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.
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 Tattooing

Jørgen Serup, MD
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Dept. of Dermatology, ”Tattoo Clinic”*

* The ”Tattoo Clinic” specialised in tattoos 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!

<table>
<thead>
<tr>
<th></th>
<th>STD Clinic Study*</th>
<th>“Beach” Study**</th>
<th>Pooled/overall</th>
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<tbody>
<tr>
<td>Total number tattooed</td>
<td>154</td>
<td>144</td>
<td>298</td>
</tr>
<tr>
<td>Complaining (vs total),</td>
<td>65/154, 42%</td>
<td>60/144, 42%</td>
<td>125/298, 42%</td>
</tr>
<tr>
<td>Photosensitive (vs complaints)</td>
<td>24/65, 37%</td>
<td>31/60, 52%</td>
<td>55/125, 44%</td>
</tr>
</tbody>
</table>

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

- **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. Ulcering 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 tattooed with culprit colour must react uniformly
• Ample 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

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

1.4 patient GH
Elevated "plateau" reactions in yellow, lichenoid

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

<table>
<thead>
<tr>
<th>Depth of dermal Infiltrate, histology</th>
<th>Number of Samples n</th>
<th>Echolucent band mm, mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1, outer</td>
<td>12, 21%</td>
<td>0.45 (0.33)</td>
</tr>
<tr>
<td>Level 2, middle</td>
<td>35, 60%</td>
<td>0.89 (0.56)</td>
</tr>
<tr>
<td>Level 3, deeper</td>
<td>11, 19%</td>
<td>1.48 (1.18)</td>
</tr>
</tbody>
</table>

* 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 till 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 remain permanently in the dermis because they are robust particles that are not easily degradated \textit{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.

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.
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
The Modern Allergen Concept:

Allergens are epitope-protein complexes 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)
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 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 TATTOOS 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