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Tattoos – more than just colored skin? Searching for tattoo allergens

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Summary

During tattooing, a high amount of ink is injected into the skin. Tattoo inks contain numerous substances such as the coloring pigments, impurities, solvents, emulsifiers, and preservatives. Black amorphous carbon particles (carbon black), white titanium dioxide, azo or polycyclic pigments create all varieties of color shades in the visible spectrum. Some ingredients of tattoo inks might be hazardous and allergenic chemicals of unknown potential.

In Germany, about 20 % of the general population is tattooed and related adverse reactions are increasingly reported. Since tattoo needles inevitably harm the skin, microorganisms can enter the wound and may cause infections. Non-allergic inflammatory reactions (for example cutaneous granuloma and pseudolymphoma) as well as allergic reactions may emerge during or after wound healing. Especially with allergies occurring after weeks, months or years, it remains difficult to identify the specific ingredient(s) that trigger the reaction.

This review summarizes possible adverse effects related to tattooing with a focus on the development of tattoo-mediated allergies. To date, relevant allergens were only identified in rare cases. Here we present established methods and discuss current experimental approaches to identify culprit allergens in tattoo inks – via testing of the patient and *in vitro* approaches.

Decorative permanent tattoos – a widespread phenomenon today

For thousands of years people have tattooed their skin for various reasons. In contrast to some cultures like Polynesian tribes, the meaning of tattoos in the Western world seemed more vague over the past centuries and was mainly associated with low social status [1, 2].

Nowadays, tattooing has become very popular worldwide. Recent polls show that about 20 % of people in Germany and 29 % in the United States of America are tattooed [2–4]. The many tattooed role models including soccer, pop and movie stars have led to a broader cultural acceptance of tattooed skin. Tattoos are applied in special parlors or in private locations. They are black or multi-colored and located on almost all areas of the human body – including mucous membranes and eyeballs. A survey showed that about 60 % of tattoos are completely or partly black [2, 5].

In this review, the term tattoo refers to permanent tattoos also including so-called permanent make-up (PMU). Permanent make-up is usually made by beauticians to decorate especially the periorbital and perioral regions [1]. Henna tattoos are not part of this article as these are not injected into the human skin.

Tattoo inks may cause a variety of health problems, many particularly related to the skin but some also relevant to other organs (Figure 1). Adverse effects comprise delayed wound healing, infections, toxic or even mutagenic processes, as well as granulomatous and allergic reactions [6].

The fate of tattoo inks in the human body

Usually, tattoo inks are passively dragged into the dermis of the skin by solid needles with the help of tattoo machines. After tattooing, a part of the injected tattoo inks leaves the

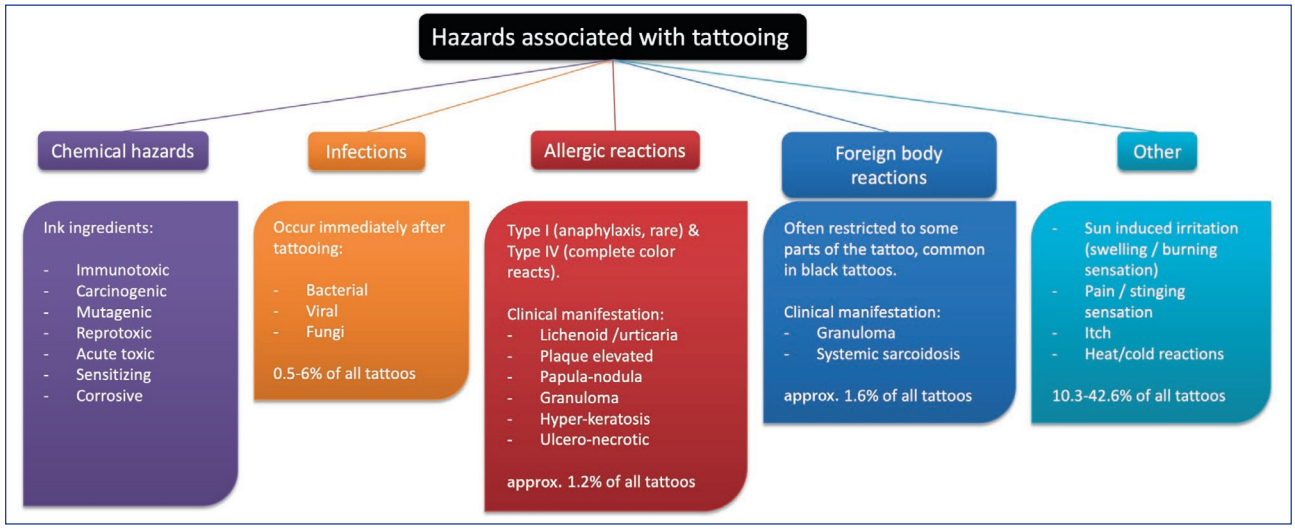

Figure 1 Possible adverse effects of tattoos. Effects may occur locally in the tattooed skin or spread systemically.

skin via the wounded surface. Pigment particles remain in the dermis and absorb light in a specific spectral range causing the color of the tattoo. Another fraction of the injected tattoo ink is removed from the skin passively via lymph or blood vessels or is actively transported by migrating cells. As a result, tattoo pigments are found in the local lymph nodes but are likely also transported to other organs such as liver, lungs or kidney [1, 2, 7, 8]. Experiments with mice suggest that pigment particles of tattoo suspensions are picked up by Kupffer cells inside the liver as indicated by the detection of particles via electron microscopy [9].

After skin healing, histological investigations showed that pigment particles are located in the cytoplasm of different cells, including fibroblasts and macrophages [1, 2, 10]. A recent investigation in mice proposed a pigment capture-release-recapture model. When tattoo pigment-laden macrophages die during the course of adult life, neighboring macrophages recapture the released pigments and seem to ensure the macroscopic stability and long-term persistence of tattoos [1, 11].

Experiments with pig and human skin revealed that the concentrations of red pigments, placed in the skin by tattoo machines, range from about 0.60 to 9.42 mg pigment per skin cm^2 [12]. In mice, about 30 % of intradermally injected red pigment disappeared from skin within six weeks after tattooing [2]. This percentage increased to 60 % when solar radiation was additionally applied to the animals' skin [13]. In general, the concentration of insoluble tattoo pigments in skin gradually decreases over the years [14]. The soluble parts of the ink will likely be excreted in the first days after tattooing.

Ingredients of tattoo inks and related chemical hazards

Tattoo inks are suspensions that may contain up to 100 different chemical compounds that may or may not be added intentionally. The coloring pigment is mixed with solvents, preservatives, and various other substances. Although they are injected into the human body, tattoo inks usually do not need to fulfil specific safety requirements in contrast to other substances that are inserted into the human body, such as medical drugs or implants. The exact list of ingredients, if known at all, depends on the practice of each manufacturer. Tattooists and beauticians purchase the tattoo inks directly from suppliers or through the internet [1].

Potential hazardous properties of chemicals in tattoo inks include carcinogenic, immunotoxic, and sensitizing properties (Figure 1). Tattoo inks containing hazardous chemicals are frequently found on the European market. The major substances of concern detected in analyzed samples are polycyclic aromatic hydrocarbons (PAH) (43 %), primary aromatic amines (PAA) (14 %), heavy metals (9 %) and preservatives (6 %) [1, 6].

Pigments

The chemical industry synthesizes pigment molecules which form tiny, solid state particles with diameters smaller than 100 nanometers (nanoparticles) up to a few micrometers [15]. Therefore, additional hazards may arise from nanotoxicological effects which should be investigated [16].

Tattoo pigments are frequently also called dyes, but the term "dyes" should be not be used since dyes are water-soluble and would not allow permanent skin decoration. The permanence of a tattoo in the skin is achieved by using insoluble pigments.

Pigments are usually sold as powder to the ink manufacturers. Wholesalers bypass a small amount of these industrial pigments as ready-to-use ink products directly to tattooists. To achieve the respective color, tattoo inks may contain different inorganic or organic pigments or a mixture of both.

Black pigments

Most black pigments are usually produced by imperfect combustion of hydrocarbons yielding soot – a mixture of black inorganic carbon particles that contain polycyclic aromatic hydrocarbon molecules (PAH). Carbon black is classified as possibly carcinogenic to humans (group 2B) by the *International Agency of Research in Cancer* (IARC) [2, 17]. This evaluation is based on the increased occurrence of lung cancer upon particle inhalation or the increased incidence of skin cancer from carbon black extracts in animal models. The latter leads to the assumption that especially impurities cause the observed carcinogenic properties. Up to 201 µg PAH per g ink were found in different commercially available black tattoo inks which is far more than the currently proposed limit of 0.5 µg PAH per g tattoo ink by the European Union [18]. PAH occur freely in the ink suspension or attached to the surface of carbon black particles [2]. PAH are metabolically activated to their respective diol-epoxides, which can bind DNA and lead to mutagenicity, resulting not only in carcinogenicity but also in effects on lymphocyte activation and macrophage differentiation [19–21].

White pigments

Titanium dioxide is an effective opacifier that is usually applied in white inks and for changing the color strength of other tattoo pigments [22]. Titanium dioxide occurs in rutile, anatase, and brookite crystal structures, but only the first two are being used as pigments. However, titanium dioxide, particularly as anatase, exhibits photocatalytic activity under ultraviolet irradiation generating reactive oxygen species, not radical. In 2010, the IARC classified titanium dioxide as group 2B carcinogen by inhalation meaning it is possibly carcinogenic to humans [1, 17]. The mechanism of carcinogenicity is thought be a so-called over-load effect when the concentration reaches a level where the particles can no longer be sufficiently cleared from the lungs. This effect is therefore unlikely to occur in other organs, such as skin.

Colored inorganic pigments

Most colored inorganic pigments are based on iron oxides in the colors yellow (FeO(OH)), red (Fe_2O_3), and black (Fe_3O_4). Since iron ore often contains heavy metals such as nickel, these display common impurities of iron oxide pigments. Nickel compounds are classified as carcinogens by the IARC [23].

Another group of pigments is based on heavy metals such as cadmium sulfide (CdS, yellow), mercury sulfide (HgS, red), chromium oxide (Cr_2O_3, green), or cobalt spinel ($CoAl_2O_4$, blue) [6]. The IARC classified these heavy metals as group 1 (carcinogenic to humans: chromium(VI), cadmium and cadmium compounds) or group 2B (organic-mercury compounds) carcinogens. The use of heavy metal-based pigments declined due to their hazardous nature.

In the European alert system for dangerous goods (RAPEX), 28 % of tattoo inks showed heavy metal contents above the threshold values defined in the *Council of Europe Resolution (CoE ResAP) (2008)1* on requirements and criteria for the safety of tattoos and permanent make-up [24]. Alerts were related in particular due to the presence of arsenic, barium, cadmium, chromium(VI), copper, lead, zinc, and nickel. In addition to toxic and mutagenic effects, some metals (nickel, mercury, chromium, and cobalt) might act as allergens eliciting cutaneous or systemic contact allergies [25]. Nickel, cobalt, and chromium are among the most common sensitizers with positive patch test reactions in about 18 %, 6 % and 3 % of patients in Europe, respectively [26].

Organic pigments

Nowadays, more than 80 % of the colored tattoo inks contain industrial organic pigments [1, 6, 22]. Organic pigments provide a huge variety of colors spanning the whole rainbow. Such pigments exhibit a strong light absorption yielding high color strength and brilliance in the skin which likely is the major reason for their application in tattoos. The polycyclic pigments are generally condensed aromatic or heterocyclic ring systems. Two important examples of heterocyclic pigments are phthalocyanine (green, blue) and quinacridone pigments (bluish red, pink, violet) [1, 22]. However, another group, the azo pigments, are still most commonly used. They span the yellow to red color range and are composed of condensed aromatic amines, which are often carcinogens or sensitizers. The synthesis of such pigments requires complex chemical processes in which also rosins, polymers, and surfactants might be added to adjust their size and dispersion properties. The resulting pigment may contain different educts, by-products, and other non-specified compounds. Due to their use during synthesis, PAA are the most important contaminants of organic pigments and their concentration is highest in inks containing azo pigments. Primary aromatic amines represent

either impurities of these inks (free aromatic amines) or degradation products after sunlight or laser irradiation [27]. Since some of these amines are classified as carcinogenic, mutagenic or reprotoxic substances they must neither be present nor released from azo pigments in tattoo and PMU products [1, 24].

Light exposed pigments and influence of laser treatment on tattoos

One important mechanism for the disintegration of particles in tattooed skin is the light induced decomposition of pigments molecules. It may continuously occur whenever tattooed skin is exposed to light sources emitting wavelengths which can be absorbed by the pigment molecules [27]. Laser treatment of tattoos is frequently performed by dermatologists in daily practice. When exposed to laser light during tattoo removal, an additional process may occur: the tattoo pigment particles may be fragmented to smaller pieces. Hitherto, the mechanisms of action have been assumed to involve heat production in the tattoo particle from absorbed light energy. Due to the high light intensity and short pulse durations applied, the heating of pigment particles is very rapid, yielding high local temperatures of up to several hundred degrees. The subsequent rapid thermal expansion causes fragmentation of the particles, accompanied by shock waves [28]. In addition, the heating and fragmentation of pigment particles may lead to cleavage of chemical bonds within the pigment molecule, producing new chemical compounds within the skin [1]. The release of hazardous decomposition products such as benzene and carcinogenic aromatic amines was reported by us and others after sunlight and/or laser irradiation [13, 29, 30].

Other ingredients

Tattooing pigment powder is almost impossible, so the pigment must be added to a fluid medium. However, pigment particles are insoluble in water and therefore require dispersion in aqueous or alcoholic solvents with the help of emulsifiers, binders (e.g. polyvinylpyrrolidone, polyethylene glycol) and thickening agents to avoid particle sedimentation [1, 6]. In addition, antifoam agents are added to avoid foam production while shaking the suspension (e.g. polydimethylsiloxane). The concentration of pigments in tattoo ink suspensions is usually between 10–30 % by volume [1].

A study from 2011 revealed that tattoo inks may contain substances like the sensitizer dibutyl phthalate, the genotoxin hexachloro-1,3-butadiene, or 9-fluorenone [31]. 9-Fluorenone is cytotoxic, generating reactive oxygen species upon light exposure [1].

In Switzerland, preservatives banned for use in cosmetics were found in up to 18 % of tattoo samples tested [32]. Among these banned substances were 1,2-benzisothiazol-3[2*H*]-one, 2-octyl-4-isothiazolin-3-one, phenol, and even the carcinogen formaldehyde [1]. Isothiazolinones are strong allergens and have a high rate of sensitization in the European population [26]. Other cases also indicate that shellac, a commonly used binder in tattoo inks, may provoke allergic reactions [33].

Furthermore, *N*-nitrosamines were found in the samples [32]. *N*-nitrosamines are impurities formed by the reaction of secondary amines with nitrite. Many *N*-nitrosamines are carcinogenic even in small concentrations which has been proven in animal testing [1, 7].

Recently, the deposition of metal debris from the tattoo needle in skin and local lymph nodes has been reported [34]. Since the tattoo needles contain high amounts of nickel and chromium, the metal debris may pose an additional risk for sensitization or allergy formation.

Adverse skin reactions to tattoos

Starting with the placement of a tattoo, a range of adverse reactions can affect human health (Figure 1). Adverse reactions may be localized in the skin or involve other organs of the body and can develop within a wide time range from immediately after tattooing to years or even decades later.

Prolonged wound healing

Tattooing leaves the skin littered with numerous holes reaching through the epidermis roughly into the mid of the dermis. The depth of the holes varies depending on the technique of the tattoo artist and the equipment used. The width of a hole depends on the tattoo needles, which frequently show diameters of 0.2 to 0.4 mm and which are assorted in bundles of up to 50 single needles. After skin injury, wound healing starts immediately to restore skin integrity.

The process of normal wound healing is divided into different phases [35]. After hemostasis, the immediate inflammatory phase lasts up to three days. Neutrophils are the first immune cells that infiltrate wounded tissues, arriving in large numbers in response to damage-associated molecular patterns (DAMPs) released from injured and necrotic cells. The neutrophils release different cytokines, chemokines, and growth factors that signal through wound-associated immune cells and epithelial cells to promote healing [36]. Neutrophils prevent infection by eradicating microbes that entered the skin.

Subsequent recruitment of circulating monocytes into sites of tissue damage occurs. Monocyte-derived macrophages or dendritic cells manage tissue repair, regulate angiogenesis, clear cellular debris, and recruit additional immune cells [36]. This late inflammation phase lasts up to ten days with

macrophages contributing to wound repair and tissue remodeling by clearing apoptotic neutrophils [37]. Such a milieu could be affected in the presence of tattoo inks, leading to an altered immune response in tattooed skin areas and a changed process of wound healing.

Skin also contains tissue-resident macrophages which are non-migratory and respond to injury or infection by sensing DAMPs [36]. Langerhans cells of the epidermis and other antigen presenting cell types may internalize antigens from the ink and migrate to lymph nodes in which these are then presented to T cells.

At last, re-epithelialization is an important step because it restores the barrier function of the skin [36]. When surgical wounds of specific depths were created in pig skin, re-epithelialization started from residual appendage structures [38]. After recovery of the skin barrier, the remodeling phase for rebuilding normal skin structures may last several months.

In conclusion, the wound healing of skin is an effective but complex process that is orchestrated by immune cells whose functions critically depend on the microenvironment in the wound. In contrast to medical procedures such as use of injection needles, microneedling, or ablative laser treatments, small tattoo wounds are filled with various cytotoxic chemicals from the ink. Unfortunately, there is a lack of studies on whether and to which extent tattoo inks might hamper normal wound healing after tattooing. Itching, crusts, and delayed wound healing are frequently reported in surveys, indicating that irritants, sensitizers or even microorganisms may disturb this process [39].

Infections

Tattooing produces numerous holes in skin facilitating the entry of microbial pathogens. Consequently, mild-to-moderate superficial skin infections may occur that should be easily treatable. Pathogens dumped into deeper skin layers can cause more severe infections requiring a more extensive medical treatment. Should pathogens enter blood vessels, systemic infections may result [40]. The severity of an infection depends on the virulence of the pathogen, the immune status of the person being tattooed, and underlying diseases [41].

A recent review summarized 67 cases of local skin infections and systemic complications with bacteria such as *Corynebacterium diphtheriae*, *Pseudomonas aeruginosa*, or *Staphylococcus aureus*, that were published between 1984 and 2015 [41]. Another review searched the literature from 1991 to 2011 and listed 13 publications reporting viral infections including vulgar warts or hepatitis C as well as 25 publications reporting bacterial infections with, for example, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, nontuberculous mycobacteria, or community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) [42]. In 2017, a case of death after septic shock was reported that was caused by a *Vibrio vulnificus* infection after bathing in seawater with a fresh tattoo [43].

Sources for microbial pathogens include the own skin surface of tattooed individuals, hands and equipment of the tattooists, and an unhygienic handling of the skin after tattooing. Another source are tattoo inks of which up to 11 % are contaminated with microorganisms [6]. Hence, even if a tattoo reaction only occurs in one color and thus points to an allergy, microbial infections should always be ruled out.

Non-allergic inflammatory reactions

From time to time, tattoos are associated with the development of long-term local immune responses manifesting as granulomatous and pseudolymphomatous reactions with the majority being foreign-body granulomas (Figures 1, 2a) [44]. However, granulomatous lesions in a tattoo can be a

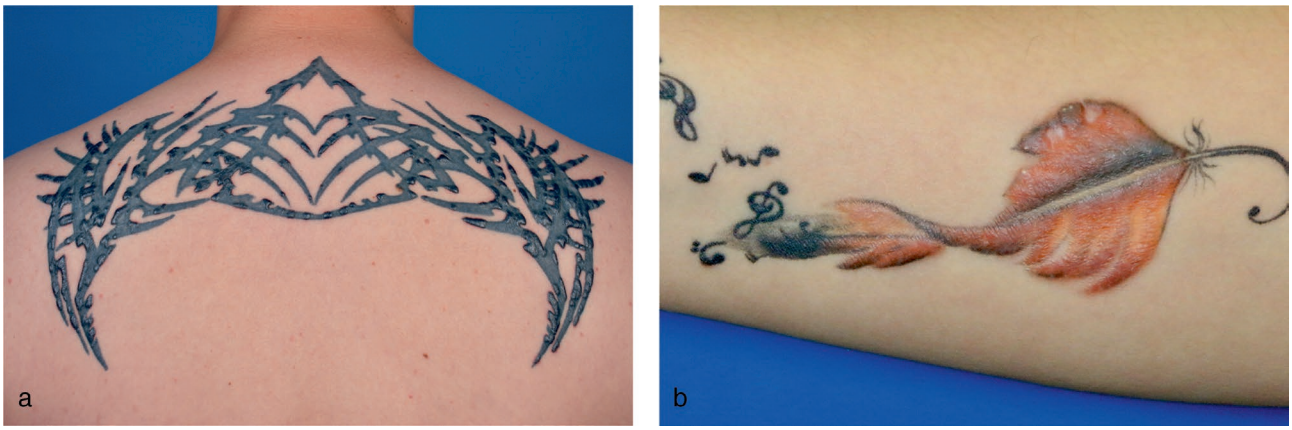

Figure 2 Common adverse tattoo reactions. Granulomatous reaction in a black tattoo (a). Type IV hypersensitivity tattoo reaction in the red colored part of a tattoo presenting as a plaque-like reaction (b).

manifestation of systemic sarcoidosis affecting the lungs and eyes (uveitis) [44–46].

Cutaneous pseudolymphoma represents a variable group of benign reactive T or B cell lymphoproliferative processes simulating cutaneous lymphoma both clinically and histologically. The pathogenesis of this disease is still unknown, but since 1903 tattoos have been reported to be associated with these reactions [44, 47].

Furthermore, tattooing can trigger certain dermatoses on the tattooed skin (isomorphic phenomenon of Koebner), including psoriasis, lichen planus, cutaneous lupus erythematous, and pyoderma gangraenosum [48, 49].

Allergic reactions

While their diagnosis remains difficult, allergic reactions to tattoos have been reported with varying degrees of diagnostic evidence.

Antibody-mediated tattoo reactions

Only few studies suggest a role for antibodies in chemical-induced hypersensitivity reactions [50]. Regarding tattoos, rare cases that likely involve antibody-mediated reactions have been reported [51–53]. A first case in the recent medical literature describes a 30-year-old woman with a color tattoo made in 1993 and a black tattoo made in 1999 [51]. One month after the last tattoo, additional color was added and twelve hours later she began to experience a "burning" sensation, which evolved into hives covering multiple areas of her body. The patient was subsequently skin prick tested to 13 different ink colors and was positive to two of them, a purple and a blue ink, indicative of an IgE-mediated hypersensitivity [44].

A second patient without any known allergies experienced anaphylaxis six hours after application of a second tattoo, which began with swelling and redness on multiple body sites [52]. A third case of delayed anaphylaxis occurred after laser treatment for tattoo removal [53]. This patient had an allergic cutaneous reaction at a distant, untreated tattoo after the first laser treatment followed by an anaphylactic episode three days after the second laser treatment.

Type IV hypersensitivity reactions

Allergic reactions to tattoos are mainly thought to be mediated by T cells, classified as delayed type IV hypersensitivity reactions according to Gell's and Coombs' historic classification. With their T-cell receptor (TCR), T cells likely recognize epitopes of chemically modified self-antigens formed in a process called haptenization. Haptenization has been especially described in the context of topical chemical exposure triggering allergic contact dermatitis (ACD) or drug exposure (drug hypersensitivity) [54, 55]. Both $CD4^+$ and $CD8^+$ T cells seem to be involved [56, 57].

The development of a type IV hypersensitivity reaction is usually separated into a sensitization phase and an elicitation phase. During the sensitization phase, dendritic cells migrate to draining lymph nodes and prime specific naïve T cells that proliferate and differentiate into circulatory and tissue-resident effector and memory populations [58, 59]. Dendritic cell migration and maturation may be triggered by the chemical allergen itself (sterile inflammation) or by concomitant heterologous immune stimulation [55]. During the elicitation phase, T cells initiate and orchestrate the inflammatory reaction involving various effector molecules, immune and other cells [59–61].

Typically, ACD manifests within approximately 1–3 days after topical chemical contact and ceases after removal of the culprit allergen. In tattoos, however, the potential allergen may be permanently present which can pose serious problems, for instance by inducing chronicity of the inflammation. The timing with which delayed-type hypersensitivity reactions occur may vary from shortly after tattoo application up to several years later [2]. Partly, this depends on whether the individual is already sensitized to a specific compound. Additionally, some allergens require metabolism, degradation or the presence additional (heterologous) immune stimulation to initiate sensitization.

The clinical patterns normally associated with allergic reactions are papulonodular, plaque-like, lichenoid, hyperkeratotic or ulceronecrotic (Figure 2b). The clinical picture also includes contact urticaria-like reactions or photo-allergic reactions [62]. Concomitant reactions in older, red-colored tattoos may be triggered.

Usually, allergic reactions occur locally with involvement of the entire tattooed area with the triggering color, but some authors describe cases of generalized rashes or eczemas, especially in previously sensitized patients. These reactions appear early (within 1–2 days after tattooing) and tend to resolve without treatment after a few weeks or months, indicating that soluble tattoo ink components may be involved. Other authors describe cases of generalized reactions upon attempting to eliminate the pigment with laser treatment and cases of photo-allergic reactions in tattoos with yellow ink containing cadmium [63, 64]. Furthermore, allergic reactions without generalized rashes can be initialized by laser treatment, and putatively by sun exposure, due to the release of sensitizers [65–67]. This so-called photoallergy should not be confused with phototoxicity which involves transient reactions during exposure of the tattoo to sunlight [68].

In some rare cases, putative allergic reactions occurring in a tattoo may also be triggered by implant materials [69, 70]. These reactions resolve with the removal of the implant.

The majority of chronic tattoo reactions are associated with red or reddish colors, for example pink, orange, violet and bordeaux [39, 42, 71]. Azo pigments were the most frequent pigments identified in skin biopsies of tattoo allergy patients with reddish colors [72].

Differential diagnosis of a type IV hypersensitivity toward a tattoo should always consider other late reactions such as granulomatous foreign body reactions, systemic diseases (for example sarcoidosis or connective tissue disease), microbial infections, and pseudo-lymphomatoid reactions.

Tattoos, medication, and (auto)immune reactions

Adverse reactions at the site of a tattoo can also occur after systemic drug treatment for different unrelated indications. Swelling, granuloma formation and pain in tattoos have been reported after treatment with BRAF and MEK inhibitors such as dabrafenib or trametinib for malignant melanomas occurring outside of the tattooed area [73]. These inhibitors prevent excessive cell growth of tumor cells but may also stimulate autoimmunity. Additionally, granulomatous reactions in the red part of a tattoo with additional symptoms in the eyes have been reported after influenza vaccination containing thimerosal, which is a mercury-containing preservative [74]. The authors suspected an allergy elicitation by thimerosal, which induced a general inflammatory response with subsequent lung sarcoidosis and hypersensitivity to the red, organic tattoo pigment. Granuloma formation from vaccines containing thimerosal have also been reported in non-tattooed persons.

Regarding the treatment with allopurinol, contradictory findings have been published. A 34-year-old patient with multiple morbidities developed systemic eosinophilia with a prominent eruption in a tattoo after the addition of allopurinol to the list of medication. Allopurinol is known to be a cause of this drug-induced hypersensitivity syndrome. On the other hand, allopurinol is a treatment option for general and tattoo-associated granuloma and sarcoidosis – putatively by either acting as radical scavenger or by preventing the formation of foreign-body giant cells [75].

In general, drugs or diseases that affect the immune system may also trigger reactions in tattoos. For example, people

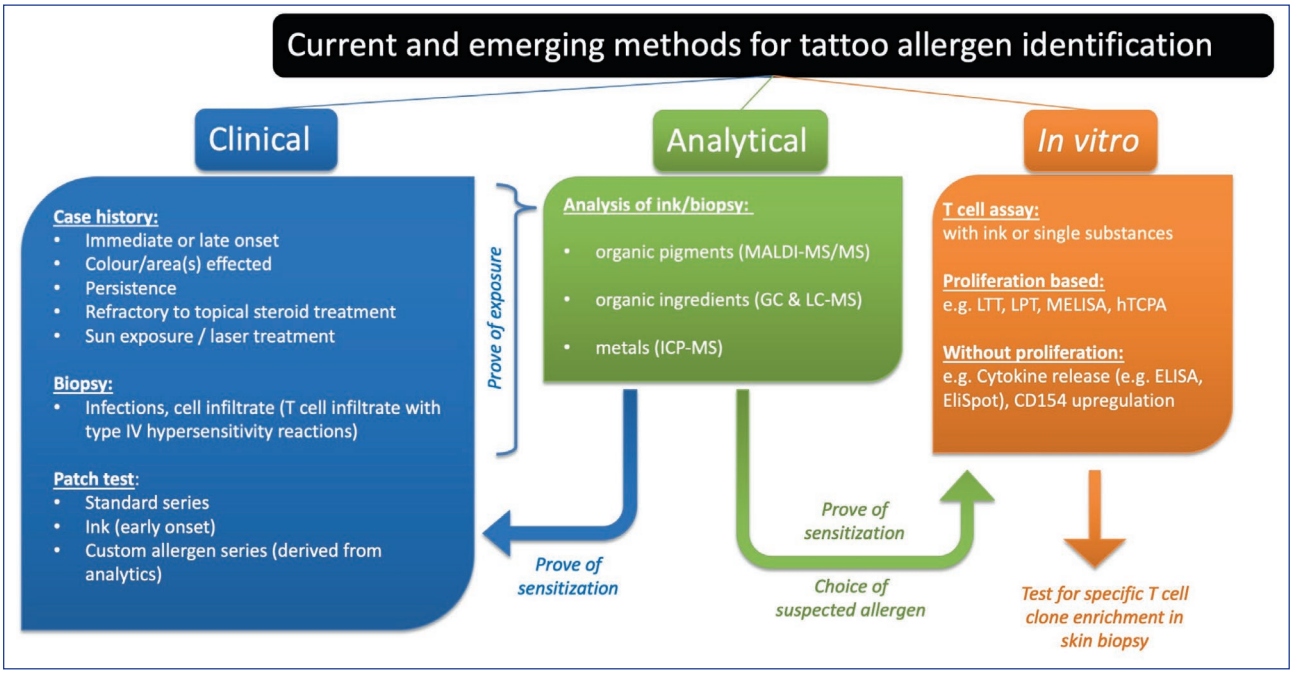

Figure 3 Potential strategy for the diagnosis of tattoo-related allergies. The clinical case history may provide information on the characteristics of the suspected allergen, for example, an association with light exposure, pigments, or soluble ink ingredients. Combined with chemical analysis, the presence of an allergen in the biopsy or ink and a positive patch test may provide a hint on the identity of the allergen. Still, the patient might be coincidentally sensitized to the substance of the positive patch test while another allergen might be causing the reaction in the skin. *In vitro* methods may show pathogenic T cell populations in the patient compared to non-allergic controls. Finding 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/ink. With the aid of high-throughput sequencing technologies, this would deliver proof that the skin reaction is indeed caused by the suspected substance.

infected with HIV may develop a type IV hypersensitivity reaction in tattoos after starting anti-retroviral therapy [76].

Diagnostic tools for allergic tattoo reactions, associated challenges, and new approaches

Diagnosing allergic reactions to tattoo inks is very challenging since diagnostic tools and knowledge about culprit allergens, not to speak of relevant epitopes recognized by the involved TCR, is very limited. Current diagnostic tests and outlines for ongoing experimental research approaches for the identification of tattoo allergens are presented in Figure 3.

Patch testing

Patch testing is the diagnostic gold standard for ACD. It is not clear whether this test is useful for diagnosing delayed-type hypersensitivity reactions provoked by allergens such as tattoo ingredients that are inoculated into the dermis. Theoretically, an intradermal test would be a more specific diagnostic tool for these reactions since it would more closely resemble the *in vivo* situation, but it is not free of risk. The applied pigments may permanently remain in the dermis after the test and chronic reactions could be triggered. As a consequence, performing these tests is considered neither appropriate nor ethical [77].

In order to investigate contact allergies to tattoo pigments, a new test block was recently introduced by the *Deutsche Kontaktallergiegruppe* (block 47). This block contains 24 substances, e.g. o-phenylphenol, iron(II) sulfate and shellac [78]. As only few studies reflect the utility of patch testing in this situation, it is not completely clear which allergens should be included in specific patch tests. Many published articles only report single clinical cases with positive patch test results and very few combine it with adequate clinical and analytical correlation.

In 2014, Serup et al. performed a patch test study of 90 patients with tattoo reactions [71]. The general outcome of the study showed that patients with reactions in their tattoos presented mainly negative or inconsistent results when patch tested with common allergens, textile dyes, problematic tattoo inks, and even individual culprit inks, if available [71]. However, if the ink was obtained weeks or months after tattooing, there is a fair chance that the product lot or composition had changed. Negative patch test results also indicate that most allergens causing tattoo reactions are formed inside the dermis, which can be slow and may require weeks, months, or years [71]. In a recent study, 37 % of allergic reactions occurred during the first month after tattooing leaving a majority of 63 % to late reactions [72]. This indicates that at least some allergens may already be present in the ink. For tattoo hypersensitivity reactions with a late onset, external factors, especially light inducing photochemical cleavage of tattoo pigments *in situ* in the skin, may contribute to allergen formation from pre-haptens or pro-haptens [27, 68, 71, 79].

The downside of patch testing is that it can only provide a correlation between sensitization and a substance the patient had contact with, for instance, nickel sensitization correlating with nickel in a tattoo ink. Patch tests are accompanied with the uncertainty that other substances that were also present in the tattoo ink may have been the main reason for the allergic reaction. They are therefore no incontrovertible proof for a causal finding.

Skin biopsy

Combining clinical aspects and histological findings from skin biopsies can help clinicians to exclude serious entities such as cutaneous infections by atypical microorganisms, systemic diseases, or lymphomatous tumor infiltrations [49]. Additionally, biopsies can prove that mononuclear cell infiltrates, for example T cells, persist in a pattern consistent with those observed in ACD. However, such histologic patterns are not definite and may also represent, for instance, irritant contact dermatitis. In 2015, Høgsberg et al. investigated 19 biopsies from chronic tattoo reactions to red pigment or mixtures or nuances of red [80]. The predominant histological pattern was interface dermatitis with increased counts of T cells and Langerhans cells which fits with delayed-type hypersensitivity reactions.

Biopsy and ink analysis

The diagnosis of allergens is particularly difficult regarding tattoo inks. As stated above, tattoo inks are complex mixtures of insoluble pigments with solvents, thickening agents, surfactants, preservatives, and other impurities or additives. In temporary and early-onset allergic reactions, soluble compounds in the tattoo ink may play a role. In these cases, the ink of the patient might give positive results in a patch test [65]. With later tattoo reactions – sometimes occurring years after tattooing – only substances still residing in the skin can trigger the allergy. In terms of pigment-derived allergens, the suspects are most likely metals, organic impurities, or decomposition products of organic pigments as well as metabolites thereof. To affirm an allergen, it is necessary to prove that the patient was exposed to this substance and is being sensitized against it. This can be done by analyzing a skin biopsy from the tattoo or the used ink. The latter is quite unreliable, especially with late onset allergies occurring months or years after tattooing with the original ink not being traceable or having an altered composition in the meantime. Past investigations

identified the metals chromium, mercury, cobalt, and nickel as likely causes of allergic tattoo reactions correlating a patch test with chemical analysis of skin biopsies [81–84]. These metals can be frequently found in inorganic pigments. The analysis of metals in skin biopsies or ink is comparably easy and standard patch test preparations exist.

With organic pigments, many structural variations can be created by chemical synthesis and might also be in use for tattooing. To date, about 104 organic pigments are known to be used in tattoo inks [85]. Without knowing the exact identity of the pigment embedded in a patient's skin, diagnostics of the underlying allergen compares to searching for a needle in a haystack.

Pigment analytics from biological tissue or inks has so far been carried out with high-performance liquid chromatography (HPLC) techniques [86] or matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) [87, 88]. HPLC methods are very much limited by the solubility of the pigments but can provide quantitative data. Only a limited amount of pigments is soluble in standard organic solvents used for HPLC analysis. MALDI-MS analysis is easily applicable to a wide range of pigments but detection limits are in the range of percentages and vary for each pigment. MALDI was proven suitable for the detection of visible amounts of pigments – such as those present in tattooed skin – in a study analyzing the pigments in 104 skin biopsies of allergic tattoo patients [72]. Here, the inks collected from the tattoo artists did not always match the pigments found in the skin of the patients.

Although the pigments suspected to cause tattoo allergies can be narrowed down by chemical analysis, possible descendent sensitizing decomposition products of these pigments as well as by-products or polymers used during their synthesis may also play a role in allergy development. For most of these substances, no standard patch test formulations exist to prove that the patient is sensitized against it. If the pigment in the patient's skin is known beforehand, a specific set of substances may be tested (Figure 3).

Detection of allergen-specific T cell responses in vitro

T cell responses to suspected tattoo allergens may be analyzed *in vitro* similar to other chemical sensitizers triggering ACD. For the development of such tests, several challenges have to be overcome. Firstly, relevant allergen-induced antigens need to be formed which are recognized by specific T cells.

Generally, knowledge on how chemicals induce T cell responses is still extremely limited [54, 55, 89]. Parkinson et al. showed for a few sensitizers that high and low abundant proteins may become modified [90] while their respective importance for T cell epitope formation remains unknown. Likely, T cells recognize allergen-modified peptides presented by major histocompatibility complex (MHC) molecules of antigen-presenting cells besides other possible mechanisms [91, 92]. This has been illustrated for the experimental model allergen 2,4,6-trinitrobenzenesulfonic acid (TNBS). This model antigen was recognized by $CD8^+$ T cells mainly if attached to a lysine at position 4 of an MHC-presented peptide in a murine contact hypersensitivity model [93]. Alternatively, chemicals may bind non-covalently to the TCR-peptide-MHC interface, termed "pharmacological interaction" in case of drugs, which sometimes occurs in a HLA allele-restricted manner [54, 94].

The importance of the different possible epitopes in the allergic immune response remains unknown and the formation of the correct epitopes can hardly be controlled by *in vitro* assays. As a pragmatic solution, antigen presenting cells and T cells from blood are incubated with chemically modified proteins or peptides. Alternatively, chemicals are added directly into the medium of the coculture [93, 95]. Since TCRs are both highly specific but also cross-reactive [96], T cell *in vitro* tests may work even if epitopes formed with skin peptides are missing.

The detection of rare activated T cells in a plethora of irrelevant bystander cells is difficult. Every individual has over 100 million mainly unique T cell clonotypes with only few being specific for a given epitope [97]. To detect rare allergen-specific T cells in blood, most labs use proliferation-based methods, for instance the lymphocyte proliferation or transformation tests LPT/LTT, the memory lymphocyte immunostimulation assays MELISA, or the human T cell priming assays hTCPA [57, 98]. To facilitate proliferation, cytokine skewing cocktails may be added or regulatory T cells may be depleted [99, 100]. Non-proliferation-based methods usually detect cytokines, for instance via enzyme-linked immunosorbent or spot assays (ELISA and ELISpot). Except for priming assays, these approaches only detect memory T cells that proliferate or express the respective cytokines. Naïve T cells and less proliferative or non-cytokine-producing memory cells are not detected, an issue that may introduce bias.

Recently, a new method providing fast and comprehensive access to antigen-specific naïve and memory $CD4^+$ T cells has been published, which is based on induced CD154 (CD40L) expression a few hours after *in vitro* stimulation [101, 102]. We and others started to adopt this method to detect, for example, nickel-specific or β-lactam-antibiotic-specific T cells [103, 104]. With those T cell assays, increased percentages of specific activated T cells after treatment with chemicals can be detected.

For a test to be diagnostic, the analysis of non-allergic control individuals is mandatory. For example, in case of *p*-phenylenediamine (PPD)-induced allergy, T cell proliferation was found increased in allergic individuals [95].

In contrast, the oxidation product of PPD, the so-called Bandrowski's base, induced proliferation in allergic and non-allergic individuals and is thus not suitable for allergy detection [95]. In case of nickel, activation of many $CD4^+$ T cells has been observed already in non-allergic donors, which we found to be due to the activation of T cells carrying a TCR with a TRAV9-2 gene segment or a CDR3 histidine [57, 104]. This activation was still topped by donors with acute nickel ACD and may therefore be used to identify some allergic patients as an alternative to patch tests in the future. Additionally, advances in high-throughput sequencing technologies may be further exploited to link antigen-specific T cell clonotypes identified in blood to those in inflamed skin [58]. An overlap of T cells from the blood reactive to the antigen with a local enrichment of these cells in the inflamed skin can causally link suspected tattoo allergens with allergic symptoms, which is not possible with patch tests.

Results from previous analytics and patch tests may help in selection of suspected chemical allergens for *in vitro* assays and sequencing approaches to achieve a causal proof for the allergen in the skin (Figure 3).

Treatment of cutaneous reactions to tattoo inks

Clinical complications toward tattoos can be managed with different strategies [44, 105]. Treatment measures may span from conservative to invasive procedures and depend on the severity and location of the lesions. A conservative treatment may include topical, oral, and/or intralesional steroids, oral anti-histamines, and protection of the tattoo from sunlight [49, 71].

Invasive methods may permanently damage the skin by scar formation. They include cryotherapy, electrosurgery, surgical excision, dermabrasion, chemical destruction via acid, or ablation with a non-Q-switched carbon dioxide laser. Q-switched laser therapy is not indicated when tattoos show signs of an allergic reaction, because the therapy itself can initiate or worsen hypersensitivity reactions. Probably, these reactions originate from the laser, which destroys the pigment-containing cells, resulting in the pigment becoming extracellular [44, 71]. The immune system may recognize allergen-induced epitopes after hapten formation from the released pigments or its breakdown products [106].

Conclusions and recommendations

Depending on its size, several grams of tattoo ink consisting of various chemical compounds are injected into the skin while applying a tattoo. Causes of the systemic effects of tattoo inks mainly remain unclear to date. Furthermore, many presentations of cutaneous reactions to tattoo inks, including allergic reactions, have been reported in the literature. It is very important for physicians to be aware of these reactions and of the possibility of eliciting further reactions through treatment with lasers or other modalities. We highly recommend pharmacological, toxicological, and epidemiological studies to clarify the possible impact of tattooing on human health.

To shed light on the matter, a collaboration between the *German Federal Institute for Risk Assessment* (BfR) in Berlin and the *Department of Dermatology of the University of Regensburg* was established. Our team develops new methods for the detection of hypersensitivity reactions in tattoos assisted by chemical analysis of tattoo allergens. Our goal is to improve diagnostics in cutaneous tattoo reactions and to enable risk assessment of potential allergens in tattoo inks that should be banned from the market. Data on patients with tattoo allergies are collected at the Department of Dermatology, University Hospital Regensburg, for subsequent analysis of the pigments in the skin and identification of the allergen via custom patch tests and potentially, by T cell assays.

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