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Investigation of Adverse Reactions in Tattooed Skin through Histological and Chemical Analysis

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Adverse tattoo reactions · Hypersensitivity · Azo pigment · Analytical chemistry · Histology

Abstract

Background: Just as the number of tattooed people has increased in recent years, so has the number of adverse reactions in tattooed skin. Tattoo colourants contain numerous, partly unidentified substances, which have the potential to provoke adverse skin reactions like allergies or granulomatous reactions. Identification of the triggering substances is often difficult or even impossible. ***Methods:*** Ten patients with typical adverse reactions in tattooed skin were enrolled in the study. Skin punch biopsies were taken and the paraffin-embedded specimens were analysed by standard haematoxylin and eosin and anti-CD3 stainings. Tattoo colourants provided by patients and punch biopsies of patients were analysed with different chromatography and mass spectrometry methods and X-ray fluorescence. Blood samples of 2 patients were screened for angiotensin-converting enzyme (ACE) and soluble interleukin-2 receptor (sIL-2R). ***Results:*** Histology showed variable skin reactions such as eosinophilic infiltrate, granulomatous reactions, or pseudolymphoma. CD3+ T lymphocytes dominated the dermal cellular infiltrate. Most patients had adverse skin reactions in red tattoos (n = 7), followed by white tattoos (n = 2). The red tattooed skin areas predominantly contained Pigment Red (P.R.) 170, but also P.R. 266, Pigment Orange (P.O.) 13, P.O. 16, and Pigment Blue (P.B.) 15. The white colourant of 1 patient contained rutile titanium dioxide but also other metals like nickel and chromium and methyl dehydroabietate – known as the main ingredient of colophonium. None of the 2 patients showed increased levels of ACE and sIL-2R related to sarcoidosis. Seven of the study participants showed partial or complete remission after treatment with topical steroids, intralesional steroids, or topical tacrolimus. ***Conclusions:*** The combination of the methods presented might be a rational approach to identify the substances that trigger adverse reactions in tattoos. Such an approach might help make tattoo colourants safer in the future if such trigger substances could be omitted.

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Introduction

The number of tattooed people has substantially increased worldwide in recent years. The incidence of tattooed individuals with at least one tattoo is estimated to be between approximately 12% and 29% [1–4]. When getting the first tattoo, the age of people is mostly less than 30 years, whereas only 10% of people are older than 50 years [5, 6]. Global interest in tattoos was investigated using Google Trends, which showed that there is significant interest in tattoos, particularly in Latin American countries [7]. Considering the high percentage of tattooed people, tattoo colourants should provide a high level of safety. Unfortunately, many countries around the world lack stringent regulations for tattooing products. In the USA, the Food and Drug Administration has the regulatory authority over ingredients in tattoo colourants, but yet no specific legal requirements apply [8]. The Council of Europe has presented its first resolution in 2003, which refers to the composition and regulation of marking and labelling of products used for tattoos and permanent makeup [9]. In the EU, a restriction on substances in colourants used for permanent body modification entered into force in 2021. It adds stricter concentration limits to all chemicals that fall within the harmonized classification and labelling, which led to a vanishing of almost all colourant manufacturers in early 2022 when the 12-month transition period of this restriction passed. About 50% of tattoos are black followed by red (14%), blue (10%), green (9%), and yellow (8%) [10]. The production of tattoo colourants requires various organic or inorganic pigments. Pigments may also contain contaminants (e.g., nickel), educts, and by-products of pigment synthesis (e.g., polycyclic aromatic hydrocarbons or primary aromatic amines), as well as substances used in pigment refinement [11]. Pigments are not soluble in water and thus a ready to use colourant product is manufactured by using additional substances such as emulsifiers, binders, or antifoaming agents [10, 12]. In general, chemicals such as polycyclic aromatic hydrocarbons (43%), primary aromatic amines (14%), or heavy metals (9%) may cause immunotoxic, carcinogenic, or sensitizing reactions [12]. The abrasion of tattoo needles may also add a health risk for tattooed persons, as the needles contain large amounts of sensitizing elements such as nickel and chromium [13].

Possible complications of tattoos are acute contact dermatitis, tattoo ink hypersensitivity reactions, chronic inflammatory black tattoo reactions, or papulo-nodular reactions in black tattoos. In addition, auto-immune diseases such as sarcoidosis or lupus erythematosus may appear in the tattooed skin [10]. When reviewing reports on such adverse reactions along with tattoos, the frequency of adverse reactions is considerably higher for coloured tattoos than for black tattoos [10]. Cancer or abnormal pigmentation may also occur in tattooed skin but possibly coincidentally [6, 14]. In addition to skin reactions, part of injected tattoo colourants is transported through cells or via blood or lymphatic vessels from the skin to local lymph nodes and likely to the liver [15–17]. At present, several studies report that allergic and granulomatous reactions represent the majority of tattoo-related complications [18–21]. These reactions may occur during or after wound healing due to a reaction of different immune cells to any ingredient of the tattoo colourant [10, 18, 22–25]. Unfortunately, the substances in the tattooing product responsible for the immune reaction are frequently unknown. Compared to other common allergic reactions, there are no specific test procedures for tattoo-related adverse reactions. Despite the high number of tattooed individuals worldwide, only a few studies have been published about the cause of tattoo-related adverse skin reactions to date [26, 27]. Detailed investigations of these reactions are needed to clarify which specific ingredients of tattoo colourants initiate the skin reaction. The knowledge of such initiators would facilitate the diagnosis and therapy of tattoo-related skin diseases. Moreover, it would be a scientific basis for banning of such problematic substances in tattoo products to reduce the incidence of such health problems. In a recent review from the authors, a possible strategy was discussed to find potential tattoo allergens. This strategy contains the assessment of clinical history and chemical analysis which might be followed by additional investigations [12]. The clinical history may provide information on the nature of the suspected substance. Chemical analysis can investigate the presence of an allergen in the biopsy or colourant. Thus, the present study aimed to combine chemical, histological, and medical examinations to identify such initiators of tattoo complications. As a first step, the present study includes 10 patients with typical adverse tattoo-related skin reactions with unknown cause.

Materials and Methods

Subjects have given their written informed consent and the study protocol was approved by the Ethics Committee of the University of Regensburg, approval number [18-1031_1-101].

Subjects

Seven women and three men were examined at the Department of Dermatology, University of Regensburg. The clinically suspected diagnosis was confirmed histologically by a dermatohistopathologist. We investigated paraffin-embedded skin biopsies that had been taken at the department of dermatology consecutively between 2016 and 2020 from 10 patients with adverse reactions in tattooed skin. Blood samples were taken upon consent of patients. The patient's sex, age, pre-existing allergies, location of tattoo, age of tattoo, onset of problems after tattooing, colours of tattoo, results of clinical inspection, histological results, and clinical diagnosis were recorded. The skin lesions in question were photographed.

Histochemistry

Biopsies of all 10 patients were fixed in formalin, embedded in paraffin, and were then stained with haematoxylin and eosin. Pictures were taken with a Carl Zeiss light microscope (Carl Zeiss Vision GmbH, Halbergmoos, Germany).

Immunohistochemistry

CD3 staining was carried out on 5-µm tissue sections. Paraffin was removed from samples with xylene followed by a decreasing ethanol-water series for each 3 min, respectively. For heat-induced antigen retrieval, sections were boiled in pre-heated 1 mM Tris/EDTA buffer (pH 9) in a pressure cooker for 3 min at maximum pressure followed by quick pressure release. Sections were blocked for 1 h in 5% foetal calf serum and 0.1% Tween in phosphate buffer saline. CD3 antibody in blocking buffer (Merck, PC630) was used in a 1:100 dilution for 1 h followed by 30 min incubation with AlexaFluor 546 secondary antibody (Thermo Fiscer, A-11035) in a 1:300 dilution. Cell nuclei were stained with Hoechst 33,342 (2 µg/mL) for 5 min. Slides were then covered with FluorSave mounting reagent (Merck KGaA, Darmstadt, Germany) and analysed using an LSM700 confocal microscope and the ZEN blue software (Carl Zeiss, Oberkochen, Germany).

Blood Samples

Out of the study population, only 2 patients agreed to take blood samples for further analysis. Depending on clinical and histological features, the blood samples of both patients (patient 2, 4) were tested for angiotensin-converting enzyme (ACE) and soluble interleukin-2 receptor (sIL-2R).

Chemical Analysis

Two patients succeeded to provide ink from their tattooists (patient 3, 5). The anonymized skin biopsies were investigated using matrix-assisted laser desorption/ionization tandem mass spectrometry [28, 29]. Colourants were analysed using matrix-assisted laser desorption/ionization tandem mass spectrometry, desorption and pyrolysis gas chromatography mass spectrometry (GC-MS) [30], X-ray fluorescence [31] analysis, and/or coupled plasma mass spectrometry as previously described [31].

Results

Clinical Characteristics and Treatment

Patient's age, sex, pre-existing allergy, location of tattoos, age of tattoo, onset of problems after tattooing, colours of tattoos, clinical inspection, histological results, and clinical diagnosis are shown in Table 1. Patient's mean age (± standard deviation) was 30.0 ± 8.5 years. We included 7 women and 3 men in this study. Tattoo-related skin lesions of all female patients occurred on their extremities. Two male study participants had adverse reactions on trunk and extremities, and the third male participant had an adverse reaction on his forearm. In the present study, 7 of the ten patients showed an adverse reaction in the red tattooed skin area. Most reactions belong to hyperkeratotic or plaque elevated type of inflammatory reactions (Fig. 1). The patients reported sudden onset of adverse reactions, causing painful pruritus. The time to first symptoms ranged from a few days up to several months or years after tattooing. Five of the patients experienced an early onset of symptoms <1 month, whereas five had an onset after 1–6 months. In patient 10, 2-year-old tattoos on both legs also started to show adverse effects in the white colour. The patients received treatment for adverse skin reactions with topical, oral, or intralesional steroids, topical calcineurin inhibitors, and fusidic acid, depending on the medical history (Table 2). The outcome of the patients was mixed. Two patients did not return for follow-up. One patient had no improvement of symptoms. Five patients had a partial remission, and 2 patients had a complete remission (Table 2). None of the 2 blood samples from patient 2 and 4 showed increased concentrations of ACE or sIL-2R. We performed chest X-rays, ophthalmologic examination, echocardiography, and abdominal sonography to exclude a systemic sarcoidosis.

Histology of Skin Samples

After the initial clinical diagnosis of the patients, biopsies were taken for confirmation. Two patients showed a granulomatous reaction with infiltrates of granulomatous arrangement containing lymphocytes, foreign body giant cells, histiocytes, and lots of eosinophilic granulocytes (patient 2 and 4). The biopsies of patient 1 (Fig. 2a) and 9 showed an atypical lymphocytic infiltrate with lots of eosinophilic granulocytes and were diagnosed with pseudolymphoma. The biopsy of patient 3 showed a dermal eosinophil-rich fixed tattoo reaction and did not allow for an unequivocal histological diagnosis (Fig. 2b). The biopsies of patient 5 (shown in Fig. 2c), 7, and 8 showed a lymphohistiocytic infiltrate, giant cells, and lots of eosinophilic granulocytes, which were diagnosed as contact dermatitis. The biopsy of patient 10 showed mild perivascular lymphocytic reaction with superficial interstitial mucin deposition.

Table 1. Demographic and clinical characteristics of the 10 patients with adverse tattoo skin reactions

No.	Sex	Age, years	Pre-existing allergy	Location of tattoos	Age of tattoo	Onset of problems after tattooing	Colours of tattoos	Clinical inspection	Clinical diagnosis	Histological findings and diagnosis
1	Male	25	None	Right shoulder, breast, right upper arm	2 years	6 months	Red, black	**Red colour** only: hypertrophic, erythematous plaques	Cutaneous granulomatosis	Pseudolymphoma
2	Male	26	None	Left lower leg, back	2 years	6 months	Red, black	**Red colour** only: circumscribed, hyperkeratotic, crusty	Delayed-type hypersensitivity	Granulomatous dermatitis
3	Female	23	Pollen	Both forearms	1 month	2 days	White, black	**White colour** only: inflammatory plaques, induration	Delayed-type hypersensitivity	Dermal eosinophil-rich fixed tattoo reaction (no clear diagnosis possible)
4	Female	38	Procaine Diclofenac	Left dorsum of the foot	1 year	A few days	Red	**Red colour** only: skin infiltration, shiny, about 2 mm elevated	Delayed-type hypersensitivity	Granulomatous dermatitis
5	Female	22	None	Right forearm	4 months	3 weeks	Red, black	**Red colour** only: circumscribed, erythematous maculae and plaques, desquamation	Allergic contact dermatitis	Contact dermatitis
6	Male	51	Grasses	Right forearm	2 weeks	1 week	Black	**Black colour** only: blurred margin of surrounding erythema, desquamation	Contact dermatitis	*No sample taken*
7	Female	24	None	Right forearm	2 years	1 week	Red	**Red colour** only: small nodules, desquamation	Contact dermatitis	Contact dermatitis
8	Female	34	None	Right forearm	11 months	6 months	Red, black	**Red colour** only: induration	Allergic contact dermatitis	Contact dermatitis
9	Female	27	None	Back of the left hand	13 months	2 months	Red, black	**Red colour** only: granulomatous plaque	Cutaneous granulomatosis	Pseudolymphoma
10	Female	30	None	Back, both lower legs	Back: 4 months Lower legs: 2 years	Back: 1 month Lower legs: 2 years	Red, black, white	**White colour** only on the back Scar-like, plaques **White colour** only on both lower legs: swelling, elevated plaques with slight ambient flush	Cutaneous granulomatosis	Mild perivascular lymphocytic reaction with superficial interstitial mucin deposition

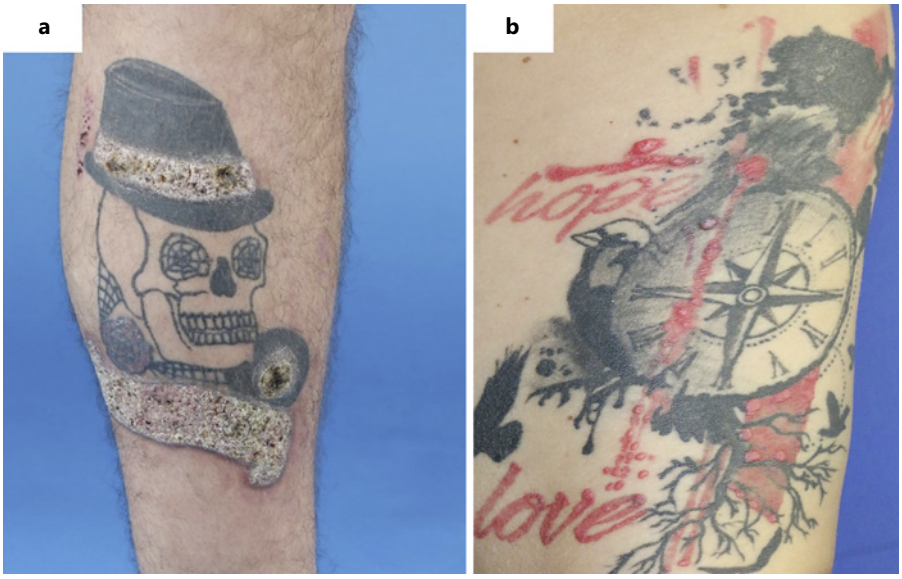

Fig. 1. Red tattoo reactions in 2 patients. **a** Hyperkeratosis on the left lower leg of patient 2. **b** Plaque elevated type inflammatory reaction on the right forearm of patient 5.

Tissue sections of selected patients were also stained by immunohistochemistry (Table 3). All showed a CD3+ T cell infiltrate. The cells showed heterogeneous immunohistochemical staining due to deformation of the sections which led to a loss of focus in some areas during microscopy (Fig. 2d, e, f).

Chemical Analysis

The punch biopsies of patient 1, 2, 3, 4, 5, 7, and 9 or the components of the 2 provided colourants of patient 3 and 5 were analysed (Table 3). X-ray fluorescence analysis of the white colourant of patient 3 identified rutile titanium dioxide as the colour-giving white pigment. Coupled plasma mass spectrometry analysis confirmed the high abundance of titanium but also provided additional information on metals like iron, nickel, manganese, chromium, cadmium, and copper (Table 3). The ink of patient 3 also contained isopropyl alcohol and 2-ethylacrolein as confirmed by desorption of the ink with GC-MS before pyrolysis. Three of the 5 analysed red biopsies contained the Pigment Red (P.R.) 170. In the biopsy of patient 5, P.R. 170, P.R. 266, and Pigment Orange (P.O.) 13 could be verified. The provided ink sample of patient 5 only contained P.R. 170 and P.R. 266 (Table 3). With help of desorption and pyrolysis-GC-MS, methyl dehydroabietate, propylene glycol, dipropylenglycol, polyethylene glycole, Triton X, and polystyrene monomer were found.

Discussion

Different surveys already unfolded many details of the tattooed population worldwide [2, 5, 32]. Women clearly have more tattoos than men, which is also reflected in the gender distribution in the present study (7 women, 3 men) [5]. Although women prefer to get tattoos on the torso (54%), while men prefer the extremities (64%) [5], tattoo-related skin lesions of all female patients in this study occurred on their extremities. Two male study participants had adverse reactions on the trunk and extremities, and the third male participant had an adverse reaction only on his forearm. The number of patients in the present study is small, but it seems that adverse reactions to tattoos are more common on the extremities, which is supported by previously reviewed studies [10]. The skin of extremities and hence the tattoo colourants are more likely exposed to solar radiation that could trigger phototoxic or photoallergic reactions [10, 33].

Skin Reactions and Clinical Diagnoses

The patients reported sudden onset of adverse reactions causing painful pruritus, which is considered to be the most common first symptom [34]. The adverse skin reactions were limited to the problematic colour in the tattooed areas. The lesions showed plaques, induration, desquamation, and nodules. The main diagnosis after histology was pseudolymphoma, granulomatous and contact dermatitis which is in accordance with other findings [14, 19]. Except for two white and one black tattoo, the reactions were related to skin lesions containing red pigments. The histological findings of the white tattoos did not confirm a common contact dermatitis but showed an eosinophil-rich infiltrate or mild perivascular lymphocytic reaction. For the black tattoo, the patient declined a biopsy, so no confirmation of the initial contact dermatitis diagnosis was possible.

Table 2. Treatment according to anamnesis and outcome in 10 patients with adverse tattoo skin reactions

No.	Anamnesis	Treatment	Follow-up
1	Enhancement, treatment with unknown topical ointment led to no improvement	Methylprednisolone acetate 0.1% cream topical 1 time per day Tacrolimus 0.1% topical 1 time per day	• After 3 months, little improvement of the adverse skin reactions and they became more flat • Tacrolimus 0.1% 2 times per day, 4 times intralesional triamcinolone acetonide 40 mg at intervals of 4 weeks • Partial remission after 2 months
2	Inflammatory reaction, no improvement under treatment with topical steroid, ACE, and sil-2R in normal range	Fusidic acid and betamethasone valerate 0.1% topical 2 times per day	• Patient failed to appear (no improvement after 6 month reported via telephone)
3	1 day after tattooing: erysipelas of the tattoo area with fever, flush, and nausea; thereafter, yellow incorporations, oozing, pre-treated with fusidic acid and betamethasone valerate	Fusidic acid and betamethasone valerate cream 0.1% topical 1 time per day	• After 2 months, adverse skin reactions almost resolved, little pruritus, little redness • Tacrolimus 0.1% 3 times a week • Complete remission after 4 months
4	Pruritus, swelling No improvement after use of unknown topical ointment, ACE, and sil-2R in normal range	Methylprednisolone aceponate 0.1% cream topical 1 time a day	• After 2 months, adverse skin reactions resolved but recurred when therapy with methylprednisolone aceponate was stopped, still thickening of red areas • Tacrolimus 1–2 times per day, methylprednisolone aceponate 2 times per day • Partial remission after 4 months
5	Blistering, severe pruritus, swelling	Diflucortolone valerate 0.1% cream topical 1–2 times per day	• After 4 months, no response to treatment, still swelling, erythema, and pruritus • Prednisolone therapy with initial 40 mg, reduction to 17.5 mg within 5 weeks • After 2 months, less induration • Recurrence of pruritus when stopping therapy • Partial remission after 4 months
6	Redness, pruritus	Mometasone furoate 0.1% topical cream 1 time a day	• Patient failed to appear (no improvement after 6 month reported via telephone)
7	Pruritus, swelling Pre-ointment with mometasone furoate 0.1% without improvement	Diflucortolone valerate 0.1% topical cream, initial occlusive application for 30 min 1 time per day for 4 days, then 1 time per day	• No improvement after 4 months
8	Pruritus, swelling, flush, thickening	Diflucortolone valerate 0.1% topical cream 1 time per day	• After 2 months, little flattening, still pruritus • Diflucortolon-21-valerate 0.1% cream, still fresh pustules • Partial remission after 4 months
9	Pruritus, swelling, thickening, pre-ointment with dimetindene gel without improvement	Mometasone furoate 0.1% topical 1 cream time a day	• Partial remission after 1 month
10	Pruritus, swelling	Fusidic acid and betamethasone valerate topical cream 1 time per day, polidocanol, and urea lotion several times a day	• Complete remission after 3 months

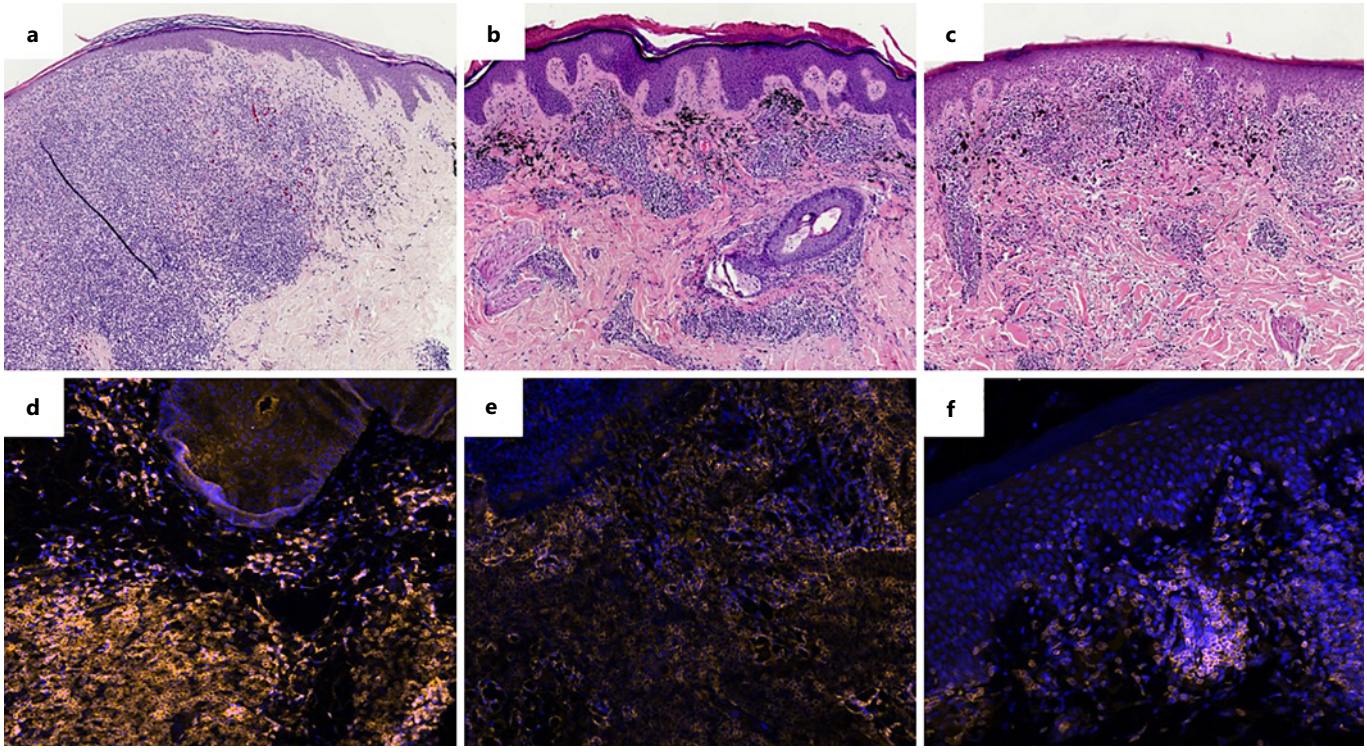

Fig. 2. Examples of H&E und CD3 staining results from punch biopsies of patient 1, 3, and 5. **a**, **d** Patient 1 diagnosed with pseudolymphoma showing CD3 clusters in the dermis. **b**, **e** Patient 3 diagnosed with dermal eosinophil-rich fixed tattoo reaction showing a more evenly distribution of CD3-positive cells in the affected areas of the dermis. **c**, **f** Patient 5 diagnosed with contact dermatitis showing smaller clusters of CD3-positive cells directly beneath the epidermis (upper part of the picture). H&E, haematoxylin and eosin.

Table 3. Chemical and immunohistochemical analysis of skin punch biopsies and/or tattoo inks

Patient No.	Colour	Biopsy analysis	Tattoo ink analysis	Immunohistochemistry
1	Red	P.R. 170 P.R. 266 P.B. 15	*No ink obtained*	CD3+
2	Red	*No organic pigment identified*	*No ink obtained*	CD3+
3	White	–	Rutile titanium dioxide (XRF analysis) titanium > iron > nickel > manganese > chromium > cadmium > copper (ICP-MS) Isopropyl alcohol, 2-ethylacrolein ([pyrolysis] GC-MS)	CD3+
4	Red	*No organic pigment identified*	*No ink obtained*	CD3+
5	Red	P.O. 13 P.R. 170 P.R. 266	P.R. 170, P.R. 266 (MALDI) methyl dehydroabietate, propylene glycol, dipropylenglycol, polyethylene glycole, Triton X, polystyrene monomer ([pyrolysis] GC-MS)	CD3+
7	Red	P.R. 170	*No ink obtained*	CD3+
9	Red	P.O. 16, *no organic red pigment identified*	*No ink obtained*	CD3+
10	White	–	*No ink obtained*	CD3+

XRF, X-ray fluorescence; ICP, coupled plasma mass spectrometry.

Half of the patients experienced an early onset of symptoms within 1 month after tattooing. Hypersensitivity reactions that occur only a few days after tattooing suggest a previous contact and clinically silent sensitization phase with the allergen, e.g., through other tattoos or products with similar ingredients. Since 2 patients with delayed-type hypersensitivity reacted within days after tattooing, they must already have been sensitized by previous exposures.

Some tattoos obviously existed for several months without causing skin problems. A time to first symptoms of several months and longer could represent an initial tolerance with a rather slow, symptomless sensitization to any chemical in the colourant. After tattooing, enzymatic reactions or (sun)light absorption might modify the pigments or other ingredients still residing in the skin [11, 34, 35, 36, 37, 38]. This may lead to the formation of new sensitizing chemicals, which might have triggered the skin reactions [39].

Also, ingestion of medical drugs may interfere with tattoos. The immunomodulatory properties of inhibitors of the mitogen-activated protein (MAP) kinase pathway and immune checkpoint inhibitors may enhance a loss of tolerance to tattoo colourants present in skin, leading to granulomatous reactions [40]. However, the 2 patients with granulomatous dermatitis diagnosed from the histology in our study had no known history of MAP kinase inhibitor treatments.

Histopathology and Laboratory Results

Histopathological findings of the adverse tattoo reactions can be assigned to lichenoid, granulomatous, and pseudolymphomatous appearance [10, 34]. The histopathological results in this study showed in particular granulomatous dermatitis, pseudolymphoma as well as features consistent with contact dermatitis. However, it was remarkable that all biopsies showed a high number of eosinophilic granulocytes. Contrary to this, a retrospective study in 74 skin biopsies of patients with allergic red tattoo reactions proved the predominant role of histiocytes and lymphocytes, whereas eosinophils were rather uncommon, which was also demonstrated by van der Bent et al. [41]. The predominantly histiocytic reaction in that study combined with interface dermatitis was the main inflammation pattern, but most biopsies showed more than one reaction pattern [41]. Another study investigated biopsies in tattooed skin of 117 patients with inflammatory reactions [42]. The most common form of inflammatory pattern associated with tattoos was fibrosis, followed by granulomatous reactions, lichenoid reactions, epithelial hyperplasia, pseudolymphomas, and spongiotic reactions. The authors found combined features of two or more types of inflammatory patterns in 64% of cases [42]. Allergic reactions to tattoos are thought to be mediated mainly by T cells and are thus classified as delayed-type (type IV) allergies [17]. With their T cell receptor, T cells recognize epitopes of chemically modified self-antigens in a process called haptenization. Chemical-induced T-cell epitopes may be abundant in tattooed skin where the colourant provides a permanent source. Both CD4+ and CD8+ T cells appear to be involved in the delayed-type hypersensitivity reactions [43, 44]. Our samples showed the presence of a prominent CD3+ T cell infiltrate in all investigated adverse tattoo reactions, consistent with the presence of delayed-type allergies. However, even in some rare cases, histological staining of a (++) positive patch test reaction did not show T-cell infiltration [45].

Neither of the 2 patients with granulomatous dermatitis showed elevated ACE and sIL-2R levels that would have indicated systemic sarcoidosis. Since sarcoidosis is also a granulomatous disease, characterized by non-necrotizing granulomas, high ACE levels and pulmonary changes should be excluded in these participants [46–48].

Skin Reactions and Chemical Analysis

The main ingredient in all tattoo colourants is the colouring pigment, mainly synthesized for industrial purposes and comprising various substances. As a first approach, such pigments can be assigned to two main groups: the inorganic (e.g., carbon black, titanium dioxide) and the organic (e.g., azo) pigments [6]. The production processes in both groups are rather different and may not only result in the desired educts but also by-products and impurities. A review of clinical complications already provided evidence that adverse skin reactions predominantly occur in coloured tattoos and less frequently in black tattoos [10]. In the present study, 7 of the 10 patients showed an adverse reaction in the red tattooed skin area. This confirms that red is still the most reported colour when it comes to tattoo reactions [11, 18, 22]. A clinical study included 301 patients with adverse reactions in tattooed skin. Allergic red tattoo reactions and chronic inflammatory black tattoo reactions accounted for 50.2% and 18.2% of all tattoo complications, respectively [20]. A smaller study with 31 tattooed individuals showed that even 75% of allergic tattoo reactions were associated with a red colour [13]. The red pigments identified in this study were P.R. 170 and P.R. 266. Additionally, P.B. 15, P.O. 13, and P.O. 16 were found and may have been used to modify the colour shade in the ink. However, as with any targeted analytical method, only known pigments above a certain concentration can be identified. In this study, paraffin-embedded

skin samples already decreased in size from histological sectioning were used. Both the paraffin residues and the small sample size may affect the detection.

Pigments may either serve as sensitizing agents themselves or smaller fragments of the pigment molecules may act as haptens, triggering an allergic reaction [49]. Interestingly, both red pigments in the biopsies belong to the group of azo-naphthol AS compounds. A recent large study investigated 104 skin biopsies from patients with adverse skin reactions to tattoos and showed that 71% of pigments represented red azo-naphthol AS pigments like P.R. 22, P.R. 170, and P.R. 266 [49]. An allergic reaction involving a red azo-naphthol AS pigment was already published more than 20 years ago [50]. The authors performed photo patch testing and (photo) prick testing with the provided tattoo ink after 10-fold tape stripping which caused positive reactions after 24 h and beyond. Pure naphthol AS caused a positive reaction upon standard patch testing. P.R. 23 was identified by mass spectrometry which is an azo pigment containing naphthol AS in its structure, but with an additional NO_2 group. A naphthol AS group can be released from such a red pigment by cleavage of the azo bond due to enzymatic or light interactions [6, 35, 51]. This chemical substance might also be involved in the adverse skin reaction in our study. The contribution of the other pigments regarding adverse skin reactions is also not fully explored yet and their potential role in the presented skin reactions remains difficult to unfold. The disazopyrazolone pigment P.O. 13 may alter cytokine secretion as proved in reconstructed human skin models [52]. 3-3′-Dichlorobenzidine, a potential decomposition product of P.O. 13, is already classified as a sensitizing substance and additionally has an IARC classification 2B, possibly carcinogenic to humans [49, 53]. González-Villanueva et al. [24] concluded from their findings that the allergic skin reaction to 3 tattoo colourants may occur when containing the azo Pigment Yellow 65, although the colourants also contained P.O. 13 and P.R. 210 (mixture of P.R. 170 and P.R. 266) according to declaration of the colourants. Azo pigments can be metabolized by cytochrome P450 as shown for Pigment Yellow 74 yielding metabolites as new substances [54].

Beside pigments, tattoo colourants may also contain different preservatives like phenol, formaldehyde, and methylisothiazolinone and impurities like nitrosamines and phthalates [6, 49, 55]. However, soluble preservatives would likely be eliminated from the skin quickly and thus only cause a transient and self-resolving reaction. In the analysis of the ink of patient 5, we detected 2-ethylacrolein which is a chemical building block and can be used for polyacrylate synthesis. Additionally, the detection of the allergen methyl dehydroabietate indicates the use of colophonium. It is insoluble in water and may therefore also reside in skin for a prolonged time period.

Patients 3 had a clinical diagnosis of a delayed-type hypersensitivity reaction and patient 10 had a clinical diagnosis of a granulomatous disease in their white tattoo, which completely disappeared upon treatment. The putative sensitizing metals nickel, chromium, and copper were found in the white colourant of patient 3. The Council of Europe ResAP(2008)1 banned the presence of nickel at high levels in tattoo colourants to protect individuals with nickel allergy. Both patients had no pre-existing allergy against nickel or other metals; 1 of these patients had a pollen allergy only. However, an even positive patch test would be considered insufficient to confirm the role of nickel in a reaction observed after tattooing since it could be coincidental [56]. Thus, in these two patients, no indication of likely allergens can be drawn from our results. However, in patient 10, white parts of an older tattoo also reacted which is a strong indicator of a true delayed-type hypersensitivity reaction. Beside the substances discussed above, other substances may occur in a tattoo colourant during shelf life, possibly due to sterilization processes of the finished products [57].

Treatment of Skin Reactions

The treatment of hypersensitivity reactions to tattoos remains difficult, and different procedures are recommended [58]. Topical and intralesional steroids appear to be only temporarily helpful, if at all [34]. In some severe cases of tattoo reactions, oral glucocorticosteroids, methotrexate, or hydroxychloroquine are used, when other methods are not effective or contraindicated. All our patients received topical steroids or tacrolimus as initial treatment. Two patients with adverse reactions to black or red tattoo colours did not show up for follow-up visits. We called these patients and they still reported the same problems in the tattooed region. Interestingly, complete remission upon treatment occurred only in the 2 patients who had adverse reactions related to the white pigment. The 6 patients with partial or no remission showed adverse reactions related to the red tattoo colour, in which the azo-naphthol AS pigments were verified in three biopsies. As with other putative allergens slowly released over time, the constant presence of the azo-naphthol AS pigment could impede the treatment of the adverse reactions in tattooed skin. A case series of 31 patients with tattoo-related skin reactions revealed that allergic tattoo reactions were treated with local corticosteroid ointments, corticosteroid infiltration, or surgical removal [22].

Laser radiation and surgical procedures might be therapeutic approaches to remove not only the tattoo but also the possible trigger substance of immune reaction [11, 59]. In general, tattoo removal is feasible by using short pulses in the range of a few nanoseconds to hundreds of picoseconds with high light intensities to selectively destroy the pigment particles in skin [60]. This selective destruction allows tattoo removal with a low risk of permanent side effects like scarring. However, laser radiation may not completely remove the colours in some cases and may cause additional degradation products. In the worst case, these might be the hapten of the local allergic reaction or lead to other immune reactions such as lymphadenopathy or local and systemic allergic reactions [11]. In contrast, the use of a vaporizing CO_2 laser enables removal of the entire tissue including the pigment particles, but this procedure carries a clear risk of scarring [61]. Another therapy approach is the fractional CO_2 laser ablation which may improve itching, burning, and impact on daily life in tattoo allergy but may change the tattoo [62].

Other surgical techniques, like excision, saving, and curettage, can remove the skin with the entire tattoo, but these techniques leave scars behind. Dermatome shaving may successfully stop the immune reaction but again with the risk of scarring [59].

Conclusion

According to the suggestions in our recent review, we combined clinical history, chemical analysis, and histological staining to identify the substances responsible for adverse reactions in tattooed skin [12]. Our data show that a detailed histological analysis and reporting could be important since putative allergic or granulomatous reactions may show atypical cell infiltrates.

The outcome of the therapy was very variable, possibly due to the limited number of cases. Only 2 patients with white tattoo reactions had a full remission. Out of 7 patients with red tattoo reactions, 5 patients at least showed a partial remission upon treatment. Chemical analysis identified the azo-naphthol AS P.R. 170 in adverse skin reaction within red tattoos. In future, a more in-depth chemical analysis of biopsies could identify not only the pigment but also other possible impurities such as naphthol AS. In addition to patch testing with the putative allergens, we will apply newly developed in vitro methods which may detect pathogenic T-cell populations in the patient's blood or biopsy compared to non-allergic controls [63, 64]. The detection of increased numbers of allergen-reactive T-cell clones in the inflamed tattoo would almost certainly indicate a type IV hypersensitivity reaction to a substance present in the biopsy and colourant used.

Limitations

The study included only 10 patients to test our suggested strategy of allergen detection.

Key Message

The main objective was to develop a joint strategy of medical diagnosis and chemical analysis to detect the cause of adverse reactions in tattooed skin.

Statement of Ethics

This study protocol was reviewed and approved by the Local Ethics Committee of the University of Regensburg, approval number [18-1031_1-101]. Written informed consent was obtained from participants to participate in the study. Written consent for the publication of patient's images was obtained.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Bernadett Kurz: conceptualization, formal analysis, investigation, methodology, validation, visualization, and writing – original draft. Ines Schreiver and Katharina Siewert: investigation, methodology, and writing – review and editing. Birgit Haslböck: conceptualization and writing – original draft. Katharina Weiß, Bianca Berner, Maria Isabel von Eichborn, Mark Berneburg, and Julia Hannemann: writing – review and editing. Wolfgang Bäumler: supervision, conceptualization, formal analysis, investigation, methodology, visualization, and writing – original draft.

Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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