



**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.

Copenhagen, Denmark • November 13-14 2013  
[www.ectp2013.org](http://www.ectp2013.org)



# Allergies and Tattooing

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)\* \*\*

Tattooed Individuals, overall:

**Of all tattooed nearly half have complaints, and 1/5 have abnormal sensitivity to light in their tattoo!**

|                                       | STD Clinic Study* | "Beach" Study** | Pooled/overall      |
|---------------------------------------|-------------------|-----------------|---------------------|
| Total number tattooed                 | 154               | 144             | 298                 |
| <u>Complaining (vs total),</u>        | 65/154, 42%       | 60/144, 42%     | 125/298, <b>42%</b> |
| <u>Photosensitive (vs complaints)</u> | 24/65, 37%        | 31/60, 52%      | 55/125, <b>44%</b>  |

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 tattoos 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

- Aquired 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; affects every part of a tattoo holding a culprit colour; if a line or writing the entire line is swollen

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. Ulcerating and invasive reactions predominated by necrosis

5. *Pigment leakage and lymphopathy 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 tattooed 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**

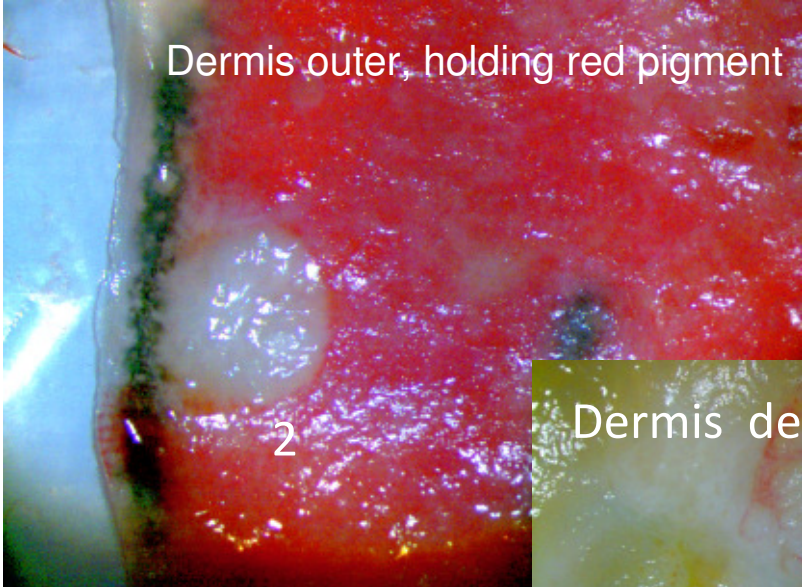
**1.6 patient BC**



Dermatome Shaving



Dermis outer, holding red pigment



2

Dermis deep

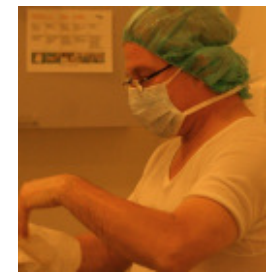


**Pattern 1. Elevated "plateau" reactions in red, scaly and "lichenoid"**

**1.4 patient GH**



## Elevated "plateau" reactions in yellow, lichenoid

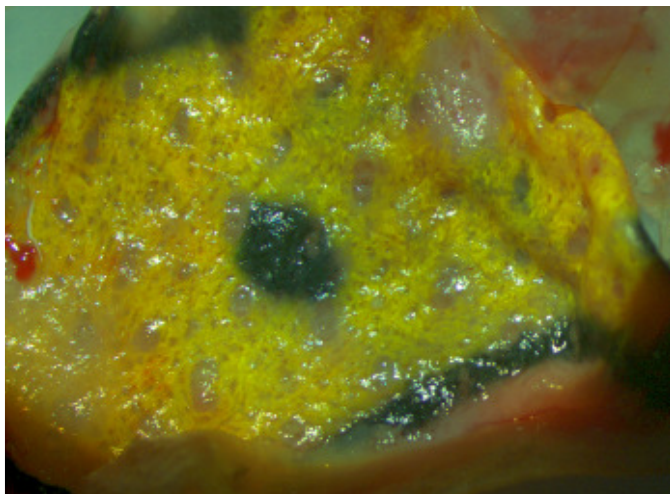


Bo Jørgensen MD

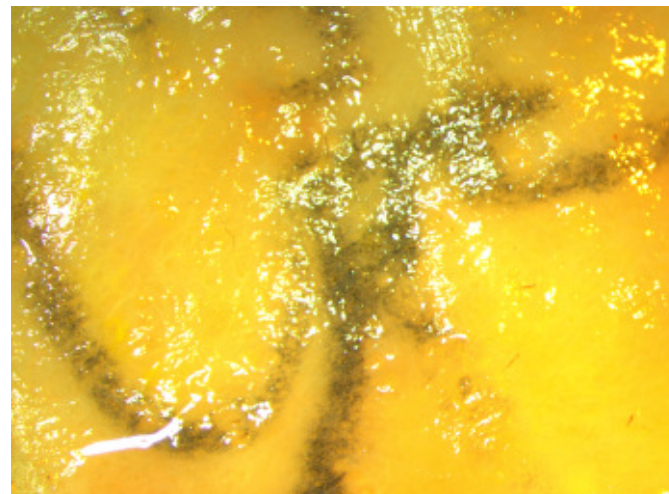
### 1.7 patient TPH – the pigment layer in the dermis on shaving



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



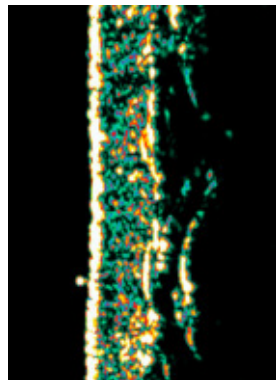
Cross reacting tattoos on leg, old and hitherto tolerated well



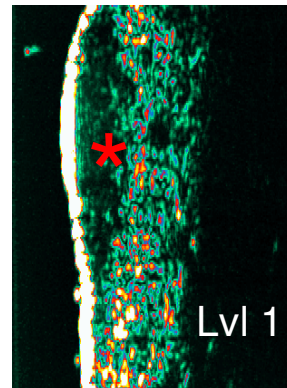
## Level of inflammation in the dermis by 20 MHz ultrasound correlates with histology. N=58\*

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

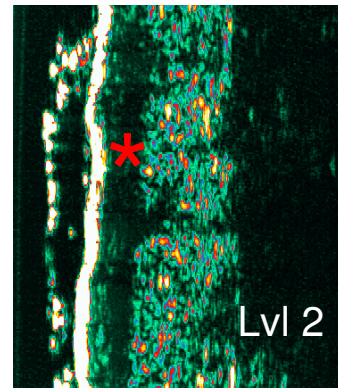
| Depth of dermal Infiltrate, histology | Number of Samples<br>n | Echolucent band<br>mm, mean (SD) |
|---------------------------------------|------------------------|----------------------------------|
| Level 1, outer                        | 12, 21%                | 0.45 (0.33)                  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



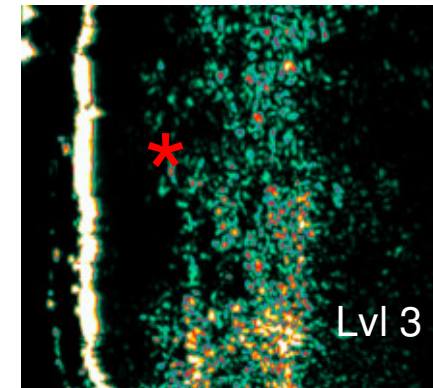
Normal



Lvl 1



Lvl 2



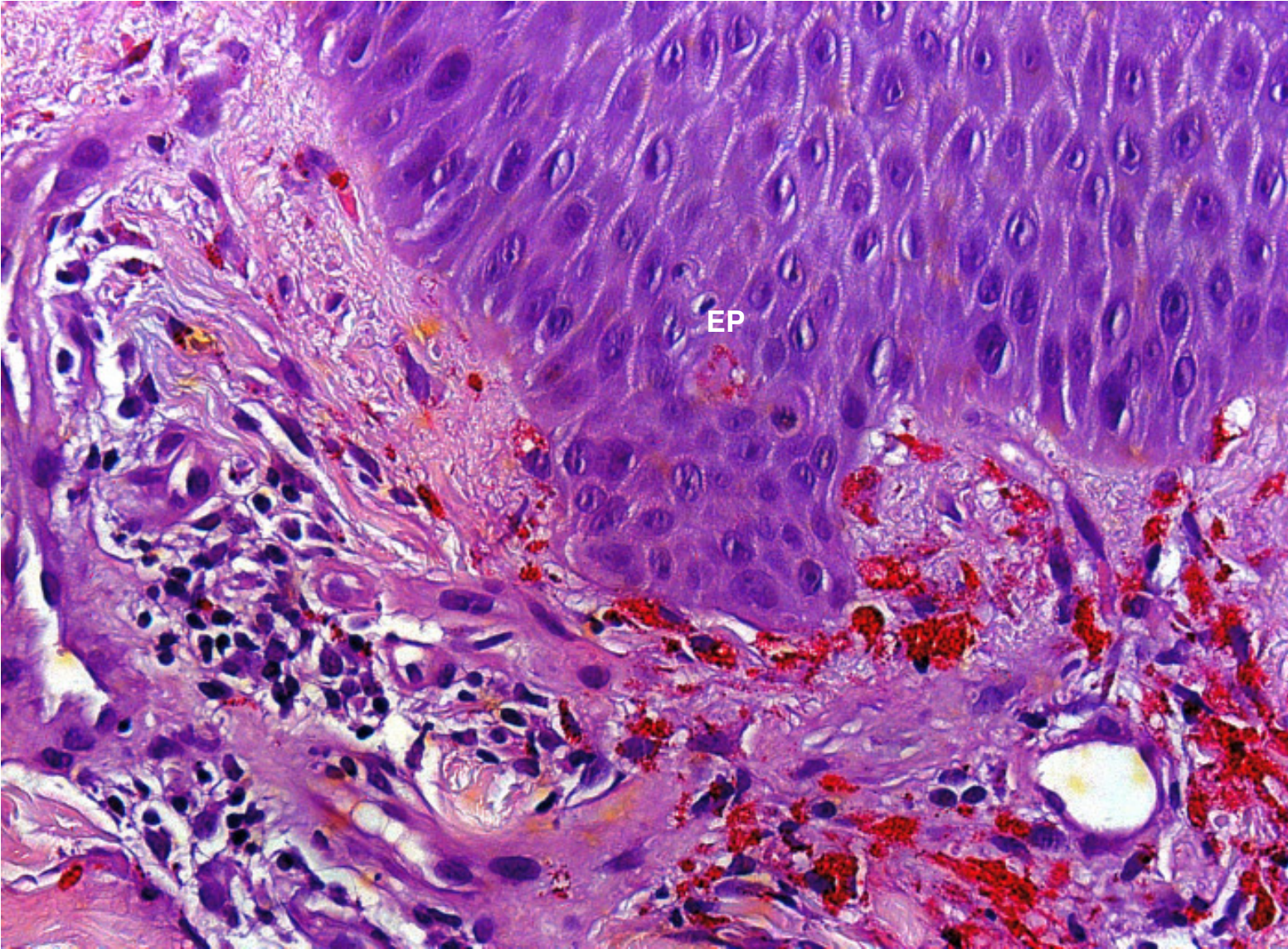
Lvl 3

\* 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 inflammation of the dermis)

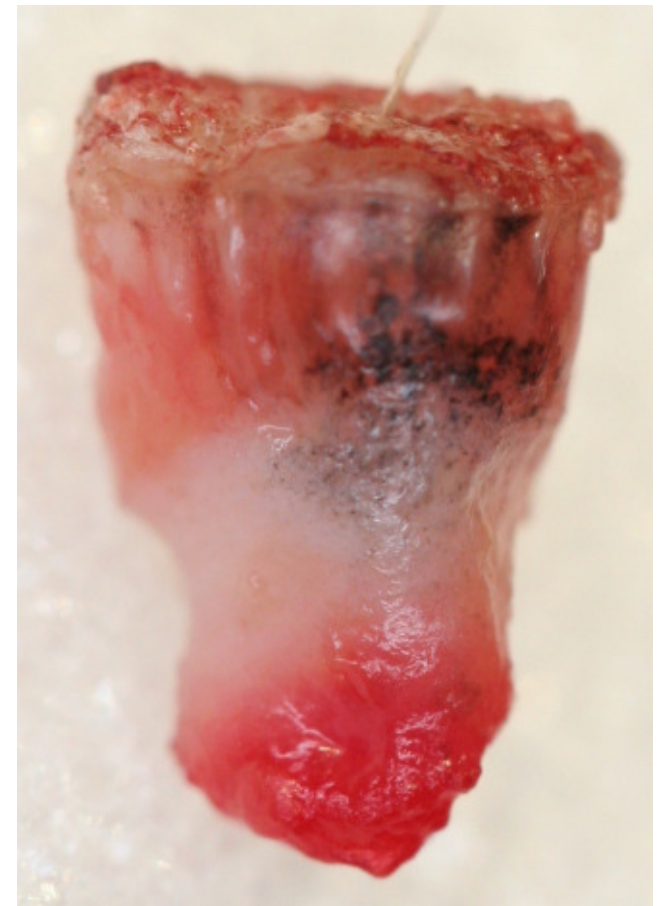


Interphase dermatitis with lymphocytic infiltration surrounding clustered red pigment in the outer dermis. Note pigment leaked to epidermis (EP)



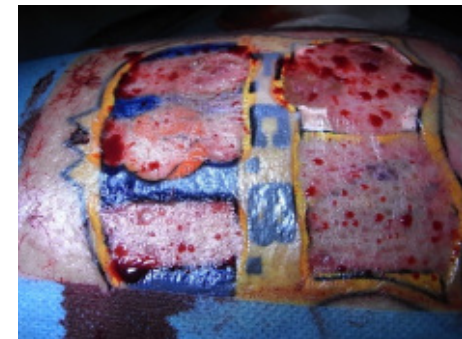
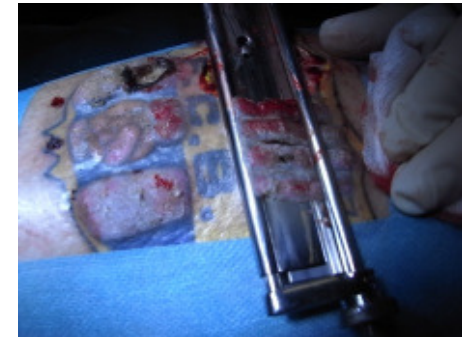
## Exophytic reactions with excessive hyperkeratosis and pigment leakage

### 3.1 patient TH



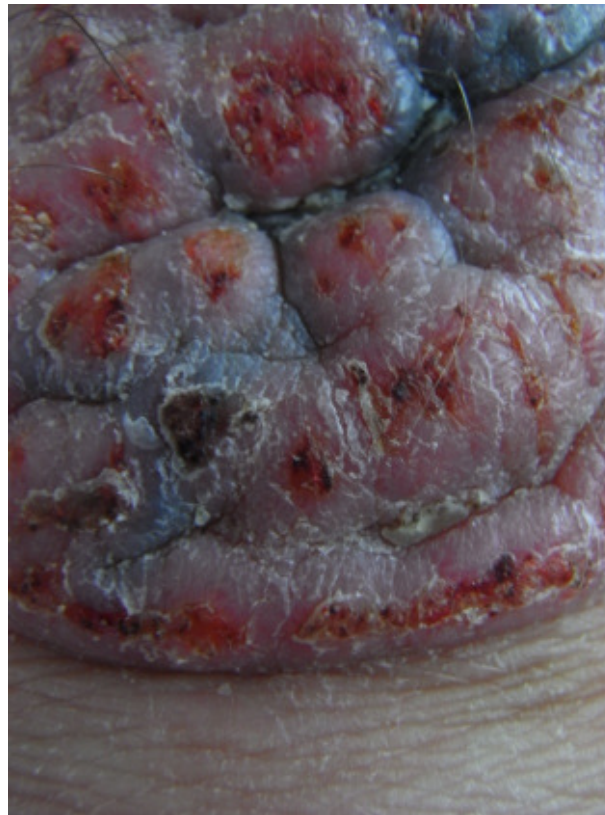
## Exophytic reactions with excessive hyperkeratosis

### 3.2 patient BL



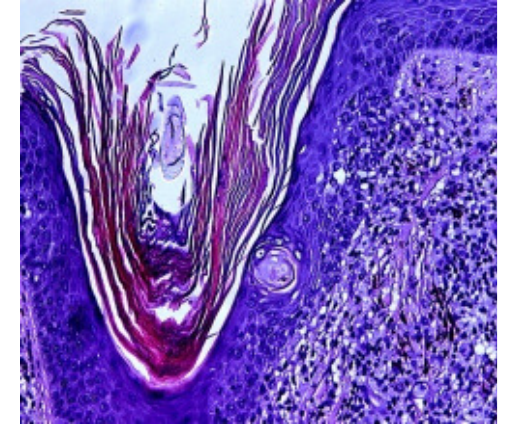
## Exophytic reactions with excessive hyperkeratosis

### 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: Pseudoepitheliomatous epidermal hyperplasia

*Pseudocanceroses in the literature*

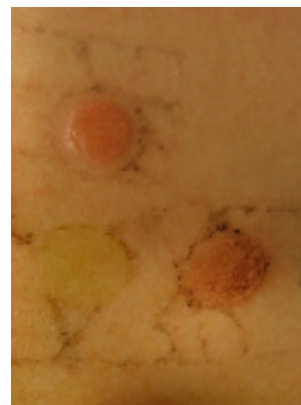
Epithelioma cuniculatum

Buschke-Löwenstein tumour

Verrucous carcinoma

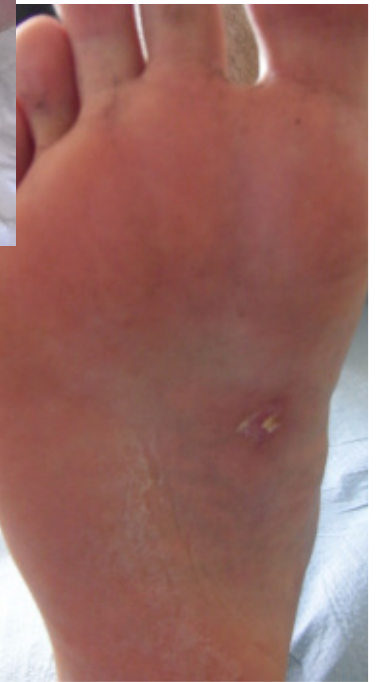
Allergic reactions going deep with ulceration, gangrena  
and, possibly, attack of the general skin  
(the immunologic "self")

**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

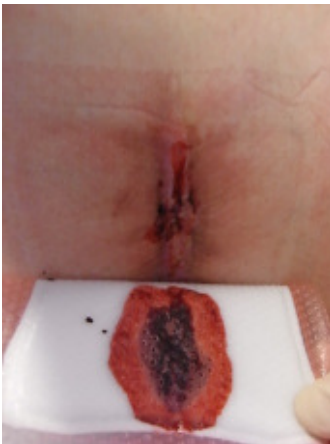


**Extensive tattoo on leg, ulcer, gangrena,**  
several operations, finally amputation.  
Entire skin now with delayed wound healing,  
Herniated disc not healed two years after surgery.

Thigh, non healing, fragile vasculature



Non healing,  
surgery of  
herniated disc

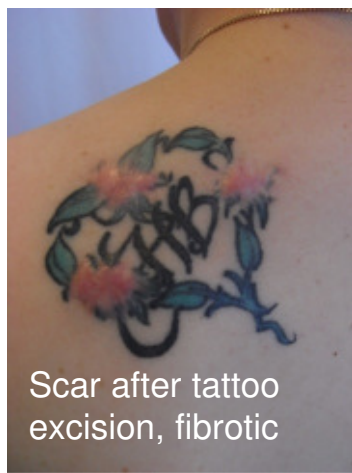
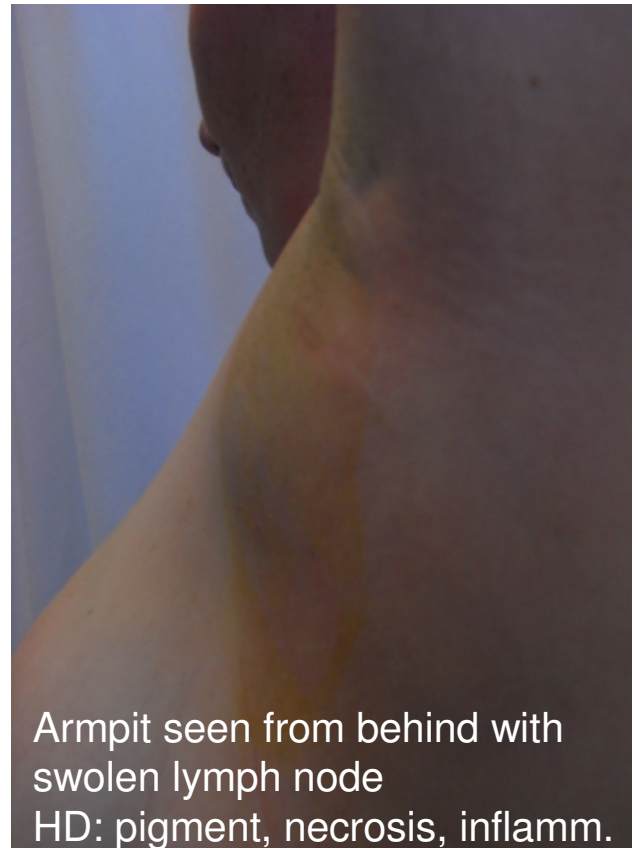


# Allergic reaction in red tattoo with lymphadenopathy with necrosis and very advanced inflammation in skin and node

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



# 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 aggressive 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, 1 chromate+  
5 others (fragrances and preservatives)  
0 PPD (para-phenylenediamine)

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)

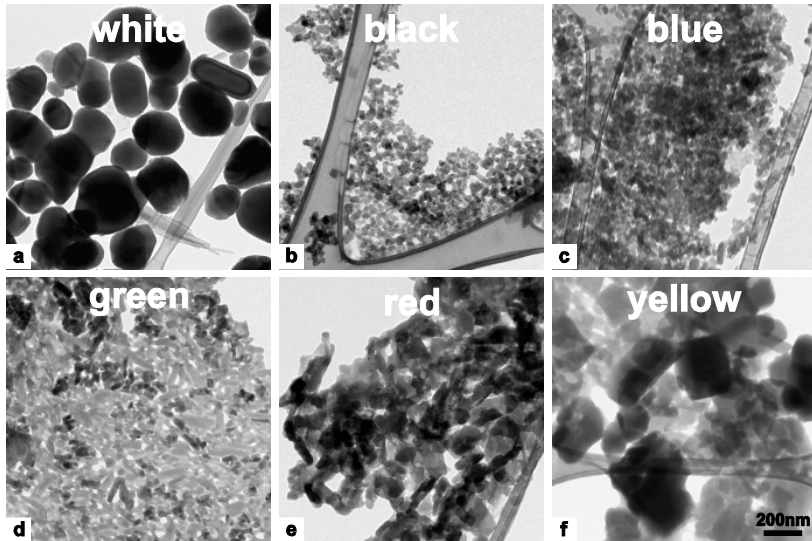
Of 10 patients tested with their culprit ink 1 (only!) had positive reading, to red.

## 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

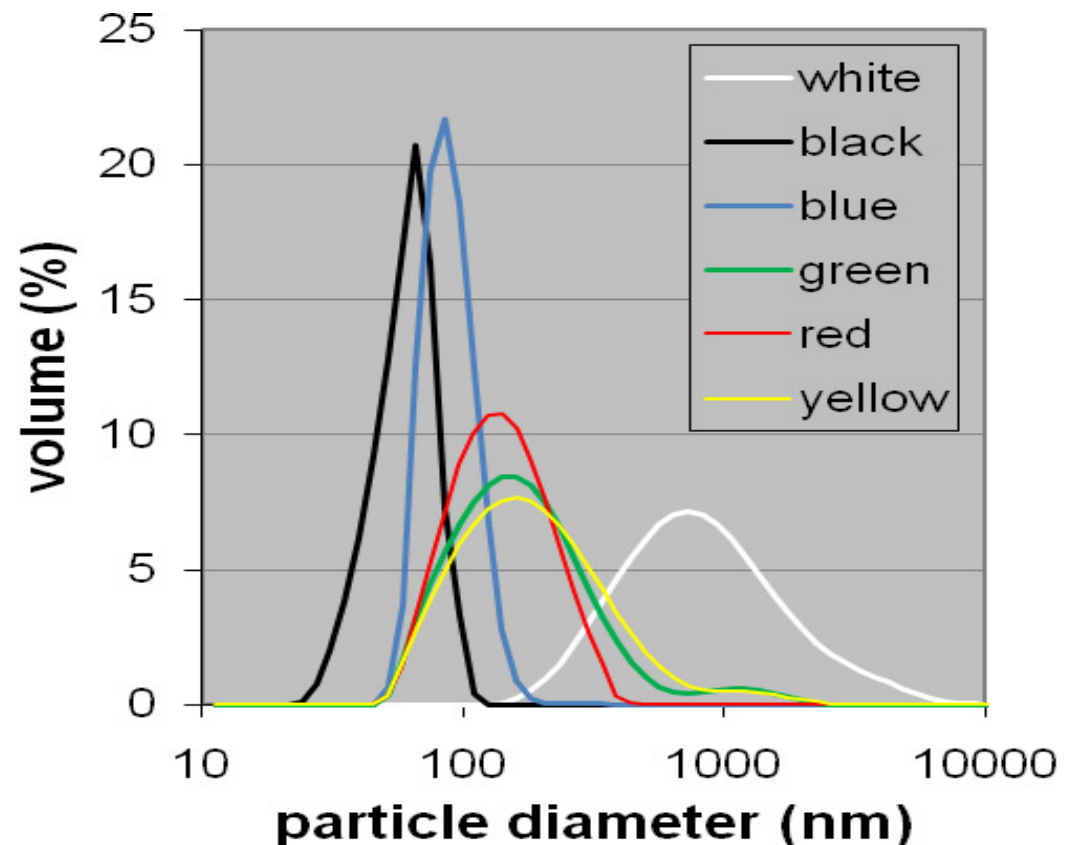


TEM of tattoo ink particles  
By Takasi Kobayasi MD  
BBH, Copenhagen

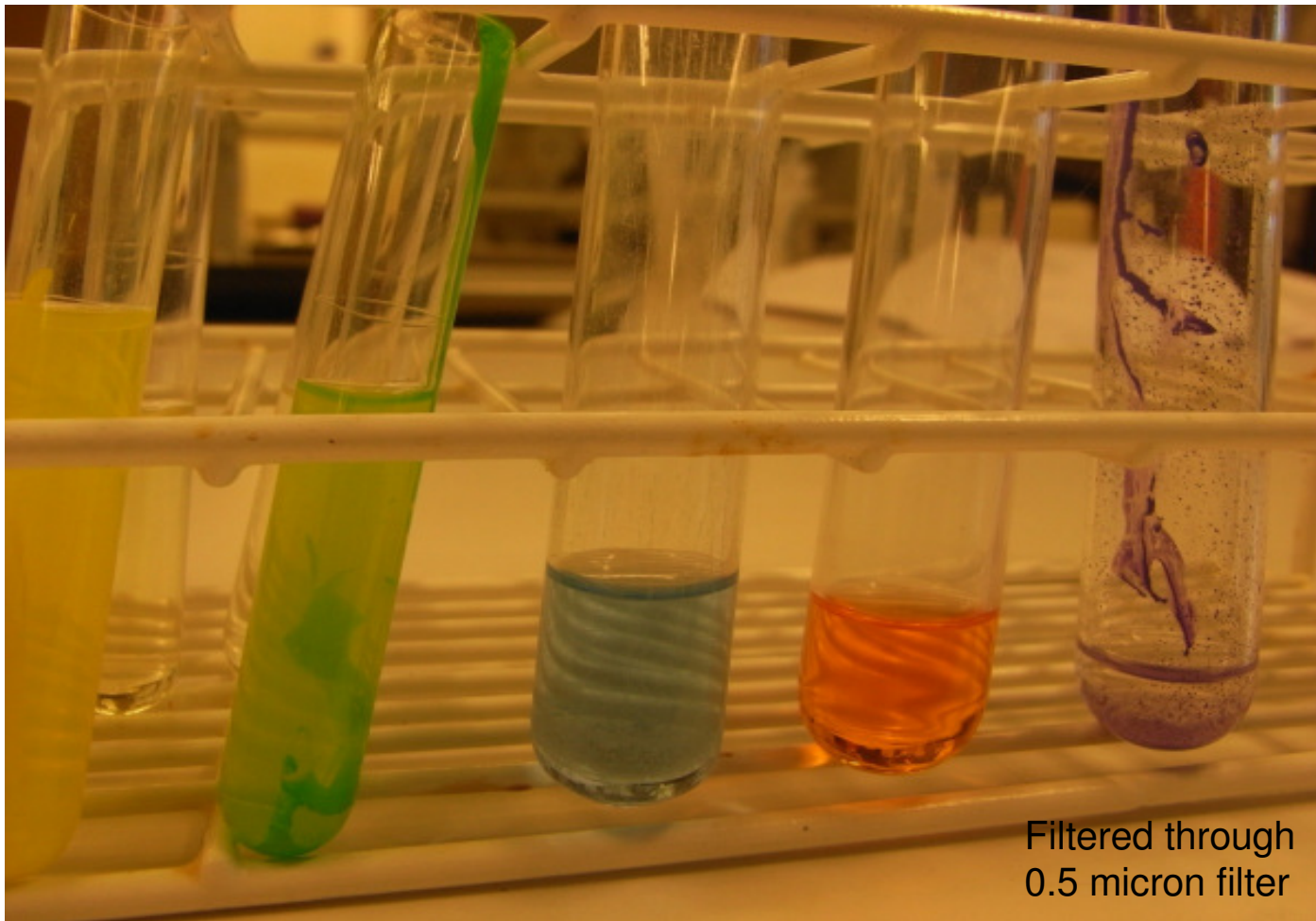
## Particle size of 58 tattoo inks by laser diffraction

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 degraded *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.

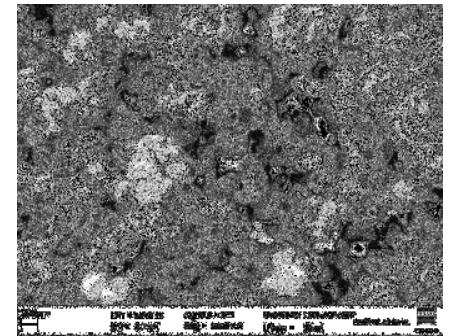


Tattoo pigments are very hard to dissolve, thus, unlikely to be available for metabolism



Harsh organic solvents

SEM of tattoo pigment



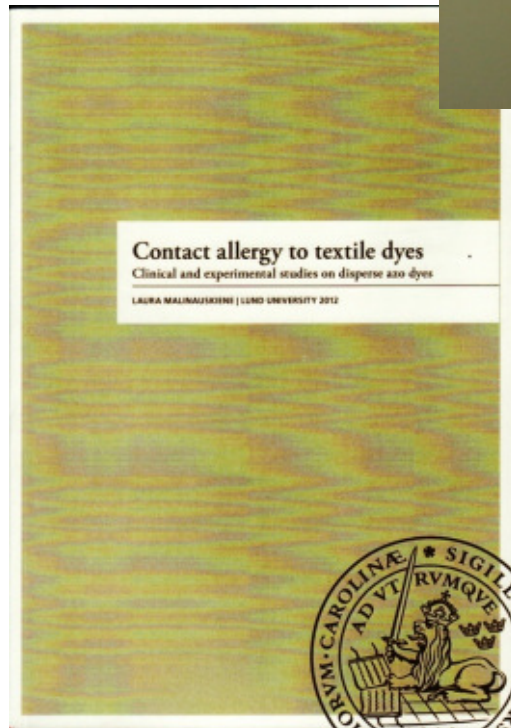
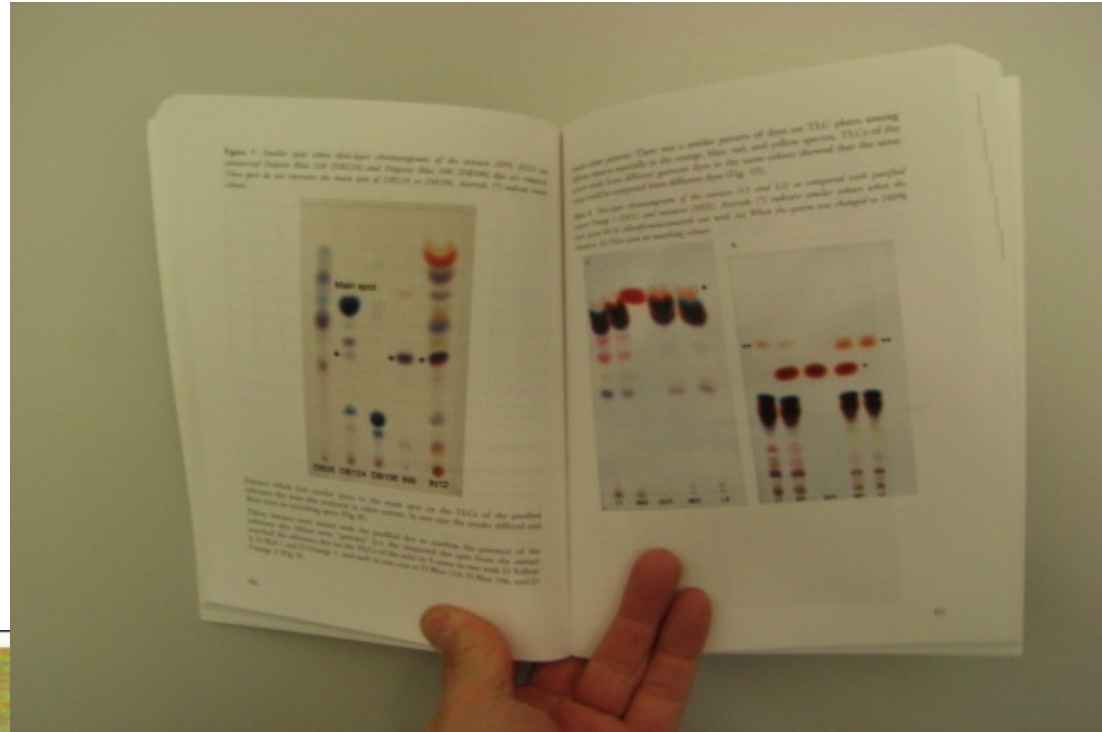
DTU/BBH, Copenhagen, DK

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. Tattoo 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.**

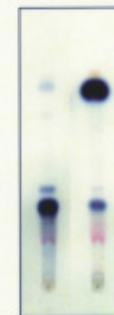
PAA in dyes or PAA metabolised from azo cannot explain allergy to dyes in textiles.

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

## Contact Allergy to Textile Dyes

Clinical and Chemical Studies on Disperse Dyes

Kristina Ryberg



Department of Occupational and Environmental Dermatology  
Malmö University Hospital  
Lund University  
Malmö 2009

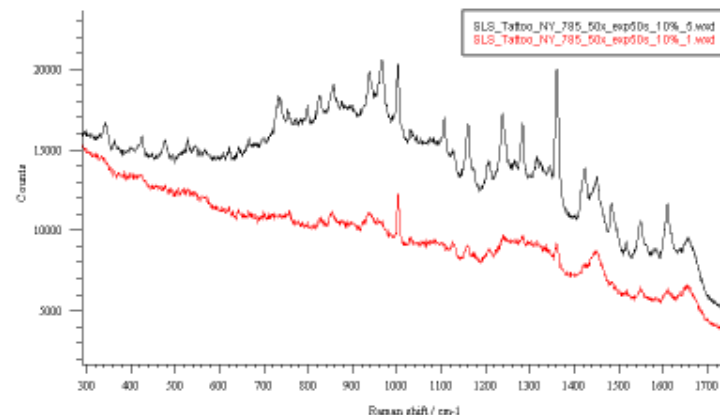
# Raman Spectroscopy of shave cut biopsies of tattooed skin reacting to red Indicates no special metabolism of pigment into the respective PAA

## Material

3 cases with known culprit ink and allergic reactions in their tattoos  
(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 tattooed 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**

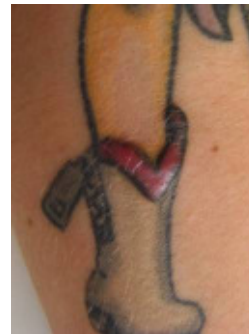






## CONCLUSION, THE CLINIC OF ALLERGIC TATTOOES

- 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 months or years
- Allergy affects every piece of skin tattooed with the culprit ink
- Cross-reactivity in old red tattoos 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.**

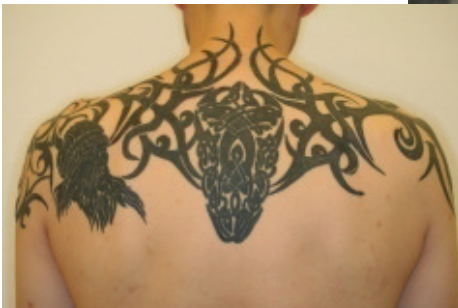


The European Council 2003/2008 resolution is nicely framed, however, made out of speculation arising mainly from ex vivo sources of questionable relevance. It remains being not validated, after 10 years. It deserves critical and fundamental reconsideration re. allergy risk prediction and prevention,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



**Elevated globoid, nodular or papular reactions, black**

**2.2 patient PIH**



**Elevated globoid, nodular or papular reaction, black**

**2.4 patient HK**

