

Are metals involved in tattoo-related hypersensitivity reactions? A case report

Christa de Cuyper¹ , Evelyne Lodewick², Ines Schreiver³ , Bernhard Hesse⁴, Christian Seim⁵, Hiram Castillo-Michel⁴, Peter Laux³ and Andreas Luch³

¹Department of Dermatology, AZ Sint-Jan Brugge-Oostende AV, 8000 Brugge, Belgium, ²Department of Dermatology, ZorgSaam Zeeuws-Vlaanderen, 4535 PA Terneuzen, The Netherlands, ³Department of Chemical and Product Safety, German Federal Institute for Risk Assessment (BfR), 10589 Berlin, Germany, ⁴European Synchrotron Radiation Facility (ESRF), 38043 Grenoble Cedex 9, France, and ⁵Physikalisch-Technische Bundesanstalt, 10587 Berlin, Germany and Technische Universität Berlin, Institut für Optik und Atomare Physik, 10623 Berlin, Germany

doi:10.1111/cod.12862

Summary

Background. Allergic reactions to tattoos are not uncommon. However, identification of the culprit allergen(s) remains challenging.

Objectives. We present a patient with papulo-nodular infiltration of 20-year-old tattoos associated with systemic symptoms that disappeared within a week after surgical removal of metal osteosynthesis implants from his spine. We aimed to explore the causal relationship between the metal implants and the patient's clinical presentation.

Methods. Metal implants and a skin biopsy of a reactive tattoo were analysed for elemental contents by inductively coupled plasma mass spectrometry and synchrotron-based X-ray fluorescence (XRF) spectroscopy.

Results. Nickel (Ni) and chromium (Cr) as well as high levels of titanium (Ti) and aluminium were detected in both the skin biopsy and the implants. XRF analyses identified Cr(III), with Cr(VI) being absent. Patch testing gave negative results for Ni and Cr. However, patch tests with an extract of the implants and metallic Ti on the tattooed skin evoked flare-ups of the symptoms.

Conclusion. The patient's hypersensitivity reaction and its spontaneous remission after removal of the implants indicate that Ti, possibly along with some of the other metals detected, could have played a major role in this particular case of tattoo-related allergy.

Key words: aluminium; chromium; titanium; contact allergy; hypersensitivity; metal implant; nickel; osteosynthesis; tattoo allergy; titanium dioxide.

Side effects after tattooing are not uncommon (1–3). Moreover, associated allergic reactions are often difficult to treat, as the (chemical) trigger usually remains in the skin. Different hypothetical pathomechanisms have been suggested in the literature, and identification of the causative allergen remains a challenge (4, 5). Tattoo

allergy mostly manifests as localized infiltration of the tattooed skin area associated with itching. When cutaneous reactions in tattoos are associated with systemic symptoms, the diagnosis of sarcoidosis can be suspected (6, 7).

Cutaneous manifestations of hypersensitivity to implant material have been described in orthopaedic and dental surgery, and have been attributed to titanium (Ti) alloys (8). However, patch testing with Ti is unreliable, and the diagnosis often relies on 'the pragmatic approach to the clinical work-up of patients with putative allergic disease to metallic orthopaedic implants', as has been proposed by Thyssen et al. (9). In the clinical

Correspondence: Dr Christa de Cuyper, Department of Dermatology AZ Sint-Jan Brugge-Oostende AV, Meiboomstraat 15, 8370 Blankenberge, Belgium. Tel: 003250417551; E-mail: christa.decuyper@outlook.be

Accepted for publication 18 June 2017

case presented, we show that specialized laboratory techniques can identify potential allergens, particularly metallic components, in reactive tattoos.

Materials and Methods

Patient's history

In April 2015, a 39-year-old man reported inflammation in several black tattoos on his extremities, which had been present for a few weeks. These tattoos had existed for >20 years without causing any health issues until the current event. Neither more recent black tattoos nor coloured parts of old and new tattoos showed any clinical symptoms. Starting a few days previously, the patient also experienced fatigue and influenza-like symptoms along with fever, which were more pronounced in the evening hours. He had suffered from lower back pain for several years, and had undergone spinal surgery. In March 2013, anterior fusion surgery (Pyramid system; Medtronic, Memphis, TN, USA) of the spine on L5–S1 in combination with a mixture of heterologous bone graft–dicalcium phosphate granules in a thermoplastic polyether ether ketone (PEEK) cage was carried out. In September 2014, a second surgical intervention with posterior fusion of L5–S1 by osteosynthesis (Revere system; Globus Medical, Audubon, PA, USA) and an identical PEEK-cage procedure was performed. There was no history of previous contact dermatitis in this patient.

The patient was heavily tattooed on his trunk and extremities. Several black tattoos on the arms and on the left leg presented with papulo-nodular infiltration and mild erythema of the surrounding skin (Fig. 1a). The black parts of some old multicolour tattoos also showed induration (Fig. 1b). All active lesions were warm and itchy. Oral corticosteroid therapy was initiated at a dose of 40 mg of prednisone daily for 4 days, yielding remission of the systemic symptoms with subsequent tapering over the next 3 weeks. However, prolonged low-dose prednisone (10 mg daily) was needed for several months to keep the symptoms under control. Several attempts to interrupt the oral steroids resulted in a flare-up of the symptoms. Local application of clobetasol propionate (0.5 mg/g cream) was added from the start to help reduce local inflammation. Because the patient also increasingly complained about back pain, his surgeon decided to remove the metal parts from his spine in October 2015 (Fig. 1c). After the removal, all skin symptoms disappeared within a week.

Diagnostic work-up, including patch testing

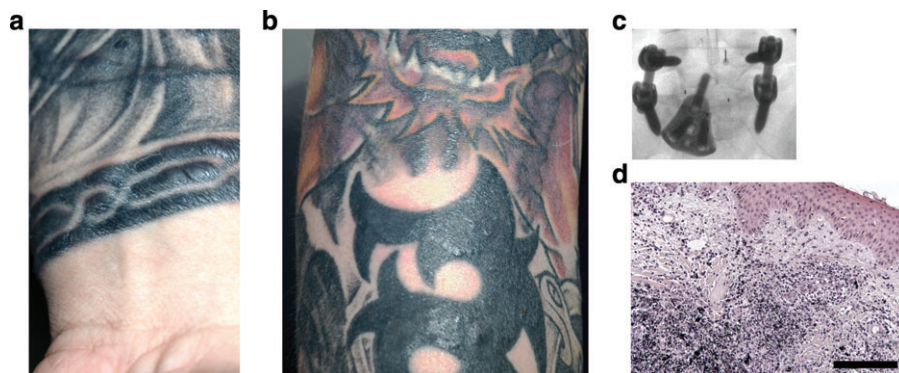
Patch testing was performed in September 2015. Prednisone treatment was paused for 4 days before skin testing. Testing on the back was carried out with the European baseline series with Belgian supplements (version 17/07/2015), and pharmaceutical, cosmetic, metal and dental series (Chemotechnique, Vellinge, Sweden). The tests were held under occlusion for 2 days with Van der Bend Chambers (Van der Bend, Brielle, The Netherlands), and fixed with Hypafix® (BSN Medical, Hamburg, Germany). Reading of the patch tests was performed on day (D) 2 to D4. Scrapings of unused metal screws and PEEK-cage material provided by the surgeon were additionally used for patch testing as described above. The original black tattoo ink could not be traced for testing. Two months after removal of the implant material, a new patch test was performed, as described before, with the intact metal screws that had been removed from the spine, and with saline extracts of these screws, obtained by keeping the screws for 7 days in saline solution (0.9% NaCl) at room temperature. Patch testing was repeated on normal and on tattooed skin in May 2017 with a new saline extract of the screws and with an extensive series of metals, including several Ti salts.

A skin biopsy was taken at the first visit of the patient in April 2015 for histological assessment of the skin reaction. The sample was fixed in formalin, embedded in paraffin, and stained with haematoxylin and eosin. A second skin biopsy of a former reactive tattoo was taken in December 2015, and sent to the German Federal Institute for Risk Assessment (BfR) submerged in Michel's transport medium for elemental analysis. Additional skin biopsies of a reactive and non-reactive tattoo and of non-tattooed skin were taken in May 2017 for elemental analysis for comparison of metallic content.

Inductively coupled plasma mass spectrometry (ICP-MS) analysis

Elemental compositions of the samples were analysed after nitric acid microwave digestion (Ultraclave; MLS, Leutkirch, Germany). Screws were extracted in plastic tubes with the minimum amount of liquid necessary to cover the surface (2–6 ml). The skin biopsies, suspended in 2 ml of deionized water, or 2 ml of the screw extracts were digested with 2 ml of 69% nitric acid and 1 ml of H₂O₂ in Teflon vessels. The microwave was run with the following temperature programme: 20–80°C (3.5 min, 100 bar, 700 W); 80–130°C (10 min, 120 bar, 1000 W); 130–200°C (6.5 min, 150 bar, 1000 W); and 200°C (30 min, 150 bar, 1000 W). Element standards for ICP-MS were purchased either from Sigma Aldrich

Fig. 1. Initial manifestation of the skin reaction. (a) Papulo-nodular reaction in a black tattoo. (b) Papulo-nodular reaction in a black tattoo and black parts of a multicoloured tattoo. (c) Radiograph of spinal screw implants. (d) Haematoxylin and eosin staining of a tattoo-reactive skin biopsy section (scale bar: 200 μ m).



(Munich, Germany) [scandium (Sc), aluminium (Al), copper (Cu), and nickel (Ni)] or from Merck (Darmstadt, Germany) [indium (In)]. For chromium (Cr), iron (Fe), barium (Ba), and Ti, 1000 mg/l standard solutions in diluted nitric acid were obtained from VWR (Darmstadt, Germany). Samples were diluted 20-fold, and 10 ppb of In and Sc were added as internal standards. An XSeries II inductively coupled plasma mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) and an ESI SC2 autosampler (Elemental Service & Instruments, Mainz, Germany) were used for sample analysis. Sample analysis was carried out in triplicate with 100 sweeps, a resolution of 0.02 amu, and a dwell time of 10 ms. Measurements were carried out with the collision cell in either -3.0 V or 0.0 V mode. H_2/He (7% vol/vol) was used as the collision gas with a flow rate of 5 ml/min. Data were processed with PLASMALAB 2.5.11.321 (Thermo Fisher Scientific). For analysis, the metal screws were first extracted in a solution of 0.9% NaCl, and subsequently in 12% HNO_3 . Each extraction was carried out for 1 week at room temperature. Acidic extraction was chosen to determine all elements possibly released by the implanted screws and to achieve an extent of release beyond the respective limits of quantification.

Synchrotron X-ray fluorescence (XRF) analysis

Cryosections of the reactive skin biopsy were freeze-dried and analysed with μ XRF and X-ray absorption near-edge structure (μ XANES) spectroscopy at the beamline ID21 located at the European Synchrotron Radiation Facility (ESRF). The experimental setup of the beamline is described elsewhere (10). The primary X-ray beam energy was set to 5.05 keV and focused down to a microprobe ($0.4 \times 0.8 \mu m^2$). The flux was 1.6×10^{10} photons/second. XRF and scattered radiation were collected with an energy-dispersive silicon drift detector, and subsequently normalized by the intensity of the primary X-ray beam. The acquisition time per point was 100 ms. The step width for collecting the XRF maps was set to 1 μ m. For

collecting Ti-XANES spectra, the energy of the incoming beam was scanned in 0.5 eV steps from 4.95 to 5.1 keV, with an acquisition time of 100 ms per energy. Depending on the concentration of the probed region, between one and 10 μ XANES spectra were collected per point, and subsequently averaged. For the collection of Cr-XANES spectra, a spectral window between 5.98 and 6.1 keV with an approximately 0.5 eV step width and an acquisition time of 100 ms was scanned.

Results

The following additional examinations were performed to exclude a possible underlying systemic disease such as sarcoidosis: blood examination, which showed mild elevation of C-reactive protein to 30 mg/l (normal: 0–15 mg/l); blood cell count and liver function tests, the results of which were within normal ranges; and a test for eosinophilia, which showed that this was absent. Angiotensin-converting enzyme showed a concentration of 37 U/l (normal: 19–92 U/l). The chest X-ray performed in June 2015 showed no abnormalities, and was found to be unchanged from a previous X-ray taken in 2008. Ophthalmological examination gave normal findings.

Histology of a skin biopsy obtained from a nodular lesion in April 2015 showed parakeratosis and exocytosis of lymphocytes in the epidermis without spongiosis (Fig. 1d). The dermis showed granulomatous dermatitis with superficial and deep infiltrates of lymphocytes and histiocytes adjacent to multifocal black pigments. Typical sarcoid granulomas were absent.

Patch testing

Extensive patch testing in September 2015, including with unused screws and PEEK-cage material (Fig. 2a) provided by the surgeon, gave negative results on D2 and D4. However, on D6 a 2+ reaction was observed in a single patch test area on the shoulder. Unfortunately, the culprit material causing this late reaction could no longer be

identified. The patient became subfebrile again, and his tattoos started to itch and swell; the itching and swelling subsided after a few days of 5 mg of orally administered prednisone. In December 2015, 2 months after removal of the implant material and complete recovery of the skin manifestations, new patch testing was performed with the metal screws that had been removed from the spine, as well as with saline extracts of the screws. Application of the screws and their saline extracts in Van der Bend chambers did not elicit a reaction on D2 and D4 on healthy skin without tattoos. However, application of the screws and extracts on a tattooed area of the left upper arm induced not only itching and swelling of this tattoo, starting after 24 h (Fig. 2b), but also swelling and itching of other, distant tattoos (Fig. 2c). The patient also became subfebrile again, recovering after ~1 week.

In May 2017, a new challenge was performed with a saline extract of the screws under occlusion for 6 days (10 May 2017 to 16 May 2017) on a formerly reactive tattoo on the left leg. The patient again experienced a feverish feeling with a warm sensation in the face within 24 h after application of the extract, lasting for 1 day. The tattoo became itchy again 24 h after application of the extract, and was slightly infiltrated at removal of the test material on 16 May, with some erythema in normal skin surrounding the tattoo. Patch testing was repeated on 16 May with a series of metals (Table 3), including Ti and Ti salts, in parallel on the normal skin of the back and on tattoos on both upper arms. The reading on D2 was negative, whereas, on D4, one single test site for metallic Ti 10% pet. was infiltrated (++) score) in a black area of an old tattoo on the right upper arm (Fig. 3a and b). On D6, all test sites were unchanged, but, shortly after a first shower, all the black parts of this old tattoo on the right upper arm started to swell; the swelling lasting for several hours, and the patient felt ill again (Fig. 3c). Within 24 h, this reaction spontaneously subsided. There was no reaction on the left upper arm where different Ti salts had been tested (Table 3).

Chemical analysis of the screws and the patient's skin biopsies

The metal screws and two skin biopsies from one of the former reactive tattoos were further analysed for their elemental compositions (Tables 1 and 2). Additional analysis was performed of a non-reactive tattoo and normal skin (Table 2). Very low levels of Ni were detected in the saline extracts of three of five samples with ICP-MS (Table 1). Ti, Fe, Cu and Ba were detected in one sample each. In all of the 12% HNO₃ extracts of the five different screws, Ni and Cr were detected. Also, high levels of Ti and Al were found

in the different implants (Table 1). To some extent, all of the metals analysed, i.e. Al, Ti, manganese, Fe, Ni, Cu, Ba, and Cr, became detectable upon acidic extraction of the five different screws removed from the patient.

In the elemental analysis of the tattoo biopsies, the levels of Al, Cr, Ni, Fe and Cu were also found to be increased as compared with non-reactive skin (Table 2). Among others, Cr, Ni and Ti were present in tattooed skin. No Ti was found in normal skin. The high Fe content in one tattoo may lead to the assumption that a mixture of black iron pigment and white TiO₂ was used to create the shades of the tattoo investigated. In order to differentiate between the photoreactive anatase and less harmful rutile TiO₂ crystal configurations, as well as Cr(III) and Cr(IV) oxidation states, we carried out synchrotron-based XRF analyses (Figure S1). A reactive tattoo skin section was analysed for element distribution and speciation (i.e. crystal configuration or binding state). Ti was highly abundant and surrounded by dermal cells (Fig. S1a). The investigation showed the rutile TiO₂ crystal structure (Figure S1b). Therefore, additional irritation by photocatalytic anatase TiO₂ is unlikely. Cr was present in small spots, and speciation revealed Cr(III) and metallic Cr(0) with no Cr(VI) present (Figure S1a and c).

Discussion

To our knowledge, this is the first case report of a systemic hypersensitivity reaction induced by metal implants in the spine that manifested with fever and cutaneous symptoms in black tattoos containing metallic pigments. Using the pragmatic approach proposed by Thyssen et al. in 2011 (9) and applying the '10-point check list that could strengthen the suspicion of clinically relevant metal allergy to an orthopaedic implant' (Table S1), we provide strong evidence for a causal relationship between the metal implants and the cutaneous and systemic manifestations in the patient investigated. In our opinion, the most important arguments are, first, that the symptoms disappeared within a week after removal of the metal screws, and second, that a patch test with an aqueous extract of the metal screws on the tattooed skin area induced not only swelling and itching in this region but also a flare-up of systemic symptoms. This provocation test was repeated more than a year later, and once again a localized and systemic reaction was seen.

Why would we suspect Ti of being a major culprit? Laboratory analysis of the metal material identified several metal components in the acidic extracts of the screws; in particular, Ti was found at very high levels (Table 1). Analysis of the skin biopsy also showed the presence of Ti. In our patient, contact allergy induced by Ni, cobalt

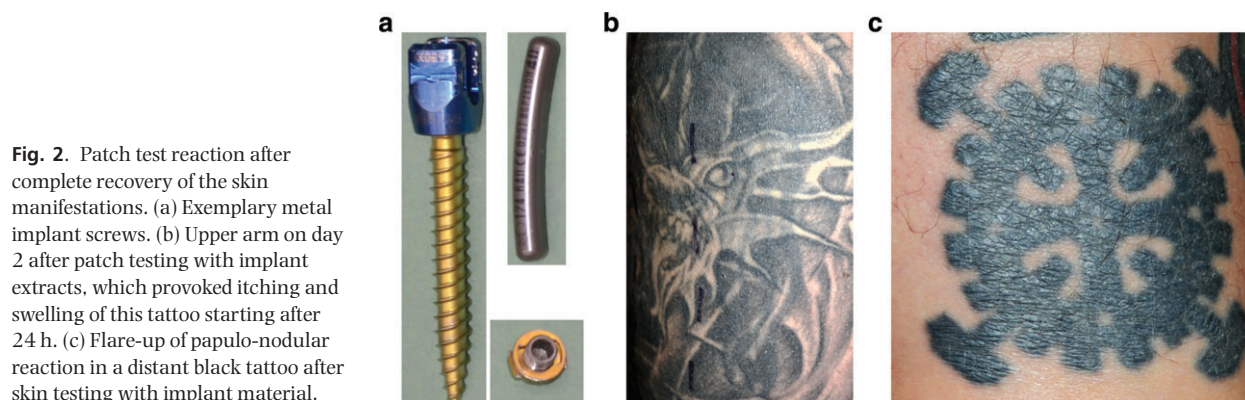


Table 1. Metal release of explanted screws after 1 week of extraction with either 0.9% NaCl or 12% HNO₃

Sample	Extract	Elements								Survey scan	
		Al	Ti	Mn	Fe	Ni	Cu	Ba	Cr	>100 000 counts	>10 000 counts
Screw 1	NaCl	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	–	–
Screw 2	NaCl	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	55.2	<LOQ	<LOQ	–	Zn
Screw 3	NaCl	<LOQ	<LOQ	<LOQ	5.7	1.0	<LOQ	<LOQ	<LOQ	–	Zn
Screw 4	NaCl	<LOQ	<LOQ	<LOQ	<LOQ	0.6	<LOQ	6.0	<LOQ	–	Zn, Sr
Screw 5	NaCl	<LOQ	7.09	<LOQ	<LOQ	0.9	<LOQ	<LOQ	<LOQ	–	Zn, Sr
Control	NaCl	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	–	–
Screw 1	HNO ₃	6370	97 200	3.70	728	17.5	430	7.30	33.7	V, Zn, W, Pb	Mo, Hg, U
Screw 2	HNO ₃	2110	29 200	1.10	581	17.5	1350	6.60	25.0	V, Zn, Pb	Mo, Sn, Ta, W, U
Screw 3	HNO ₃	1570	31 400	2.70	762	16.5	43.1	46.7	26.8	V, W, Pb	Zn, Sr, Gd
Screw 4	HNO ₃	1510	23 600	3.40	571	6.90	86.8	88.4	14.3	V, W, Pb	Zn, Sr, Sn, U
Screw 5	HNO ₃	1620	23 300	5.30	674	21.4	163	84.0	32.7	V, Pb	Zn, Sr, Gd, U
Control	HNO ₃	<LOQ	<LOQ	<LOQ	6.50	<LOQ	<LOQ	<LOQ	<LOQ	–	–

Al, aluminium; Ba, barium; Cr, chromium; Cu, copper; Fe, iron; Gd, gadolinium; Hg, mercury; LOQ, limit of quantification; Mn, manganese; Mo, molybdenum; Ni, nickel; Pb, lead; Sn, tin; Sr, strontium; Ta, tantalum; Ti, titanium; U, uranium; V, vanadium; W, tungsten; Zn, zinc. All results are shown as nanograms per millilitre of extract.

(Co) and Cr seems to be rather unlikely in the light of the negative patch test results and the absence of a typical and traceable medical history of allergy to these elements. Moreover, Cr in the skin and in the metal implants was present at oxidation state III but not at oxidation state VI. In general, the sensitizing effects of Cr are predominantly attributed to Cr(VI). Nevertheless, Cr(III) has also been shown to be able to trigger dermatitis, although with slightly less potency (11). Patch testing with different Ti salts, including TiO₂, as well as with many other metals gave negative results, with the exception of one single test with metallic Ti 10% pet. showing infiltration on tattooed skin on D4 (Table 3). TiO₂ is a common component of tattoo inks. It has very little light absorption ability, and reflects white light almost unchanged because of its high scattering power. It is therefore used to create lighter shades of colourants in tattoo inks (12). Although TiO₂ is omnipresent in consumer products, in particular in cosmetics and sunscreens, primary cutaneous problems attributable to Ti allergy are rare. Larese Filon et al.

stated that ‘the hazard associated with TiO₂ nanoparticles with regard to permeation and penetration is very low’ (13). Ti alloys are widely used in cardiovascular, reconstructive, orthopaedic and dental surgery because of their biocompatibility and corrosion resistance. When exposed to air and biological fluids, they spontaneously form a stable oxide film primarily composed of TiO₂, providing high resistance to corrosion. However, this protective layer can be compromised, and dissolution of alloy elements may thus occur. In the medical literature, allergic reactions to Ti implants, used in orthopaedic surgery and dentistry, have been reported for many years (8). In some cases, patch testing was helpful to prove the relationship, but was not always conclusive. According to the review by Fage et al. (8), patch tests seem to be unreliable and insufficiently sensitive to show type IV hypersensitivity reactions against Ti. The memory lymphocyte immunostimulation assay (MELISA), a novel lymphocyte stimulation test, is proposed by some authors as an alternative method to prove metal allergy, and has been used to identify and

Table 2. Metal concentrations in the skin biopsies obtained from the patient

	Elements					Survey scan	
	Al	Cr	Ni	Fe	Cu	>100 000 counts	>10 000 counts
Reactive tattoo 1	4.3	0.9	0.4	21	5.9	Ti, Zn	Mn, Ba, Rb, Pb
Reactive tattoo 2	<LOQ	0.3	<LOQ	5.3	0.2	Ti	–
Non-reactive tattoo	<LOQ	0.2	<LOQ	0.9	0.4	Ti	–
Non-tattooed skin	0.7	0.2	0.2	3.2	3.1	–	–

Al, aluminium; Ba, barium; Cr, chromium; Cu, copper; Fe, iron; LOQ, limit of quantification; Mn, manganese; Ni, nickel; Pb, lead; Rb, rubidium; Ti, titanium; Zn, zinc.

Results are shown as micrograms of element per gram of biopsy weight.

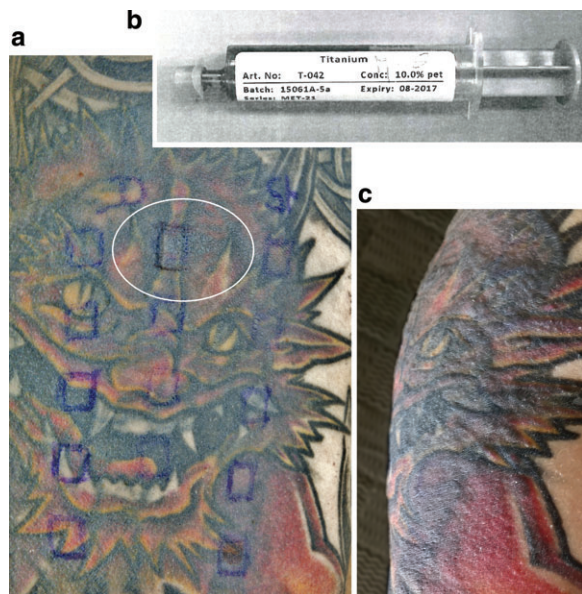


Fig. 3. Patch test reaction in May 2017 with (a) a 2+ reaction on day (D) 4 to (b) titanium 10% pet. and (c) flare-up with papulonodular reaction on D6 in the black parts of an old tattoo on the right arm.

differentiate metal-induced sarcoid-like reactions in pulmonary disease (14). However, this test is not yet approved as a routine method for testing Ti allergy; moreover, it is costly and not easily available in daily dermatological practice. The same applies to the diagnostic approach that we propose for suspected tattoo allergy (Table S2).

In our patient, only metallic Ti elicited a positive patch test reaction on D4. We would suggest several explanations. It is unclear whether sensitization was induced by the tattoos or by Ti released from the implants, which seems to be more probable, considering the late onset. Although we showed that Ti in the reactive tattoo was mainly TiO_2 rutile pigment, it cannot be excluded that traces of metallic Ti were present in the old tattoos and provoked a reaction. If metallic Ti was not present, or was

present to a lesser extent, in more recent black tattoos, which were probably made with different inks, this could explain why only old tattoos reacted. Another explanation might be that a combination of several elements is needed, and that association with one of the other metals could play a role. Most interestingly, the presence of metallic components from the screws seems to be a key factor in provoking the hypersensitivity reactions and exciting lymphocytes at a level sufficient to lead to reactions to the tattoos. We can presume that, after surgical removal of the screws, the metallic release from the tattoo pigments in the skin was insufficient to maintain the reaction, explaining why the manifestations calmed down spontaneously after surgery. However, a challenge with an extract was sufficient to reproduce the local and systemic symptoms in 2015 and again 1.5 years later. Moreover, a pronounced flare-up occurred on the right upper arm on D6 of the most recent patch testing. This was probably induced by the positive test reaction to Ti in the same area, which triggered memory T cells in the surrounding old reactive tattoo (Fig. 3c).

In summary, different mechanisms are likely to contribute to the disease process, which might be a reason why there are so many different clinical presentations of tattoo allergies. For instance, lichenoid, eczematous, psoriasiform, pseudolymphomatous, hyperkeratotic and ulcero-necrotic clinical presentations of lesions have been described. There is often no correlation with the histological pattern, which may even overlap in the same biopsy. For instance, in biopsies, lichenoid, granulomatous and sarcoid-like, and, less frequently, epidermal alterations such as acanthosis or spongiosis can be seen (3, 4, 5). In our case, there was a dermal infiltrate of lymphocytes and histiocytes, but no spongiosis. In tattoo allergies, red ink is often involved, although reactions to other colours have also been reported (4, 5, 15). Mercury-based and cadmium (Cd)-based pigments were replaced by organic pigments decades ago, and should no longer be responsible for allergic reactions in red and yellow tattoos (12, 15, 16). However, these elements can still be found in

Table 3. Patch testing in May 2017 with different metals, including forms of titanium (in bold), in parallel on the tattooed skin of the upper arms and on normal skin of the back

Test materials May 2017 – Right side						
Product no.	Product name	D2		D4		
		Arm	Back	Arm	Back	
A0013	Ammonium tetrachloroplatinate 0.25% aq.	–	–	–	–	
	Amalgan metals Allergeaze 20% pet.	–	–	–	–	
C022	Copper(II) sulfate pentahydrate 2% pet.	–	–	–	–	
P015	Potassium dicyanoaurate 0.1% aq.	–	–	–	–	
P001	Palladium(II) chloride 2% pet.	–	–	–	–	
T042	Titanium 10% pet.	–	–	++	–	
A021	Aluminium 100%	–	–	–	–	
V002	Vanadium 5.0% pet.	–	–	–	–	
M030	Molybdenum 5.0% pet.	–	–	–	–	
Baseline series						
P014A	Potassium dichromate 0.5% pet.	–	–	–	–	
C017A	Cobalt(II) chloride hexahydrate 1% pet.	–	–	–	–	
N002A	Nickel sulfate hexahydrate 5% pet.	–	–	–	–	
C009	Methylisothiazolinone 0.01% aq.	–	–	–	–	
MX 30	Textile dye mix 6.6% pet.	–	–	–	–	
Test materials May 2017 – Left side						
No.	Product no.	Product name	D2		D4	
			Arm	Back	Arm	Back
1	G005B	Gold(I) sodium thiosulfate dehydrate 2% pet.	–	–	–	–
2	T0040	Titanium dioxide 10% pet.	–	–	–	–
3	M031	Manganese chloride 2% pet.	–	–	–	–
4	T043	Tungsten 5% pet.	–	–	–	–
5	I016	Ferric chloride 2% pet.	–	–	–	–
6	A038	Aluminium hydroxide 10% pet.	–	–	–	–
7	N008	Niobium(V) chloride 0.2% pet.	–	–	–	–
8	Z009	Zirconium dioxide 0.1% pet.	–	–	–	–
9	Z008	Zirconium(IV) chloride 1.0% pet.	–	–	–	–
10	Z001	Zinc 2.5% pet.	–	–	–	–
11	A022	Aluminium(III) chloride hexahydrate 2% pet.	–	–	–	–
12	T039	Titanium(III) nitride 5% pet.	–	–	–	–
13	T041	Titanium(III) oxalate decahydrate 5% pet.	–	–	–	–
14	T049	Calcium titanate 10% pet.	–	–	–	–

tattoo inks as impurities, and allergies to Ni, Cd and Co are still being reported in the literature (17–19). Although, in some rare cases, the culprit ink component has been identified, experts agree that skin testing is rather disappointing (4, 5, 17, 20). Serup et al. tested a series of 90 patients with chronic tattoo reactions against common allergens, textile dyes, a battery of tattoo inks considered to be potentially harmful, and even identified culprit inks from patients. They concluded that patch test reactions mainly lead to negative or inconsistent results (5). In general, currently, tattoo allergy remains unpredictable, and skin testing with tattoo inks prior to tattooing is useless in terms of having no predictive value. Tattoo allergy has been confirmed through positive patch

testing of the suspected ink in only a few cases (4, 17). Therefore, it is thought that, in the majority of cases, the allergen is not present in the ink as such, but could be formed in the skin, subsequently leading to haptenation, resulting in delayed-type contact allergy. The pigments in the inks could be considered to be pro-haptens modified by chemical transformation in the skin (5).

Sunlight exposure could be an influencing factor. We observed a severe localized pseudolymphomatous reaction associated with generalized dermatitis in a patient with a large red tattoo induced during a sunny holiday in Thailand (15). Many observations underline the hypothesis of *in vivo* decomposition or alteration of tattoo colourants. Indeed, the effects of natural sunlight and

laser light on tattoo pigments have been shown (21–24). Decomposition products with carcinogenic properties resulting from photochemical cleavage have been identified (23). Also, laser-induced pigment cleavage *in vitro* has been examined and shown by different authors (23, 24). Severe allergic reactions after laser tattoo removal have also been reported (25). Accordingly, we observed localized vesicular to bullous dermatitis in a patient with a black tattoo occurring 24 h after the third treatment with a Q-switched Nd:YAG laser at a wavelength of 1064 nm. A skin biopsy of this patient showed spongiosis and an infiltrate with eosinophils and lymphocytes, suggesting allergic contact dermatitis. Patch testing with the black ink lasered with the same wavelength elicited a strong positive reaction (3+), whereas the pure ink did not elicit any reaction (3). Serup et al. stated that the papulo-nodular pattern in black tattoos might not be an allergic reaction, and should rather be explained by other causes, such as needle trauma or as a foreign body reaction resulting from a heavy load of carbon black nanoparticles (5). In a recent report, they concluded that papulo-nodular reactions should be seen as a marker of sarcoidosis (7). This could be accepted for a number of papulo-nodular tattoo reactions, but in our opinion it is certainly not applicable to all of them. Even in the presence of widespread reactions in other black tattoos, the diagnosis of sarcoidosis should be confirmed by histology, and should even then be considered with caution. Granulomatous and sarcoid-like reactions to metals such as gold (Au) and palladium (Pd) in body piercings and also to Al in tattoos and in vaccines are well documented and considered to represent allergy (4, 26–29). Our case presented as papulo-nodular lesions in black tattoos. It is obvious that we dealt with a hypersensitivity reaction induced by implant material and not with sarcoidosis.

As shown by ICP-MS and synchrotron XRF-based analysis of the skin biopsy of our patient, Ti and a range of metal ions were present in his tattoos. The mechanism and pathogenesis of allergy triggered by heavy metals such as Ni, Au and Pd are still poorly understood. Granulomatous reactions in other organs such as the lungs as the consequence of exposure to inorganic substances, for example beryllium, are well described in the literature, but have to be differentiated from sarcoidosis. As mentioned before, Fireman et al. used MELISA testing to

identify metal sensitization in sarcoid-like metal-exposed patients with lung disease and to differentiate it from true sarcoidosis (14). Perhaps it could be speculated that a similar phenomenon can take place in tattoos.

Conclusion

The hypersensitivity reaction in our patient and the spontaneous remission after removal of the metal implants indicate that metals could play a major role in tattoo allergy, which is a health issue of increasing relevance that has to be explored in more depth. The detection of corresponding metals in the implants and the tattooed skin provides support for this hypothesis, although many questions remain open. Moreover, it remains unclear whether the different manifestations of tattoo allergies might have comparable or different underlying pathomechanisms. Both further investigation and reliable and affordable test methods are urgently needed.

Acknowledgements

The authors thank the ESRF for allocated beam times on ID21. This work was supported by the intramural research project (SFP #1322-604) at the BfR.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. 10-Point check list that could strengthen the suspicion of clinically relevant metal allergy to an orthopaedic implant.

Table S2. Diagnostic approach for suspected tattoo allergy.

Figure S1. XRF and XANES analyses of the tattooed skin biopsy isolated from the patient presented. (a) XRF-map of a thin section of the skin biopsy taken after remission of the symptoms. Red = titanium (Ti); green = chromium (Cr); blue = phosphor (cell nuclei). Epidermal layer on the right, distinguishable by high nuclei density. Scale bar = 50 μ m. (b) Ti-XANES shows a clear peak at about 4.997 keV being specific for rutile TiO₂. (c) Cr-XANES with a peak-plateau at about 6 keV and a peak at 6.02 keV being specific for metallic Cr(0) and Cr(III), respectively.

References

- 1 Laux P, Tralau T, Tentschert J et al. A medical-toxicological view of tattooing. *Lancet* 2016; **387**: 395–402.
- 2 Serup J, Hutton Carlsen K, Sepehri M. Tattoo complaints and complications: diagnosis and clinical spectrum. In: *Tattooed Skin and Health*, Current Problems in Dermatology, Vol. **48**, Serup J, Kluger N, Bäuml W (eds): Basel, Karger, 2015: pp. 48–59.
- 3 Pérez-Cotapos M L, de Cuyper C, Cossio L. Tattooing and scarring technique and complications. In: *Dermatologic Complications with Body Art. Tattoos, Piercings and Permanent Make-Up*, de

- Cuyper C, Perez-Cotapos M L (eds.): Berlin, Heidelberg, Springer-Verlag, 2010: pp. 29–42.
- 4 Aberer W, Snauwaert J, Render U M. Allergic reactions to pigments and metals. In: *Dermatologic Complications with Body Art. Tattoos, Piercings and Permanent Make-Up*, de Cuyper C, Perez-Cotapos M L (eds.): Berlin, Heidelberg, Springer-Verlag, 2010: pp. 61–89.
 - 5 Serup J, Hutton Carlsen K. Patch test study of 90 patients with tattoo reactions: negative outcome of allergy patch tests to baseline batteries and culprit inks suggest allergens(s) are generated in the skin through haptenization. *Contact Dermatitis* 2014; **71**: 225–263.
 - 6 Kluger N. Sarcoidosis on tattoos: a review of the literature from 1939 to 2011. *Sarcoidosis Vasc Diffuse Lung Dis* 2013; **30**: 86–102.
 - 7 Sepehri M, Hutton Carlson K, Serup J. Papulo-nodular reactions in black tattoos as markers of sarcoidosis: study of 92 tattoo reactions from a hospital material. *Dermatology* 2016; **232**: 679–686.
 - 8 Fage S W, Muris J, Jakobson S S, Thyssen J P. Titanium: a review on exposure, release, penetration, allergy, epidemiology, and clinical reactivity. *Contact Dermatitis* 2016; **74**: 323–345.
 - 9 Thyssen J P, Menné T, Schalock P C et al. Pragmatic approach to the clinical work-up of patients with putative allergic disease to metallic orthopaedic implants before and after surgery. *Br J Dermatol* 2011; **164**: 473–478.
 - 10 Salomé M, Cotte M, Baker R et al. The ID21 scanning X-ray microscope at ESRF. *J Phys Conf Ser* 2013; **425**: 182004.
 - 11 Hansen M B, Johansen J D, Menné T. Chromium allergy: significance of both Cr(III) and Cr(VI). *Contact Dermatitis* 2003; **49**: 206–212.
 - 12 Dirks M. Making innovative tattoo inks products with improved safety: possible and impossible ingredients in practical usage. In: *Tattooed Skin and Health*, Current Problems in Dermatology, Vol. **48**, Serup J, Kluger N, Bäuml W (eds.): Basel, Karger, 2015: pp. 118–127.
 - 13 Lares Filon F, Mauro M, Adami G et al. Nanoparticles skin absorption: new aspects for a safety profile evaluation. *Regul Toxicol Pharmacol* 2015; **72**: 310–322.
 - 14 Fireman E, Bar Shai A, Alcalay Y et al. Identification of metal sensitization in sarcoid-like metal-exposed patients by the MELISA® lymphocyte proliferation test – a pilot study. *J Occup Med Toxicol* 2016; **11**: 18.
 - 15 Naeyaert C, de Cuyper C. Rood pigment de grote boosdoener in Tattoos. *Ned Tijdschr Dermatol Venereol* 2013; **29**: 563–564.
 - 16 Gaudron S, Ferrier-Le Bouedec M C, Franck F, D'Incan M. Azo pigments and quinacridones induce delayed hypersensitivity in red tattoos. *Contact Dermatitis* 2015; **72**: 97–105.
 - 17 Morales-Callaghan A M, Aguilar-Bernier M, Martinez-Garcia G, Miranda-Romero A. Sarcoid granuloma on black tattoo. *J Am Acad Dermatol* 2006; **55**: S71–S73.
 - 18 Forte G, Petrucci F, Cristaudo A, Bocca B. Market survey on toxic metals contained in tattoo inks. *Sci Total Environ* 2009; **407**: 5997–6002.
 - 19 Jungmann S, Laux P, Bauer T T et al. From the tattoo studio to the emergency room. *Dtsch Arztebl Int* 2016; **113**: 672–675.
 - 20 Wenzel S M, Welzel J, Hafner C et al. Permanent make-up colorants may cause severe skin reactions. *Contact Dermatitis* 2010; **63**: 223–227.
 - 21 Bernstein E F. Laser tattoo removal. *Semin Plast Surg* 2007; **21**: 175–192.
 - 22 Hauri U, Hohl C. Photostability and breakdown products of pigments currently used in tattoo inks. In: *Tattooed Skin and Health*, Current Problems in Dermatology, Vol. **48**, Serup J, Kluger N, Bäuml W (eds.): Basel, Karger, 2015: pp. 164–169.
 - 23 Vasold R, Naarman N, Ulrich H et al. Tattoo pigments are cleaved by laserlight, the chemical analysis *in vitro* provide evidence for hazardous compounds. *Photochem Photobiol* 2004; **80**: 185–190.
 - 24 Schreiver I, Hutzler C, Laux P et al. Formation of highly toxic hydrogen cyanide upon ruby laser irradiation of the tattoo pigment phthalocyanine blue. *Sci Rep* 2015; **5**: 12915.
 - 25 Eklund Y, Troilius Rubin A. Laser tattoo removal, precautions and unwanted effects. In: *Tattooed Skin and Health*, Current Problems in Dermatology, Vol. **48**, Serup J, Kluger N, Bäuml W (eds.): Basel, Karger, 2015: pp. 88–96.
 - 26 Psaltis N M, Gardner R G, Denton W J. Systemic sarcoidosis and red dye granulomatous tattoo inflammation after influenza vaccination: a case report and review of literature. *Ocul Immunol Inflamm* 2014; **22**: 314–321.
 - 27 Lindblad E B. Aluminium compounds for use in vaccines. *Immunol Cell Biol* 2004; **82**: 497–505.
 - 28 McFadden N, Lyberg T, Hensten-Petersen A. Aluminum-induced granulomas in a tattoo. *J Am Acad Dermatol* 1989; **20**: 903–908.
 - 29 Erdohazi M, Newman R L. Aluminium hydroxide granuloma. *Br Med J* 1971; **3**: 621–623.

Online supplemental table 4. 10-Point check list that could strengthen the suspicion of clinically relevant metal allergy to an orthopaedic implant.

Symptom	Observation in the patient
1. Chronic dermatitis beginning weeks to months after metallic implantation	yes
2. An eruption overlying the metal implant	not demonstrated but increasing back pain at the site of the implant reported by the patient
3. A morphology consistent with dermatitis (erythema, induration, papules)	yes
4. In rare instances, a systemic allergic dermatitis reaction	yes multiple tattoos/fever
5. Histology consistent with allergic contact dermatitis	yes, granulomatous type
6. Positive patch test reaction to a metal used in the implant	yes to extract of screws and metallic titanium
7. Serial dilution patch testing giving positive reactions to low concentrations of the metal under suspicion	not done
8. Positive <i>in vitro</i> test results for metals, for example the lymphocyte transformation test	not done
9. The dermatitis reaction being therapy-resistant	yes
10. Complete recovery following removal of the offending implant	yes

Online supplemental table 5. Diagnostic Approach for Suspected Tattoo Allergy

A. skin testing with ink (if available) and additional test series

1. positive skin test to the ink: allergen is present in the ink

a) can be an additive such as preservative e.g. methylisothiazolinone

b) can be an element of a colourant identified with positive specific patch test e.g. mercury

c) can be an element of a colourant identified by positive photopatch test e.g. cadmium

d) can be a pigment identified with positive specific prick test e.g. pigment red

e) can be an unidentified component > do element/pigment analysis of the ink > repeat skin test with identified components.

2. Negative skin test to the ink and additional test series

a) can be an allergen released or formed in the skin after decomposition of the colourant by

-metabolisation

-sunlight > test with UV and if available UV-radiated ink?

-laser irradiation > test with lasered ink

Two possibilities:

b) Released element or decomposition product is the allergen

c) Decomposition product is hapten, needs protein binding for sensitisation

b)&c) go to B. for element/pigment analysis of skin biopsy

B. Skin biopsy

1. Histology is suggestive for allergy

> go to A. skin testing

> do element analysis of skin biopsy > specific skin test with identified components

> consider lymphocyte transformation tests

2. Histology dubious > needs to be differentiated from

-aspecific granulomatous reaction (reconsider allergy)

-foreign body reaction/trauma

-sarcoidosis --> do chest X-ray and ACE

