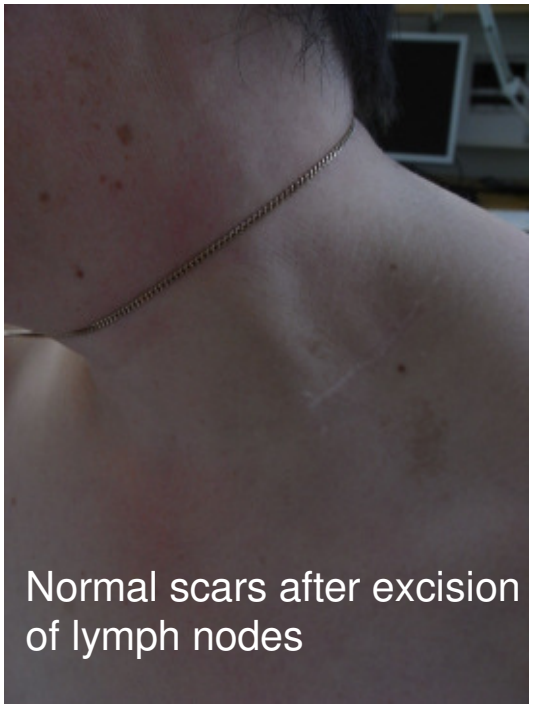
Patch test to culprit tattoo ink (brand ”Tattoo”, Taiwan) was negative, non-necrotic



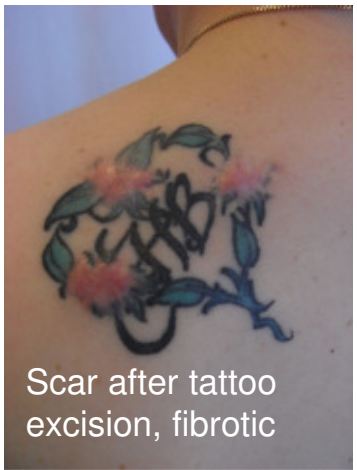
Reaction in red Tattoo, initially



Armpit seen from behind with swolen lymph node
HD: pigment, necrosis, inflamm.



Normal scars after excision of lymph nodes



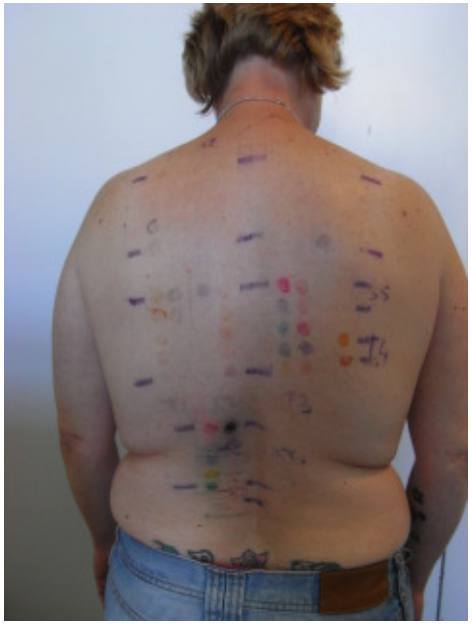
Scar after tattoo excision, fibrotic

Allergy Patch Test in Tattoo Reactions

MATERIAL

58 tattoo reaction sufferers tested with
- Standard allergen battery of 43 common allergens (includes nickel and PPD)
- Battery of 32 disperse textile dyes (European standard)
- Tattoo ink battery, 8 samples (brand ”Tattoo”, Taiwan, known to produce agressive reactions)
- Samples of individual culprit inks in 10 cases

Applied in occlusion chambers for 48 hours, read after 48 and 72 hours (and one week)



RESULTS in 58 patients, positive patch test readings by number

Standard allergens: 16 nickel*, 5 cobalt, 1chromate+
5 others (fragrances and preservatives)
0 PPD (para-phenylendiamine)

Dispersed textile dyes: 1 Disperse yellow, 1 urea formaldehyde

Tattoo ink battery: 4 Tattoo red, 1 also pos. to white, green, purple**

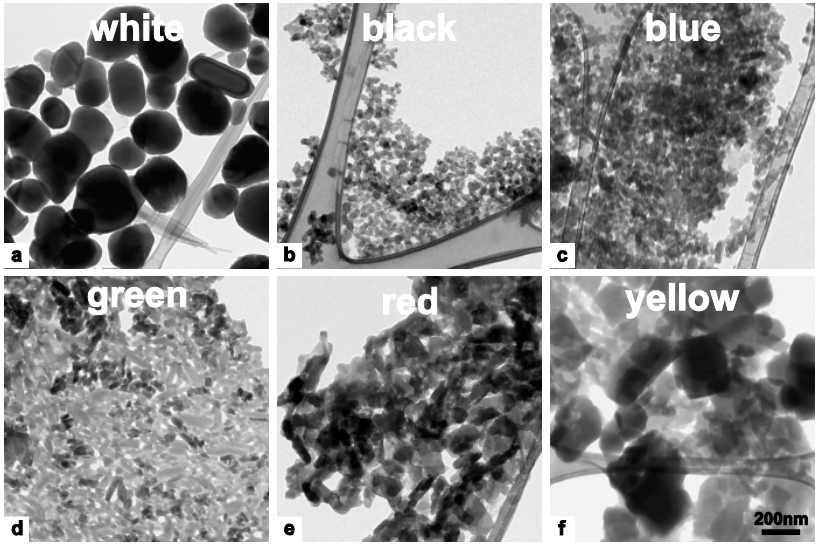
*Nickel, 33% positives vs 11% in the test routine (233/2071)

Of 10 patients tested with their culprit ink 1 (only!) had positive reading, to red.

Conclusion

Patients with tattoo reactions by allergy patch test failed to react to a tattoo ink battery, to textile dyes and even to their respective culprit ink stock product.
However, reactions to nickel were more frequent contrasting no special reactions to other common allergens including preservatives and PPD.

Tattoo pigments are robust particles



TEM of tattoo ink particles
By Takasi Kobayasi MD
BBH, Copenhagen

Particle size of 58 tattoo inks by laser diffraction

Høgsberg T, Loeschner K, Löff D, Serup J. Tattoo inks of general usage contain nanoparticles. Br J Dermatol 2011;165:1210-18

Tattoo pigments remain permanently in the dermis because they are robust particles that are not easily degradated *in vivo* by enzymes such as azo-reductases needed for metabolism into PAA chemical splits.
Bacteria on the skin surface and in the gut may produce significant (?) amounts of reductase.

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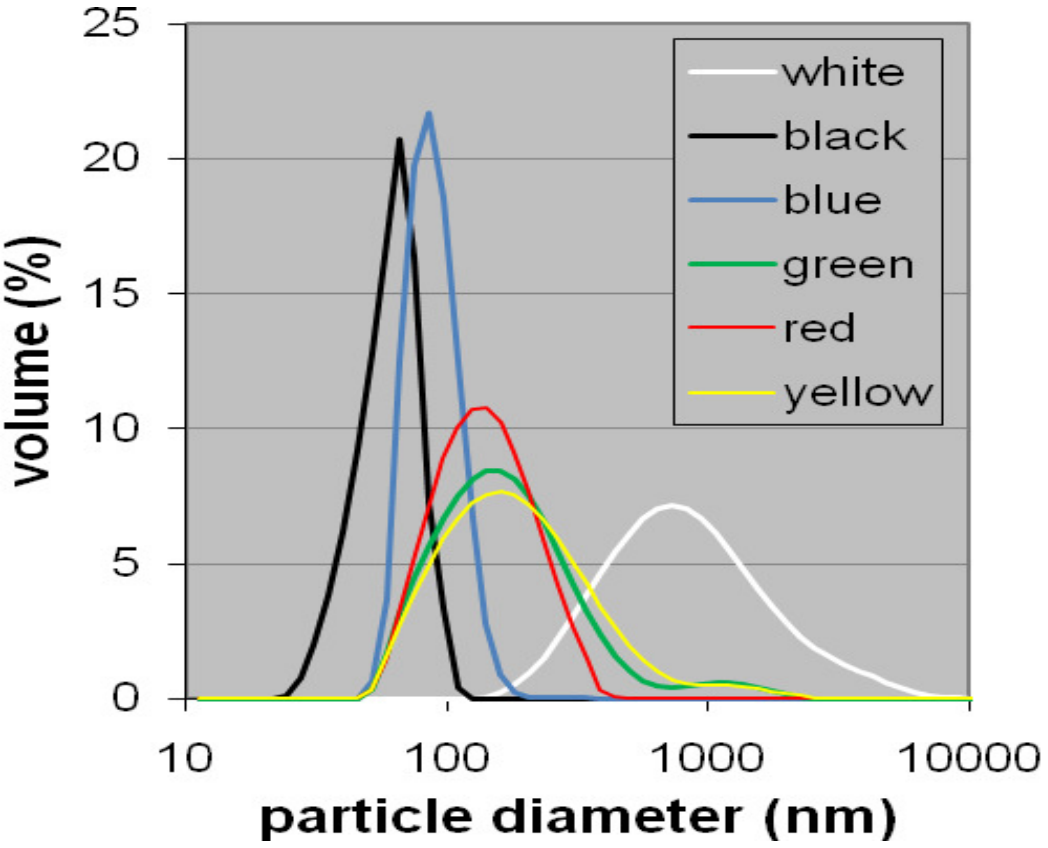
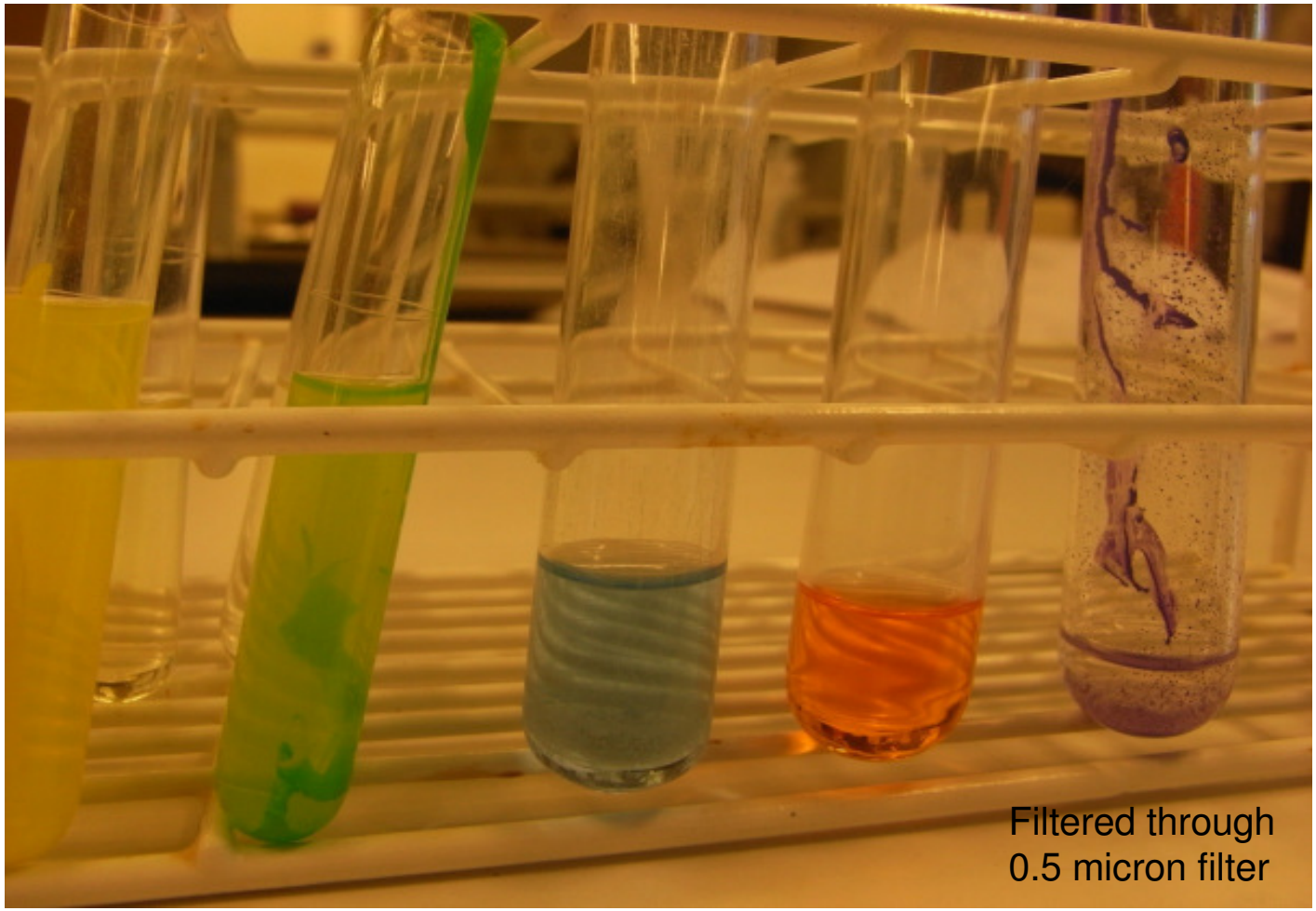
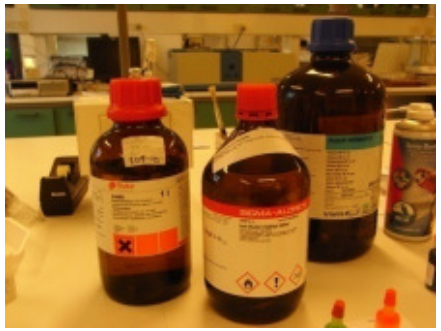


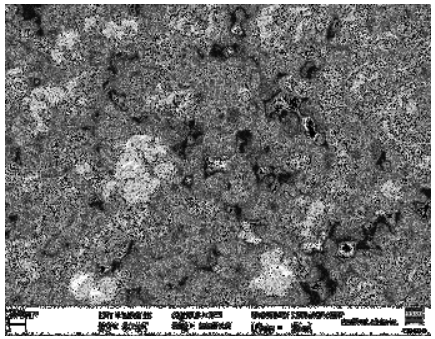
Tatto pigments are very hard to dissolve, thus, unlikely to be available for metabolism





Harsh organic solvents

SEM of tattoo pigment

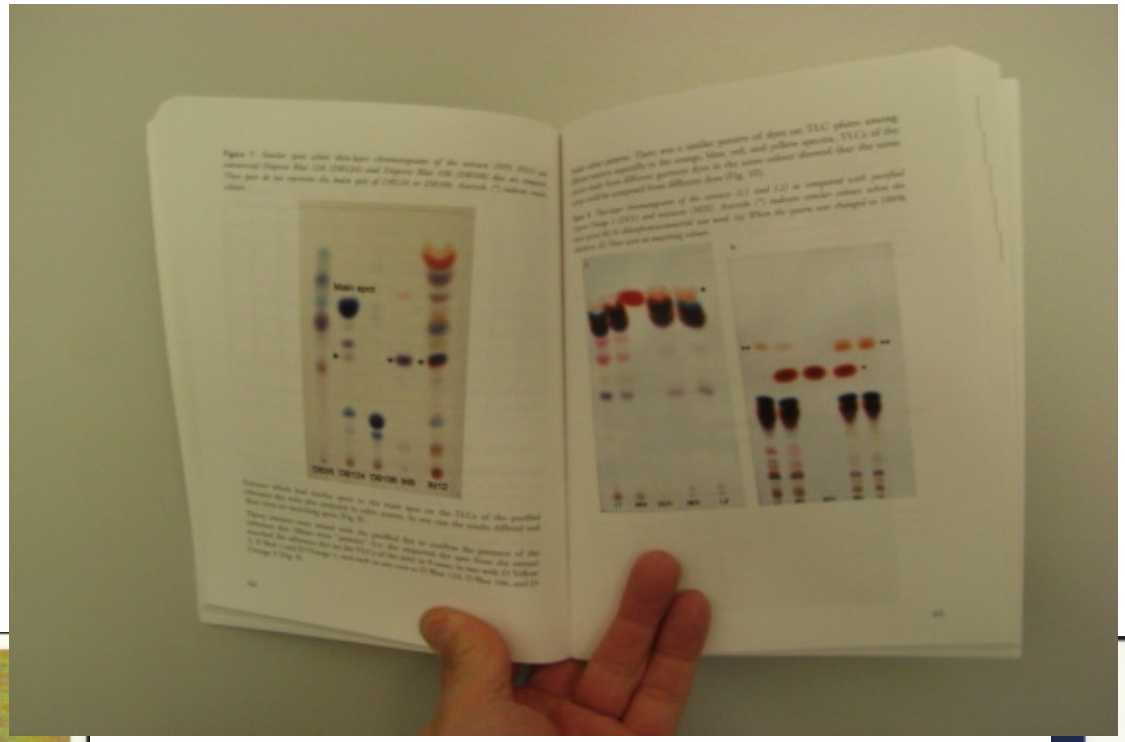


DTU/BBH, Copenhagen, DK

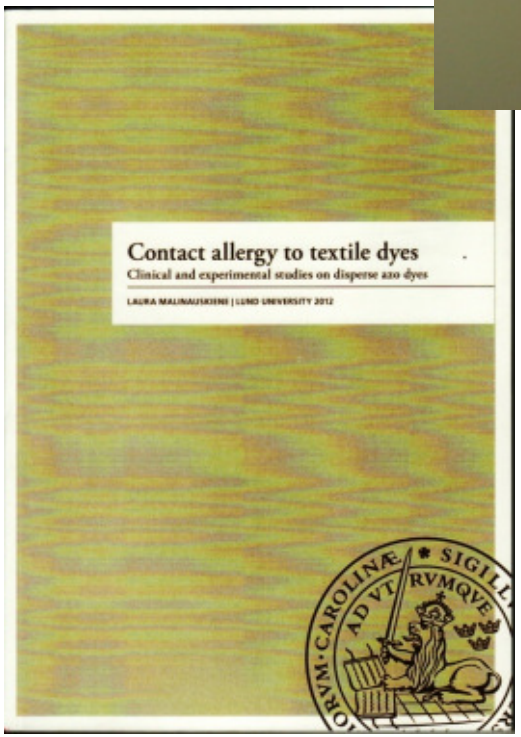
Ink stock products are hard to dissolve in harsh solvents and thus unlikely to be bioavailable, not obvious source of PAA split metabolites in the skin.
Free chemicals and free PAA are likely to be washed out swiftly, i.e. in days.

Sample preparation/analytic methods for textile dyes are not directly applicable to tattoo ink products. Tatto pigments, being robust particles, are very special.

Textile dermatitis, azo dyes, PAAs – failure of PAA to be concluded culprit allergen



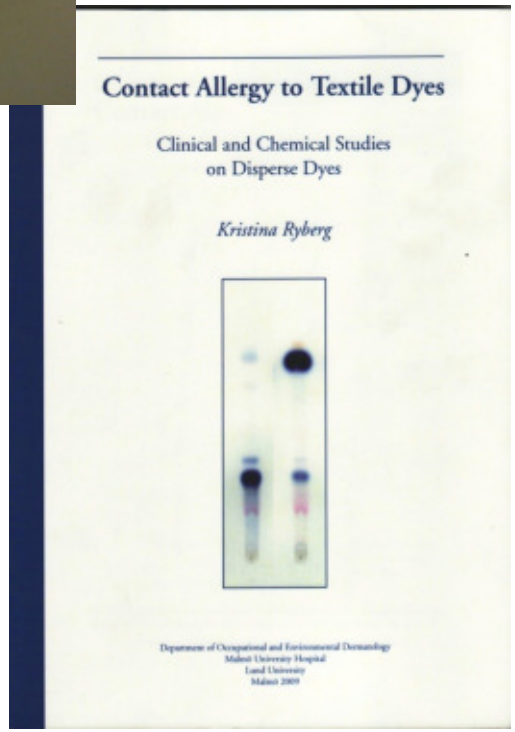
Textile dyes can be dissolved. They comprise several fractions by chromatography. Likely to be bioavailable.



Two Swedish dissertations on disperse textile dyes have rejected the metabolism theory.

PAA in dyes or PAA metabolised from azo cannot explain allergy to dyes in textiles.

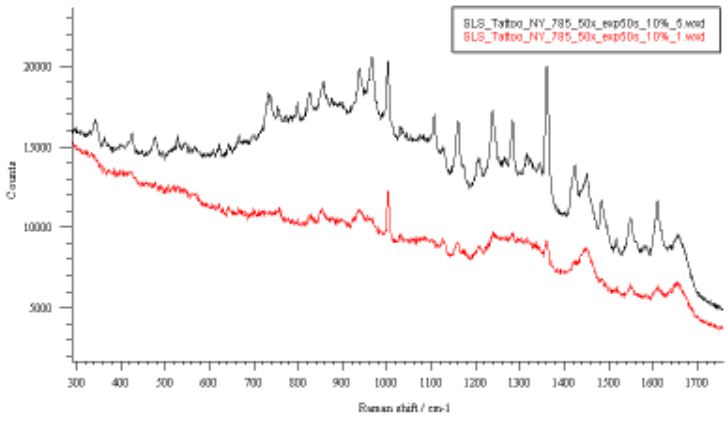
In azo allergy negative patch test to respective PAAs, Swedish Study



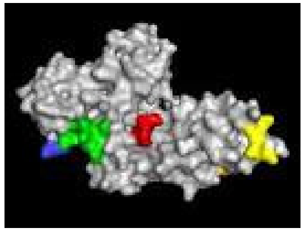
Raman Spectroscopy of shave cut biopsies of tattooed skin reacting to red Indicates no special metabolism of pigment into the respective PAA

Material
3 cases with known culprit ink and allergic reactions in their tattooes
(Brands ”Tattoo” red, Intenze Bright Red, Starbrite Grimson Red, all azo)
Shave biopsy samples of outer dermis, reactions and normal skin reference
Ink samples, before and after daylight exposure
PAA standards aniline, o-anisidine, 3,3`-dichlorobenzidine, known from the inks

Result
- very different spectra in tattoed skin vs normal, due to the pigment
- PAAs identified in stock inks as fingerprints only
- One ink sample, brand ”Tattoo”, by Raman showed aniline after 24 h daylight
- **No shave biopsy showed peaks representing the expected PAA**



BBH/Danish Institute of Technology
unpublished data onfile



Epitope(s)-protein complex

The Modern Allergen Concept:

Allergens are epitope-protein comlexes formed *In vivo (**haptenation**).*

Thus, the culprit allergen in tattoo reactions is not found in the ink stock product. Proteins inside skin contribute.

PPD (para-phenylene diamine) allergene exemplified (PPD documented epitope in "henna "allergy, allergic Patients always react to patch test with PPD solution although the true allergen is a PPD-protein complex generated in the skin)

REVIEW PAPER

Contact Dermatitis
Review Article

Hapten–protein binding: from theory to practical application in the in vitro prediction of skin sensitization

... review, we consider some of the theoretical aspects of protein haptenation, how mechanisms of protein haptenation can be investigated experimentally and how we can use such knowledge in the development of novel, alternative approaches for predicting skin sensitization potential in the future.

Key words: hapten–protein binding; in vitro assay; peptide binding; skin sensitization. © Blackwell Munksgaard, 2005.

Accepted for publication 27 June 2005

Contact Dermatitis • Review Article
COD
Contact Dermatitis

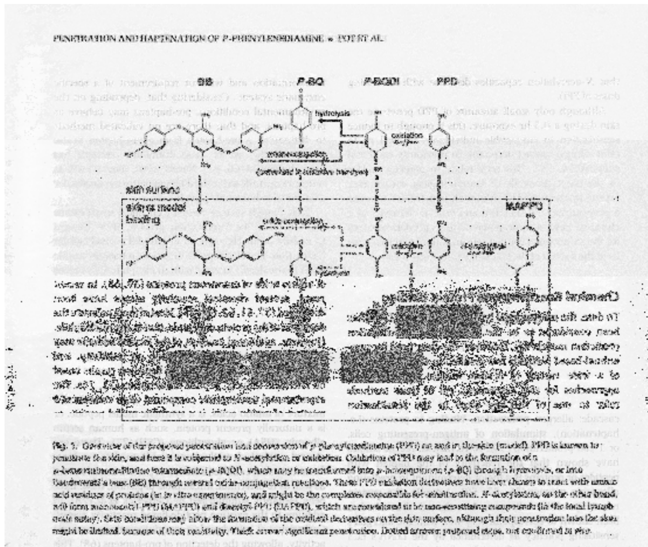
Penetration and haptenation of *p*-phenylenediamine

Laura M. Pot[1], Simone M. Scheitza[2], Pieter-Jan Coenraads[1] and Brunhilde Blömeke[2]
[1]Department of Dermatology, University Medical Centre Groningen, University of Groningen, 9700 RB Groningen, The Netherlands and [2]Department of Environmental Toxicology, University Trier, Universitätsring 15, 54286 Trier, Germany

Summary

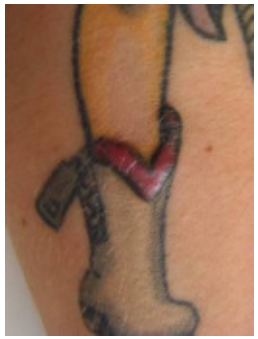
Although *p*-phenylenediamine (PPD) has been recognized as an extreme sensitizer for many years, the exact mechanism of sensitization has not been elucidated yet. Penetration and the ability to bind to proteins are the first two hurdles that an allergen has to overcome to be able to sensitize. This review is an overview of studies regarding PPD penetration through skin (analogues) and studies on the amino acids that are targeted by PPD. To complete this review, the auto-oxidation and *N*-acetylation steps involved in PPD metabolism are described. In summary, under normal hair dyeing exposure conditions, <1% of the applied PPD dose penetrates the skin. The majority (>80%) of PPD that penetrates will be converted into the detoxification products monoacetyl-PPD and diacetyl-PPD by the *N*-acetyltransferase enzymes. The small amount of PPD that does not become *N*-acetylated is susceptible to auto-oxidation reactions, yielding protein-reactive PPD derivatives. These derivatives may bind to specific amino acids, and some of the formed adducts might be the complexes responsible for sensitization. However, true *in vivo* evidence is lacking, and further research to unravel the definite mechanism of sensitization is needed.

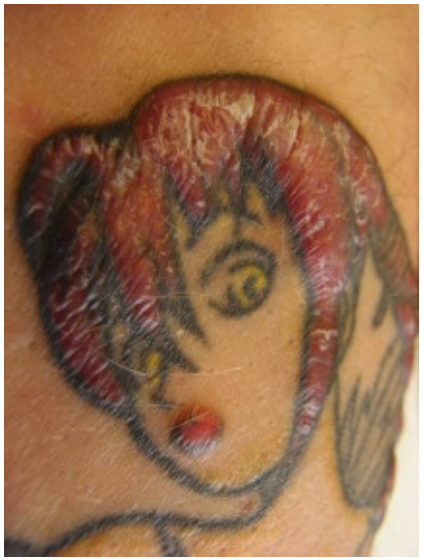
Key words: haptenation; penetration; *p*-phenylenediamine; protein binding; sensitization.



CONCLUSION, THE CLINIC OF ALLERGIC TATTOOES

- Red azo is the ”high risk” group
- Allergy is (in contrast to complaints) quite uncommon and typically arise late after remarkably long time, i.e. after moths or years
- Allergy affects every piece of skin tattooed with the culprit ink
- Cross-reactivity in old red tattoes hitherto tolerated may occur
- Allergy may be aggressive and cause ulceration and attack of normal skin of distant location.Thus, may attack immune ”self” tissue.
- Histology: T-lymphocyte inflammation of the outer dermis, inter-phase dermatitis as predominant pattern, with basement membrane leaks and hyperplastic epidermal reactions





CONCLUSION AND STATUS ON ALLERGENs CAUSING ALLERGY OF TA AND FUTURE SCREENING OF TATTOO INKS

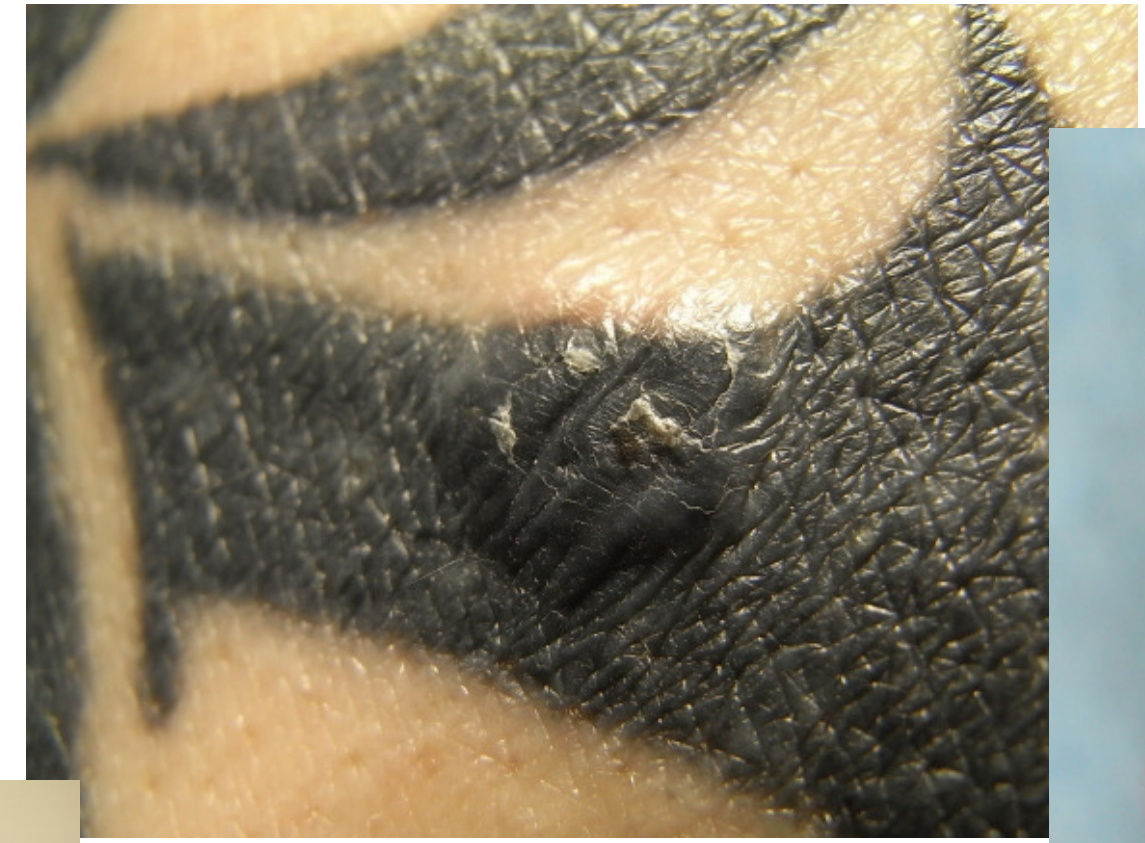
- The allergen is not present in the tattoo ink stock bottle, as allergy patch testing concludes!!!
- The allergen, thus, is unlikely to be a simple chemical split product such as PAAs, which is found in free form in many inks
- PAA was not found in shave biopsies of reactions to red, by Raman
- The PAA metabolism theory was shown to fail in textile dye/azo allergy
- The allergen is most likely formed in the skin as a complex of epitope(s) and protein, which may also explain aggressive allergy with attack of the general skin organ independent of tattooing
- The epitope(s) is entirely unknown but likely to be somehow originated from the red azo pigment (often of dubious purity)
- **PRACTICAL CONSEQUENCE: lack of knowledge and lack of rationale and validation makes separation of allergy-safe and allergy-risky tattoo ink stock products in the marketplace impossible.**

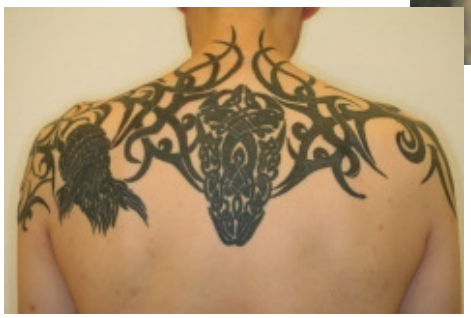
The European Council 2003/2008 resolution is nicely framed, however, made out of speculation arising mainly from ex vivo sources of questionable relevance. It remains being not validated, after 10 years. It deserves critical and fundamental reconsideration re. allergy risk prediction and prevention, as for other themes.

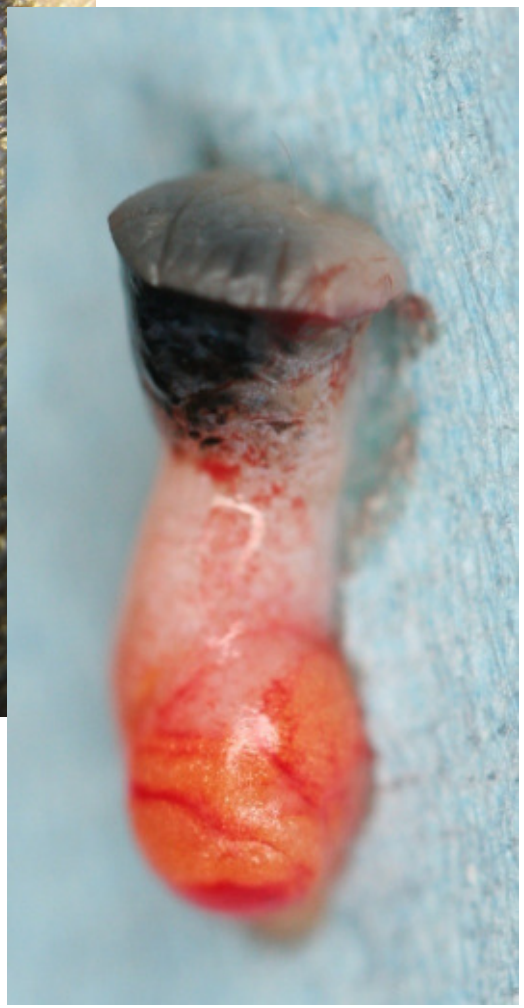

THE SAW IS FAMILY
THE DEAD WALK

Elevated globoid, nodular or papular reactions, black

2.2 patient PIH







Elevated globoid, nodular or papular reaction, black

2.4 patient HK