

Diagnostic approach in allergic and irritant contact dermatitis

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Contact dermatitis is a highly frequent disease with a significant impact on the quality of life of the affected patients and a relevant socioeconomic impact. According to the pathophysiological mechanisms involved, two major types of contact dermatitis may be recognized: irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD). The two types may, and often do, coexist. Differentiating between ICD and ACD is often difficult in the clinical setting. The basis for a diagnosis of either ICD or ACD is mainly established by a comprehensive clinical history and physical examination, as well as by performing appropriate diagnostic patch testing. The only useful and reliable method for the diagnosis of ACD remains the patch test. Positive patch test results, the current and/or past relevance of which has to be assessed, are confirmative of contact sensitization. Additional tests, such as the repeated open application test or the provocative use test, are sometimes necessary to confirm a causal relationship. This algorithmic diagnostic approach will allow the adoption of rational measures of allergen or irritant avoidance and the implementation of realistic patient information and education.

KEYWORDS: allergens • allergic contact dermatitis • contact dermatitis • irritant contact dermatitis • irritants
• patch testing • skin testing

As the main interface with the environment, the skin is placed in the hazardous position of routine exposure and assault from exogenous chemicals and physical agents. Fortunately, most of these exposures result in no clinically apparent disease. However, in some circumstances, an exposure results in a cascade of pathogenic events leading to ongoing inflammation and clinical contact dermatitis (CD). CD is highly prevalent, representing more than 90% of occupational skin disorders, giving good reason for its relevant socioeconomic impact [1]. In addition, it usually adopts a chronic and refractory clinical evolution, determining a considerable degree of physical handicap and compromise in the quality of life of the affected subjects. According to the pathophysiological mechanisms involved, two major subtypes of CD may be recognized: irritant CD (ICD) and allergic CD (ACD). Differential diagnosis between ICD and ACD may cause considerable problems in clinical practice. The two types may, and often do, coexist, thereby further complicating matters. This represents a considerable predicament, in view of the high frequency of these entities and their impact on the patient's quality of life. Making a correct diagnosis and identifying the causative agent(s) is of the utmost

importance for the institution of appropriate therapeutic and preventive measures. The identification and avoidance of triggers will help to avert the distress and suffering of CD.

Pathomechanism of ICD & ACD

Irritant CD is the clinical result of direct inflammation arising from the release of proinflammatory cytokines from skin cells (principally keratinocytes) in response to chemical (in most cases) or physical harmful stimuli [2]. Depending on the type of irritant contacted, there is a broad range of disruptive effects on the epidermis. The three main pathophysiological changes of irritant dermatitis are: skin barrier disruption, cellular epidermal change and mediator release, all of which are interconnected [3]. Disruption of the epidermal barrier function results in the release of proinflammatory cytokines from damaged cornified cells and from viable keratinocytes that become activated [4,5]. Skin irritants are able to activate the skin's innate immunity independently of the antigen presentation pathway, by the induction of proinflammatory mediators that directly recruit and activate T lymphocytes, without the induction of antigen-specific memory T cells [6]. Most of the cytokines/factors and cell adhesion molecules previously associated

with ACD, such as ICAM-1, lymphocyte function-associated antigen (LFA)-1, IL-1 α , IL-1 β , TNF- α , granulocyte macrophage-colony-stimulating factor (GM-CSF) and IFN- γ , have been found in the epidermis and dermis in irritant reactions [3]. By contrast, ACD is the clinical result of a delayed contact hypersensitivity (CHS) reaction elicited by immunogenic substances (allergens); hence, the inflammatory response is orchestrated by clonally expanded allergen-primed memory T lymphocytes. The current paradigm of delayed contact sensitivity follows a two-step mechanism, comprising a sensitization and an elicitation phase. In the sensitization phase, low-molecular-weight chemicals in contact with the skin (haptens) will activate the skin's innate immunity and induce an inflammatory response, recruiting and activating leukocytes and dendritic cells (DCs). The small size of these chemicals allows penetration through a skin barrier that is otherwise impermeable to large molecules under physiological conditions. The haptens or conjugated hapten-peptide complexes are internalized through pinocytosis or receptor-mediated endocytosis and processed by DCs, which upregulate the expression of surface molecules such as MHC molecules and costimulatory factors including IL-1 and TNF- α . Concomitantly, DCs migrate to the draining lymph nodes, where they present the haptenated peptides together with the MHC class I and II molecules to specific MHC class I-restricted CD8 $^{+}$ and MHC class II-restricted CD4 $^{+}$ T cells. These cells will have effector and regulatory functions on the delayed hypersensitivity reaction, respectively, and the development of a skin inflammatory reaction against specific allergens will probably depend on the balance between the effects of effector and regulatory/suppressor T cells. **There is new evidence** that innate immune lymphocytes, such as invariant natural killer T cells and possibly natural killer cells, may also play an important role [6,7].

Recent studies have demonstrated that allergen sensitization induces the development of distinct CD8 $^{+}$ T-cell subpopulations that produce IFN- γ (Tc1) or IL-17 (T_{IL-17}). It is currently assumed that both IFN- γ and IL-17 are required for optimal CHS responses [8]. CD4 $^{+}$ cells, by contrast, produce the Th2 cytokines IL-4 and IL-10 [9]. T-cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: IFN- γ -producing (Tc1) effector CD8 $^{+}$ T cells and IL-4/IL-10-producing (Th2) regulatory CD4 $^{+}$ T cells. Allergen-specific, clonally expanded T cells leave the lymph nodes and circulate in the blood, secondary lymphatic organs and tissues, including the skin. By expressing skin-homing factors, such as cutaneous lymphocyte-associated antigen or CCR4, they can preferentially recirculate into the skin. In the elicitation phase, following subsequent contact with the same chemical, allergen-specific T cells already present on the skin will orchestrate an inflammatory response, which is the basis of the clinical dermatitis. Therefore, the mechanisms at the origin of the clinical lesions are dissimilar in the two types of dermatitis, at least in which concerns the early stages of the inflammatory response. ICD follows the activation of innate immunity, while in ACD both the innate and acquired immunity are activated and, as a result, antigen-specific effector T cells will drive the inflammatory response.

The later stages giving rise to an eczema lesion may, on the other hand, be very similar in ACD and ICD. In practice, irritancy and allergy, similar to innate and acquired immunity, are almost always associated and closely linked. Both types of dermatitis share the same effector pathways and involve the same cytokines, chemokines, apoptosis phenomena and cellular necrosis, as well as the recruitment of a polymorphic inflammatory infiltrate [10–19]. In addition, most strong allergens also have irritant properties, frequently disregarded because their allergenic potential dominates their toxicity profile [20,21]. Irritancy is believed to play a crucial role in the development of ACD. Skin cell damage and cytokine release induced by irritants may represent a 'danger signal' for the immune system, activating antigen-presenting DCs and recruiting DC precursors, which are blood monocytes, into the skin, thus predisposing to contact sensitization [22–25]. In the absence of activation of innate immunity, the maturation of skin DCs is incomplete and there is no appropriate activation of pro-inflammatory effector T cells. Conversely, immature DCs are capable of activating anti-inflammatory regulatory T cells. This knowledge can be applied directly to clinical practice: the early recognition and opportune treatment of ICD will contribute to preventing the development of ACD.

Clinical diagnosis of ICD & ACD

Differentiating between ICD and ACD is often difficult in the clinical setting, thus misleading the physician with respect to cause and preventative strategy.

The diagnosis of CD depends on patient history, clinical examination, exposure assessment (including hazard identification, estimation of dermal exposure and risk characterization), analysis of all predisposing and contributory factors, and comprehensive diagnostic testing [26]. Even though a preliminary working diagnosis of CD may often be made after a thorough clinical assessment, deciding whether the dermatitis primarily depends on irritancy or allergy is not always straightforward. No pathognomonic clinical signs and symptoms can unambiguously discriminate between ACD and ICD.

Clinical history

The first step in the diagnosis of CD is a comprehensive and standardized anamnesis that covers the clinical evolution of the dermatitis and all possible etiological factors (Box 1) [26].

Assessment of exposure

Clinical history should investigate all possible sources of allergenic or irritant exposure that the subject is exposed to. This includes a meticulous consideration of the working activities and occupational milieu. It is important to identify the nature of the patient's current and previous jobs, exposure to irritant or allergenic products, and physical environmental factors, such as temperature, humidity, airflow or exposure to ultraviolet light. Material safety data sheets can help identify materials and their side effects. If occupation appears causative, an occupational history must be obtained and a workplace visit may be required. We should also scrutinize nonoccupational sources,

including hobbies and leisure activities, and domestic exposures, such as personal skin care products and fragrances, clothing and accessories, plants, and previous and current treatments. In addition, potential cross-sensitizers the patient may have come in contact with previously, have to be carefully considered and assessed. The assessment of exposure includes the determination of the intrinsic allergenic or irritant potential of the suspected agent(s) together with relevant quantitative data, including the dose, frequency and duration of exposure, and the extent of the exposed skin surface area. In addition, we have to consider simultaneous exposure to several allergens and/or irritants and concomitant exposure factors, such as occlusion, friction, trauma and heat, among others, which might enhance the percutaneous penetration, as well as the existence of inflamed or otherwise damaged skin. Contact sensitization or irritation may result from a single exposure to a strong allergen or irritant, respectively. However, in most cases, several exposures are required for sensitization or irritation to develop. This is the case for cumulative ICD, where multiple sub-threshold skin insults due to multiple weak irritants may surpass the healing capacity of the skin, resulting in clinical dermatitis. In addition, exposure to chemical and mechanical irritation may lead to increased percutaneous penetration of contact allergens. Therefore, the clinical scenario will often reveal that an ACD is preceded by an ICD.

Sometimes, even when a comprehensive history has been taken, the source of exposure remains concealed. Patients often fail to recognize a causal agent when exposure is infrequent or sporadic. Some sources of exposure remain hidden due to insufficient chemical identification in many household or industrial products [26]. In addition, we should be aware that there are different possibilities of exposure to consider, including: intentional application to the skin; contact with allergen-contaminated items; transfer of an allergen with the hands to the face, neck or other sites, which produces 'ectopic' CD; exposure to a product that is used or has come in contact with a partner or another person who is close to the patient – what is called 'connubial' or 'consort' dermatitis, or dermatitis 'by proxy'; transfer of the allergen through the air – gasses or vapors, droplets, or powders – which gives rise to an 'airborne' dermatitis; systemic exposure to the allergen (or to a cross-reacting substance) after previous sensitization of the skin,

which may produce a lesional flare-up at the previous contact sites or a diffuse or generalized eczematous reaction, usually called 'systemic CD-type reaction'. Finally, there is the possibility of photoallergic or phototoxic CD (the consequence of exposure to a photoallergen or a phototoxic substance and sunlight) or protein CD whose culprit is not a low-molecular-weight hapten but a proteinous substance.

Clinical symptoms

Symptoms of ICD may include burning, itching, stinging, soreness and pain, particularly at the beginning of the clinical course, whilst pruritus is the cardinal symptom in ACD.

Box 1. Clinical history for the assessment of contact dermatitis.

Clinical characteristics of the dermatitis

- Characteristics of initial lesions and clinical evolution
- Time of onset and possible relationship with exposure to allergens or irritants
- Dermatitis area corresponding to exposure site
- Dermatitis morphology suggesting specific contactants

History of occupational exposure

- Job description; occupational gestures and characteristics of the working milieu
- Potential allergens and irritants in the working environment
- Characteristics of the exposure: dose, frequency and site
- Concomitant exposure factors: temperature, humidity, occlusion, friction, and so on.
- Time relationship to occupation; effect of holidays and time off work
- Personal protective measures at work (gloves, masks and barrier creams)
- Other workers similarly affected?

History of nonoccupational exposure

- Domestic products: cleansers and detergents
- Skin care products, fragrances, nail and hair products
- Pharmaceutical products (under prescription and over the counter)
- Personal protective measures at home (gloves)
- Jewellery and clothing
- Homework and hobbies

Variants on exposure

- Direct contact with the agent
- Contact through fomites or contaminated surfaces
- Combination of contact with the causative agent and sun exposure, resulting in a photocontact or photoaggravated dermatitis
- Contact with spouses, partners, relatives or friends who convey the agent, which results in connubial or consort dermatitis
- Transfer from other body sites, generally by the hands, to more sensitive areas, such as eyelids or neck, resulting in an ectopic dermatitis
- Exposure to gases, droplets or particles in the atmosphere, which results in airborne dermatitis
- Systemic exposure in previously sensitized patients

History of previous dermatitis, atopy and other skin/general diseases

- Past contact dermatitis (occupational or not)
- Previous patch testing
- Other exogenous or endogenous dermatitis: atopic dermatitis, stasis dermatitis, psoriasis and sensitive skin
- Mucosal atopy (asthma and rhinoconjunctivitis)
- Family history of atopy and other skin diseases

Time course of the dermatitis

After detecting one or many putative agents, it is mandatory to determine whether a time relationship exists between the attributable exposure and the clinical course of the dermatitis. The patient's description of events may be important in the investigation, including the date of onset of the dermatitis, its clinical characteristics and initial affected sites, the rapidity of onset after exposure and description of the clinical course. Features claimed to be helpful in distinguishing ICD include skin reaction upon first exposure – at least with strong irritants – and rapid onset of dermatitis after exposure. In ACD, two phases are required: an initial phase, during which sensitization is acquired, followed by elicitation of a cutaneous inflammatory reaction. Except for very potent allergens, the primary sensitization does not result in clinical skin lesions, probably due to the low numbers of responder T lymphocytes in this phase. Subsequent challenges, resulting in clonal T-cell expansion and representation of the antigen to already primed memory T cells, may result in cytokine release and cytotoxicity, generating the clinical lesions. However, it is almost impossible to substantiate this difference in clinical practice. Low-grade irritants will induce dermatitis only after multiple exposures. Moreover, many allergens have cross-reactants that can give a reaction on first exposure, without a period of induction. Concerning the time course for the onset of clinical CD, the classical study by Fregert [27] in newly exposed workers with occupational dermatoses demonstrated no significant time differences in the development of ACD and ICD.

The acute irritant reaction usually reaches its peak quickly, within minutes to a few hours after exposure, and then starts to heal. However, certain irritants may elicit a delayed inflammatory response, and visible inflammation is not seen until 12–24 h or even longer after exposure [28,29]. Even sodium lauryl sulphate, the most studied irritant substance, may give a more intense inflammatory reaction at 48 h after exposure, a time course more characteristic of allergic reactions [28]. In ACD, the elicitation time depends on the characteristics of the sensitizer, the intensity of exposure and degree of sensitivity. Lesions usually appear 24–72 h after the exposure to the causative agent and reach their peak at approximately 72–96 h. However, they may develop as early as 5 h or as late as 7 days after exposure. Often, ACD improves more slowly than ICD when exposure ceases, and recurs faster (in a few days) when exposure is re-established. However, cumulative ICD to several weak irritants usually requires many days or even weeks to improve when the exposure is discontinued. A clinical course characterized by iterative, sudden flares of dermatitis frequently indicates ACD. A feature of ACD that may be a source of confusion is the fact that development of a delayed hypersensitivity may suddenly occur after years of contact with a substance. While it is true that many cases of ACD develop following recent (weeks to months) exposure to an allergen, ACD may also develop after years of repeated exposure.

History of other previous or concomitant dermatitis & family skin disorders

History should provide insights in differentiating CD from other exogenous or endogenous dermatitis. Furthermore, most of the time, we are dealing with multifactorial dermatitis, and we have

to consider all contributory factors. Therefore, we must examine all possible susceptibility factors, especially personal and family atopy as well as other skin diseases. Those patients who had a history of childhood atopic dermatitis are likely to suffer ICD later in life, mainly in the hands [30]. Atopy may also predispose to other types of contact reactions, such as protein CD or contact urticaria.

The genetic basis for increased susceptibility to CD is being intensely studied. Recently, it was demonstrated that a large proportion of individuals with AD have an epidermal expression deficiency of filaggrin (*FLG*). Different studies have shown that between 16 and 56% of patients with atopic dermatitis carried one or more *FLG* null alleles, in comparison with 5–10% of subjects in the general European population. Meta-analysis of data from these studies demonstrated an odds ratio (OR) of 3.6 for carriers of a *FLG* null allele for risk of atopic dermatitis [31]. The induced alterations in the skin barrier function may predispose to CD by allowing a greater penetration of chemical irritants or haptens. de Jongh *et al.* [32] observed that *FLG* null alleles were associated with increased susceptibility to chronic ICD (OR: 1.91; 95% CI: 1.02–3.59); however, they did not determine whether or not the *FLG* null allele was an independent risk factor. On the other hand, the prevalence of one or more contact sensitizations was not statistically different in carriers of a *FLG* null allele (16 out of 37; 43%) than in noncarriers (160 of 259; 62%). Lerbaek and colleagues did not find an association between contact allergy and *FLG* null alleles in a population-based study [33]. However, an association was found between *FLG* null alleles and a positive patch testing for nickel (Ni) in combination with adverse skin reactions to jewellery, but not with contact sensitizations to other allergens [34]. This finding may have important implications for our understanding of Ni sensitization because Ni is chelated in the epidermis and perhaps to FLG [35]. Therefore, more research is needed to determine whether or not the hypothesis of increased risk of allergic sensitization in carriers of *FLG* null alleles is valid.

History of psoriasis is important, as sometimes it may be difficult to distinguish some subtypes of psoriasis, especially eczematous psoriasis or hand psoriasis, from CD. In many psoriasis patients, local triggering factors such as repeated friction or trauma, as well as chemical contactants, play a provocative role in the initiation of psoriasis lesions (Köebner phenomenon). Besides, patients with hand psoriasis tolerate cutaneous irritants poorly and are more susceptible to develop ICD [36].

Clinical examination

A systematic consideration of the clinical features of the skin reaction is needed to make a correct diagnosis of CD. CD is characterized by eczematous inflammation. However, the physician should keep in mind that, rarely, ACD may adopt other clinical patterns [37,38], such as erythema multiforme-like [39,40], urticarial papular plaques, lichen-planus-like and lichenoid eruptions [41], purpuric petechial reactions [42], dermal reactions [43], lymphomatoid CD [44], granulomatous and pustular reactions [45], pigmentation disturbances [46], and even pemphigoid lesions [47]. In most cases, the eczematous nature, the shape and pattern of distribution of the lesions, often primarily confined to the site of contact, suggest the diagnosis. The distribution is usually the single most

important clue to the diagnosis of CD but can also lead to confusion when presentation is not typical. It is possible for CD to occur unilaterally, even though exposure is bilateral, or to occur at sites distant from the actual exposure due to transfer of the agent with the hands or fomites. In sites where the *stratum corneum* is thinner, thereby allowing greater penetration of the contactant – as in the case of eyelids – the rash may be more severe than at other contact points. Sometimes, especially in the early stages of the condition, clinical examination may reveal clinical clues that point to a specific exposure (e.g., a linear configuration or a cut-off lesion at sites of contact with gloves or shoes). However, in the evolutive course, the original pattern of the dermatitis is frequently modified by secondary dissemination, infection or treatment. Therefore, early diagnosis is of the utmost importance.

Differences in the clinical morphology between ICD & ACD

It is often impossible to unambiguously discriminate between ICD, ACD or even endogenous eczema on clinical grounds, as erythema, edema, scaling and vesiculation in acute dermatitis, and fissuring, lichenification and hyperkeratosis in the chronic phase, are largely nonspecific signs.

Skin irritation was formerly considered a monomorphous process. However, it represents, from both a clinical and a mechanistic perspective, a multifarious disease, depending on different endogenous and exogenous factors. ICD is now understood as a complex biological syndrome with a diverse pathophysiology and a broad range of morphological macro- and microscopic features (TABLE 1) [48]. Irritant reactions were believed to be nonspecific and reproducible in all exposed subjects, in contradistinction with the uniqueness and specificity of allergic reactions. Yet the effects of irritants on cutaneous targets are highly variable, depending on several factors, such as the type of agent, concentration, amount applied to the skin surface, surface area, regional variations, length of exposure, mode of exposure, concomitant environmental factors and individual susceptibility, including age, sex, race, genetic background and concomitant disease [49–55]. Irritant thresholds and dose responses vary considerably among individuals and also in the same individual over time [49]. Consequently, a particular episode of irritant dermatitis may be the outcome of a multitude of factors. The clinical presentation may be highly polymorphic and may vary depending on the characteristics of the triggering agent and the reactivity of the subject. As stated by Rietschel [55], in ICD it is common to observe vesicles juxtaposed closely to patches of erythema, erosions, bullae, or other morphological changes, which suggest that small differences in irritant concentration or contact time produce large differences in skin damage. The substantial variations in clinical morphology and evolutive course of ICD pose a veritable diagnostic and classification challenge. ICD lesions are usually sharply demarcated and confined to the contact area, while in ACD, lesions are less circumscribed and frequently disseminated. However, ICD lesions may disseminate depending on the characteristics of the exposure. The existence of a gravitational influence, such as a dripping effect, points to irritancy. Irritant reactions due to wet work and exposure to detergents often involve the finger webs, concentrate under rings, and may extend over the dorsum of the

hands in an apron-like distribution. Irritancy may produce a dry, glazed or scalded appearance of the epidermis. Pustules, necrosis and ulceration may also be seen after contact with strong irritants, whereas they are rarely observed in ACD. Vesiculation, edema and weeping are usually more prominent in ACD, but certain irritants may induce a polymorphous dermatitis with vesiculation mimicking ACD (TABLE 2). Furthermore, ACD in certain body areas, such as the eyelids, penis and scrotum, may exhibit only erythema and edema without vesiculation. The diagnosis of acute ICD to strong skin irritants is usually straightforward, because the rapid onset of skin lesions after exposure indicates the causative agent. On the other hand, subacute or chronic ICD or ACD frequently appear as an eczematous condition with erythema, excoriations, lichenification, scaling and/or hyperkeratosis, and the causative agents are not always readily apparent. The distinction may be even more complicated because many allergens have irritant effects and/or both types of contact agents act jointly.

Patch testing

Patch testing is currently used in clinical practice as the most important investigative and diagnostic method available for studying delayed contact hypersensitivity. It constitutes – together with a detailed clinical history and a complete physical examination – a fundamental step in the diagnostic work-up of ACD. By contrast, the diagnosis of ICD is made on clinical grounds alone, mostly by exclusion of ACD: a negative patch test result points towards irritation or endogenous disease. However, this is clearly insufficient for making a diagnosis of ICD. A negative patch test may represent not testing with the causal agent or, even when the causative allergen has been tested, the reaction may have been false negative. On the other hand, a true positive patch test result does not eliminate the possible coexistence of both diseases.

Indications for patch testing

Patch testing is indicated when an allergic component of the dermatitis is suspected. It has been shown to be significant both in confirming contact sensitivities suspected from the clinical history and in unveiling unsuspected sensitivities. Several studies have shown that history and physical examination alone are not adequate to consistently and fully evaluate a patient's contact allergens [56–59]. Cronin studied 1000 patients by thorough clinical investigation and patch testing and demonstrated that the accuracy of the clinical prediction varied depending on the characteristics of the clinical dermatitis and the causative allergen [57]. For Ni, the most frequent sensitizer in women, the allergy was anticipated in 64% of the subjects, whilst chromate, the most common sensitizer in men, was suspected only in 40% of cases. For other common allergens, such as lanolin and neomycin, sensitization was predicted in only 16 and 8% of the cases, respectively. Similarly, Fleming *et al.* demonstrated that clinical questions were accurate to predict the causative allergen in only 29–54% of ACD cases, depending on the allergen involved [59]. Reliable identification of causative allergens by history alone represents an overwhelming task in which we are usually unsuccessful.

Table 1. Clinicopathological spectrum of skin irritancy.

Type of irritation	Onset and type of exposure	Clinical characteristics
Acute ICD	Acute; often single exposure to strong irritant	Erythema, edema, weeping, vesicles, bullae, necrosis, burning, pain
Acute-delayed ICD	Delayed onset of clinical lesions: 8–24 h or longer after exposure; induced by special irritants	Erythema, papules, vesicles, bullae
Irritant reaction	Develops in weeks; often seen in individuals involved in wet work and appears after multiple exposures	Erythema, papules, dryness, scaling; it may resolve or, with continued exposure, may progress to a full-blown ICD
Cumulative ICD	Develops in months to years; multiple exposures to different agents; often weak irritants, capable of inducing a reaction only after repetitive exposure	Erythema, papules, dryness, scaling, fissuring, lichenification. Burning, itching, soreness
Traumiterative ICD	Develops in months to years; multiple exposure to a single agent	Erythema, papules, dryness, scaling, fissuring, lichenification
Traumatic ICD	Develops in weeks to months after skin trauma	Erythema, papules, dryness, scaling, fissuring, lichenification, callus
Friction ICD	Develops in weeks to months by repetitive microtrauma	Erythema, papules, dryness, scaling, lichenification
Pustular and acneiform CD	Develops in weeks to months; exposure to special agents (comedogenic-pustulogens), frequently occurs with occlusion	Papules, pustules, comedones
Exsiccation Eczematide	Develops in weeks; multiple exposure	Dryness, scaling, fissuring, itching, soreness
Nonerythematous (sub-erythematous) irritation	Variable onset	Dryness, scaling, stinging or itch sensation
Subjective (sensory) irritation	Variable onset (frequently acute onset); exposure to special agents	Nonvisible changes, stinging or itch sensation

CD: Contact dermatitis; ICD: Irritant contact dermatitis.

In addition to the investigation of probable ACD, patch testing should also be performed to uncover unsuspected ('occult') contact allergies in patients with chronic or nonresponsive eczematous dermatitis, especially those with hand or hand-and-foot dermatitis (dyshidrotic, hyperkeratotic and even *pustulosis palmaris et plantaris*), stasis dermatitis, atopic dermatitis, nummular eczema and unclassified eczema. Patch testing may also be considered in patients with eczematous psoriasis [60,61], essential pruritus and suspected drug eruptions [62]. When these patients are assessed clinically but without patch testing, they may not be suspected of having an allergic component. Moreover, in many cases, the offending agent is present in the topical products prescribed – or self-administered – for the treatment of the primary disease. Although patch testing is primarily conducted according to the clinical history and physical examination, the diagnostic process is bidirectional and test results will guide further questioning and investigation.

Reconsidering the history in the light of the test results can lead to recognition of many concealed sources of causative exposure [63].

Patch testing materials & procedure

Patch testing involves the application of specific allergens directly to the skin under controlled conditions, causing a local allergic reaction in a susceptible (sensitized) person. It employs the agent that causes the disease; it applies that agent to the target organ, and it reproduces locally the pathogenic and immunologic mechanisms and morphological changes of the disease itself, thus reproducing the dermatitis 'in miniature'. The allergens are usually incorporated in petrolatum, although for some allergens a different vehicle, such as water, acetone, ethanol 70%, or olive oil, must be used. Allergens are applied in hypoallergenic chambers, which are mounted on adhesive tape and attached to the upper back of the patients. There is also a ready-to-use test system (TRUE Test™ [Allerderm, AZ, USA]) [64], in which the allergen is dissolved in an aqueous or ethanol solution and then incorporated in a dried-in-gel vehicle such as polyvidone or a cellulose derivative. A thin layer of this gel is then coated onto a polyester sheet and dried to form a patch. The sheet is cut into 9 × 9 mm² patches and arranged in panels on strips of tape. The TRUE Test produces an exact dosage, even surface spread and high bioavailability for the allergens. This solves the problems of low bioavailability, uncertain dosage and uneven surface distribution, which are commonly seen when petrolatum is used as the vehicle [65–67].

The present standards for patch test methodology were established by the International Contact Dermatitis Research Group (ICDRG) in the 1960s and 1970s, but numerous variations have been introduced by the different groups. The test is generally kept in place for a period of 48 h, although in some centers it is applied for only 24 h. Furthermore, there are variations in reading times and even in the score grading of patch test reactions. Until a general agreement is reached, a complete description of the patch test methodology—including the source of allergens and the chambers used, the duration of patch test application, reading times and score grading of patch test reactions—should be included, allowing for inter-laboratory comparisons of test results.

Patch test standardization

During the last decades of the 20th Century, great effort was devoted to optimization and standardization of the patch testing materials and methodology [68–72]. Significant research on

Table 2. Clinical differences between irritant contact dermatitis and allergic contact dermatitis.

ICD	ACD
Morphology	
• Acute ICD includes erythema and edema and sometimes vesicles or bullae, oozing and pustules • Necrosis and ulceration may also be seen with corrosive materials • ICD is mostly characterized by dryness, roughness, glazed or scalded appearance of the skin • Chronic ICD may have hyperkeratosis, desquamation, lichenification and fissuring • Lesions are characteristically sharply circumscribed to the contact area • Usually there is absence of distant lesions, but sometimes dermatitis may be generalized, depending on the nature of the exposure	• Pustules, necrosis or ulceration are rarely seen • ACD is mostly characterized by edema, vesicles and oozing, however, these features are usually not present in subacute or chronic ACD • Chronic ACD may display hyperkeratosis, desquamation, lichenification and fissuring • Clinical lesions are more intense in the contact area, but they usually exceed this area and their limits are ill defined • Dissemination of the dermatitis with distant lesions may occur
Symptoms	
• Symptoms of acute ICD are burning, stinging, prickling, pain and soreness of the skin (pruritus may be present)	• Pruritus is the main symptom of ACD
Clinical course	
• Acute ICD may appear after first exposure (at least with strong irritants) • In acute ICD, lesions appear rapidly, usually within minutes to a few hours after exposure, but delayed reactions can be seen • Irritant reactions are characterized by the 'decrecendo phenomenon' • The reaction reaches its peak quickly, and then starts to heal	• Sensitizing exposure(s) is required • Clinical lesions appear after subsequent challenges with re-presentation of the antigen to already primed (memory) T cells • Lesions usually appear 24–72 h after the last exposure to the causative agent, but they may develop as early as 5 h or as late as 7 days after exposure • Allergic reactions are characterized by the 'crescendo phenomenon' and the kinetics of resolution may be slower

ACD: Allergic contact dermatitis; ICD: Irritant contact dermatitis.

chemical and toxicological aspects of test allergens, appropriate vehicles and skin penetration all contributed to the development of reliable and consistent patch test techniques. Yet, systematic studies for several important aspects are still lacking, and patch testing still confronts inherent methodological problems. Several factors may influence patch test reactions and many sources of unreliability still exist, including variations in patch test materials, technique and methodology, as well as inherent biological variability of patch test responses. Variations in the amount of material applied can lead to erroneous results. The ideal test situation is a test area completely covered with the test preparation without any spreading outside that area. Excessive amounts can provoke spillover and irritant reactions, while inadequate dosing may, conversely, result in false-negative and doubtful reactions. The amount of allergen applied with the Finn Chamber technique should approximate to 20 mg [73] but, as a manually dispensed system, the amount of allergen applied is potentially variable depending on the technique [74,75]. This variation was reported to be higher when testing allergens in solution [76]. Preprepared patch test systems, such as TRUE Test, prevent the inconsistencies in the concentration of the tested allergen, the amount of material applied and the possibility of error in the sequence of allergens. The use of an appropriate vehicle is crucial as it influences the bioavailability and subsequent percutaneous penetration of the allergen [77–79]. Petrolatum remains the standard vehicle for most allergens, with the exception of the TRUE

Test. Earlier studies demonstrated that patch test suspensions in petrolatum contained undispersed allergen particles, and both the particle size and number differed significantly between different test substances and different manufacturers [80–83]. This was frequently seen with metal salts [84] such as Ni sulphate [85]. Other test substances, such as disperse dyes [86–88], also produced a number of problems. Ryberg *et al.* analyzed commercial patch test preparations of eight different disperse dyes from different suppliers and observed wide variations in concentration compared with the label, impurities and even the presence of a different dye allergen in the final preparation [86]. Frick *et al.* performed chemical analyses of 14 commercial test preparations of diphenylmethane -4,4'-diisocyanate in petrolatum and observed a poor correlation between the stated and observed concentrations [89]. Furthermore, many studies have found poor stability for some allergens [89–93]. Allergenic degradation products can be formed during storage, mostly by oxidation, as in the case of terpenes, such as limonene and linalool [91]. This may have several implications, including inappropriate diagnosis and incorrect counseling for the individual patient, as well as unfeasibility in the comparison of patch test results from different departments. Advances are being made in the optimization of patch test preparations and the dispersion of allergens, and the quality of these materials has significantly improved in the last 15 years [94], but not much significant research has been carried out on alternate vehicles in patch testing [95].

Patch test reading

The readings of the patch test results are performed 48 h after application, approximately 30 min after the removal of the patches and again after 72 h and/or preferably 96 h after application. Unfortunately, the timing of the reactions to different allergens does not necessarily follow the timing of the readings. Delayed readings, 1 week after application, are highly recommended, especially for some slow-reacting allergens, such as neomycin or corticosteroids, among others; even Ni may be a slow-reacting allergen [96–98].

The classification and score grading of patch test reactions depends on descriptive morphology. Typical morphological features of an allergic test response are erythema, edema, papules and vesicles (or bullae). At least an erythematous infiltration and/or papules should be present for a reaction to be considered allergic, while reactions that show only erythema without infiltration – called doubtful reactions – are frequently nonspecific or correspond to irritancy. Allergic patch test reactions are traditionally scored in terms of intensity, and a grading scale from 1+ to 3+ is currently accepted for ranking these allergic reactions (TABLE 3) [99]. However, there is still controversy concerning patch test readings and the grading scale. For instance, there are discrepancies in the reading of the 1+ reaction between the different CD groups. Some groups define the 1+ reaction as homogeneous redness in the whole test area with scattered papules, while others only require redness and homogeneous infiltration in the whole test area. No real consensus has been reached in this matter so far. Menné and White have proposed introducing an extra grade of patch test reaction in the scoring [100]: (+) homogeneous redness in the test area with scattered papules; (++) homogeneous redness and homogeneous infiltration in the test area; (+++) homogeneous redness and infiltration with vesicles; and (++++ homogeneous redness and infiltration with coalescing vesicles. However, it is debatable whether this distinction has any practical benefit. By contrast, other authors have suggested that a simplified score may reduce the inter-individual variations in patch test readings [101].

The substantial challenge in diagnostic patch testing is that reading is subjective, based on inspection and palpation of the test responses; therefore, there exists considerable inter-individual variation in how patch tests are both read and then interpreted by clinicians. The reader's background knowledge and experience can greatly affect the results [102].

Validity of patch testing results

The validity of any test system is its intrinsic ability to detect which individuals have the target disease and which do not, relying on the test capability to detect both true positive and true negative reactions, while minimizing the number of false-positive and false-negative reactions.

False-positive & false-negative patch test reactions

A false-negative reaction can occur for a number of reasons. One easily correctable mistake is the failure to perform a delayed test reading after allergen removal and evaluation at 48 h. This

is especially important for allergens known to elicit delayed reactions and in elderly patients, who may present a protracted immunologic response. Furthermore, false-negative reactions may be present when the allergen concentration is too low to elicit a response; the vehicle may not have released a sufficient amount of the allergen; the test site might have been inappropriate, or the patient's skin is unresponsive by prior sun exposure, concurrent immunosuppressive therapies or other causes; or because of methodological flaws, such as insufficient occlusion or inadequate replication of the clinical dermatitis conditions by the test. When patch testing with a particular substance is negative in a patient who has an evident dermatitis from contact with that substance, the putative allergen should be retested (perhaps in a different concentration, with a different vehicle or with a different testing method, such as open or semi-open tests, provocative use tests (PUT) and/or the repeated open application test (ROAT). Likewise, the investigator must be aware of the pitfalls of false-positive reactions. A false-positive reaction may be due to several causes, such as testing with allergens that are marginally irritant, such as metals, formaldehyde or epoxi resin; testing with allergens at concentrations that exceed their irritancy thresholds; spill-over reaction from a nearby true-positive reaction; multiple simultaneous positive reactions; or testing patients with active dermatitis or otherwise sensitive or irritable skin. It is difficult sometimes to discriminate between false-positive reactions and true allergic reactions; in such cases, the true nature of false-positive reactions can sometimes be unveiled by repeat patch testing of the individual allergen with lower concentrations or serial dilutions, repeated patch testing with 24 h occlusion, or by performing additional tests such as the ROAT [103,104]. Irritation reactions in ROAT are very limited compared with patch tests but, for some patients, open tests are less sensitive than classical patch tests. False-negative reactions are more difficult to detect and require high levels of suspicion to unveil. Even if the allergic nature of a positive reaction as read by international guidelines cannot be taken for granted, for most common allergens a positive patch test reaction is predictive for contact sensitization [105,106]. The validity of patch testing may, therefore, be considered as good for many allergens, if tested under controlled conditions and at the proper concentration, and if performed and evaluated according to the international guidelines [105,106].

Sensitivity, specificity & predictive value

The significance of a patch test result is determined not only by the sensitivity and the specificity of the test itself, but also by the prevalence of the condition in the studied population. If the rate of contact allergy in the population tested is low, then the negative predictive value increases and the positive predictive value decreases. Conversely, when the rate of allergic persons tested increases, then the positive predictive value will increase at the same test sensitivity, while the negative predictive value will decrease. [104,105,107,108]. This substantiates the importance of achieving a high prevalence rate of truly sensitized patients. We should critically consider all the information about clinical history and physical examination and generate precise

pretest probabilities of meeting the case definition for ACD before patch testing [104,105,108,109]. In other words, the technique of patch testing is most effectively utilized as a confirmatory tool in those patients in which a diagnosis of ACD was made based on strict clinical criteria. Performing patch test as a 'last recourse' for managing refractory patients who otherwise do not meet the clinical criteria for ACD would not be expected to yield the best results.

Testing with the standard allergen series

With the premise of increasing the sensitivity of the patch test procedure and detecting as many clinically relevant allergic subjects as possible, we commonly employ arrays of many test substances grouped as tests series in the routine evaluation of patients with suspected ACD. Habitually, the patch test evaluation starts with one of the standard screening series of allergens, such as that proposed by the ICDRG [110], the European and Environmental Contact Dermatitis Research Group (EECDRG) and the North American Contact Dermatitis Group (NACDGD) [111], which comprise a variety of chemically defined compounds and mixes of allergens, both natural and synthetic, found in industrial and domestic settings. Their constitution is based on the statistics of allergens and they are periodically revised to adapt to changes in exposure, introduction of new environmental allergens onto the market, and information regarding irritation, active sensitization and so on. Requirements to include a chemical in a standard series have been formulated by Bruze *et al.* [112]. Demands on a sensitizer in the standard series are: being a common sensitizer and being common in the environment; having a high prevalence of contact allergy with a rate exceeding 0.5–1% of true allergic reactions in routinely tested patients with suspected contact dermatitis; and giving reliable patch test results with a high degree of clinical relevance and minimal adverse effects, particularly patch test sensitization. Minor variations are due to differences in cultural customs and practices, industrialization and use in different countries. It was believed that testing with the standard series detected up to 80% of all contact allergies [113]. However, this percentage is currently deemed too high [114]. In a multicenter study including 4824 consecutive patients from five European CD departments, the sensitivities detected by the standard series alone ranged from 37 to 73%, depending on the testing institution [115]. Sherertz and Swartz found that 36% of positive reactions occurred to allergens in the standard series exclusively and, overall, 76% reacted to one standard allergen [116]. Most patients should be tested to a standard series, and also to additional series or individual allergens that are believed to be associated with the clinical situation and will be selected depending on the clinical history and the distribution of the dermatitis, the patient's occupation or the geographic area where the patient resides.

Table 3. Scoring of patch tests according to the International Contact Dermatitis Research Group.

Score	Reaction
- (0)	Negative reaction
?+	Doubtful reaction; erythema only
+ (1+)	Weak (nonvesicular) positive allergic reaction; erythema, infiltration and possibly papules
++ (2+)	Strong (vesicular) positive allergic reaction; erythema, infiltration, papules and vesicles
+++ (3+)	Extreme positive allergic reaction; bullous reaction
IR	Irritant reaction

In series, chemicals are tested in well-defined concentrations in order to reduce the chance of false-positive and false-negative reactions. It should be taken into consideration, however, that many substances are tested simultaneously, which may, because of multiple testing, give rise either to false-positive reactions or to reactions not relevant to the specific situation. On the other hand, if only a small panel of chemicals selected on the basis of history of exposure is tested, relevant allergies may be missed.

Testing with additional allergens

Testing with additional allergens depending on the exposure gives a substantial number of additional positive reactions [104,105]. Furthermore, testing with the products used by the patient in the workplace or at home, as well as the ingredients and/or product extracts, is often required. However, testing with nonstandard allergens, other than those of the recommended series, should be undertaken with caution. These may be chemically pure substances, but they are often compound products and may contain unknown components. Even some components can be irritants, such is the case for many industrial products, and special prudence should be exerted when testing with them. Besides producing false-positive irritant reactions, the injudicious use of undiluted industrial substances in patch testing increases the risk of active sensitization. Therefore, it is better not to use these materials in patch testing unless there is a definite clinical suggestion of their role in the causation of the dermatitis. Specialized textbooks regarding test concentrations and vehicles for many nonstandardized materials are currently available and may constitute a starting point when testing with these substances [117]. Should any substance be considered potentially irritant, an open use test may be envisaged. It should be performed with diluted substances, whose concentration can be progressively increased as far as no response, either allergic or irritant, appears. In addition, before any human test is performed, complete toxicological information of the material must be procured.

Assessment of clinical relevance

Patch testing results require biological and clinical interpretation. The fact that contact allergy to certain allergen(s) has been reliably demonstrated by careful patch testing does not prove that such allergen(s) is responsible for the patient's ACD. A true

positive patch test reaction only indicates that the patient has been previously exposed and sensitized to the substance. Patients may suffer major changes in their lifestyle on the basis of patch testing results, therefore it is crucial to establish that the positive reaction is actually linked to the clinical dermatitis, either as a primary cause or as an aggravating factor. Based on the presence of a putative allergen in materials that come into contact with the skin, either occupationally or during leisure activities, the pattern of distribution of the skin lesions, and the effect of elicitation by exposure and healing of the dermatitis by avoidance, positive patch test results are judged as possible, probable or certainly relevant. According to the ICDRG criteria [99,118], we consider that a positive patch test reaction is 'relevant' if the allergen is traced. If the source of a positive patch test is not traced, we consider it as an 'unexplained positive'. We use 'current' or 'present' relevance if the positive patch test putatively explains the patient's present dermatitis. Likewise, when the positive patch test explains a past clinical disease not directly related to the current symptoms, we refer to this as 'past' relevance. From a practical perspective, establishing that a positive reaction has past relevance or possible relevance does not direct the clinician to intervene directly for the very problem for which past testing was performed. However, reporting not currently relevant data serves an important epidemiological role and may be useful in preventing further outbreaks of ACD in a patient. The determination of relevance primarily depends on the expertise of the investigator and the possibility of detecting the allergen in the environment of the patient. In many cases, a positive reaction is judged as nonrelevant owing to insufficient environmental information. If the results of the patch tests are negative for a patient in whom a diagnosis of ACD has been proposed, one has to go back to the beginning – that is, to a thorough anamnesis and physical examination. The assumed allergens should be retested (perhaps in another concentration, with another vehicle or with another testing method) and additional allergens should be tested [119,120]. Often, a visit to the patient's workplace proves rewarding. We must perform a rigorous environmental evaluation, investigating the existence of allergenic exposures, characteristics of this exposure and possible concurrent factors. Relevance scores and accuracy of the assessment are significantly improved by a comprehensive knowledge of the patient's chemical environment. In Box 2 we propose practical guidelines and additional testing procedures for assessment of relevance.

Besides patch testing, other types of skin tests, such as open and semi-open tests, tests with product extracts, ROAT and PUT, may be required to establish a definite causative relationship between the positive patch test result and the clinical dermatitis [121–123]. ROAT has significant potential in refinement of the evidence-based diagnosis of clinical relevance. This test is not standardized to the same extent and it is time-consuming, but mimics some real-life exposure situations. However, for general validation, a standardized measurement of the results of use testing, such as the ICDRG scoring system for patch testing, is required [123]. Several studies have shown a significant relationship between the patch test threshold and the ROAT threshold [124–126], but

the amount of allergen required to elicit a reaction for these two study methods is not the same. Fischer *et al.* demonstrated that the dose per application eliciting a reaction in the ROAT is substantially lower than the dose required to elicit a reaction in the patch test [125,126]. This could be explained by the accumulation of allergen in the skin from repeated exposure and/or the repeated stimulation of the immune system. The stronger response in the ROAT compared with the patch test threshold is relevant for risk evaluation of the elicitation potential of allergens in final products, since the same dose per unit area eliciting a negative patch test result might elicit a reaction when applied repeatedly in an open test [124].

Once the patch testing procedure is completed, the allergens have been identified and relevance has been established, the next step is education. Even when properly conducted and interpreted, patch testing loses its value if education is not systematic and comprehensive [126]. It is imperative that the patient fully understands the test and complies with the procedure and resultant avoidance regimen. Providing the patient with written information on specific allergens, synonymous names and crossreactors, in addition to suggestions on how to avoid the allergens, is the final step of patch testing.

Immunological testing

The combination of clinical history and patch test results constitutes the two cornerstones in the diagnosis of delayed hypersensitivity. However, a significant drawback with patch testing is the possibility of inducing changes in the patients' immune status. Patch testing may reactivate earlier dermatitis, boost up previous hypersensitivities – clinically silent due to a high activation threshold – or, worse, it may actively sensitize the patients to the applied haptens. Another disadvantage is that it is often necessary to postpone the test in patients with disseminated or severe dermatitis. In addition, patch testing may produce equivocal results. Therefore, the development of *in vitro* immunological procedures for delayed contact sensitivity testing may be valuable. The aim of *in vitro* methods is to assess the antigen-specific T-cell activation. Several *in vitro* tests have been used in the diagnosis of contact sensitivity. At present, the most used *in vitro* tests – although primarily in the experimental setting – involve challenging lymphocytes with allergen *in vitro*, and either cellular changes related to T-cell activation and proliferation (lymphocyte transformation test [LTT]), or the amount of lymphokines produced (lymphocyte stimulation test), is assessed as a parameter of T-cell activation [127,128]. In the 1960s and 1970s, the LTT was established in order to determine metal and drug allergy *in vitro* [129,130]. However, the problem of nonspecific proliferation of lymphocytes in the presence of Ni was already evident at that time and most studies showed an overlap in lymphocyte proliferation between Ni-allergic and nonallergic subjects [129,131]. Varying degrees of Ni-induced T-cell activation have been reported in individuals with negative patch tests to Ni, suggesting that the occurrence of Ni-reactive T cells in the peripheral blood is not always associated with the manifestation of clinical disease [132–134]. Lisby *et al.* noted no difference in IFN- γ release

Box 2. Assessment of relevance of patch test reactions.**1. Perform a complete and standardized history (including sex, age, occupation, occupational and domestic exposures, adverse reactions, time course, effect of elicitation of the dermatitis by exposure and healing by avoidance)**

- The patch test results will guide further interrogation

2. Carry out a detailed physical examination

- Consider the primary site of dermatitis and the pattern of distribution of the skin lesions
- Look for clinical clues of specific exposure and the possible correlation with products containing the allergen in question

3. Determine the existence of allergenic exposure

- Qualitative exposure assessment (shows the presence or absence of the allergen in question – or a cross-reacting substance – in the patient's occupational or domestic environment)
 - Product information (product labeling, material safety data sheets, information from manufacturers or suppliers, textbooks and product databases)
 - Chemical analysis of suspected products
- Quantitative exposure assessment
 - Removal techniques, such as skin washing and wiping
 - Surrogate skin techniques, where a chemical collection medium is placed on the skin
 - Fluorescent tracer techniques
 - Biological monitoring

4. Assess all exposure parameters

- Consider all possible types of exposure: direct, indirect, sporadic and concealed
- Consider the possible route of exposure
- Evaluate the specific site(s) of contact and the presence of skin damage or previous dermatitis in the contact area(s)
- Determine the concentration of the substance in the suspected product
- Appraise the intensity of exposure (i.e., dose, duration, frequency and total surface area)
- Determine the existence of simultaneous exposure factors: humidity, occlusion, temperature and mechanical trauma

5. Repeat the patch test and/or perform additional testing procedures

- Test the suspected allergen(s) with a different concentration (including serial dilution), vehicle, occlusion time or other variables
- Test with products brought by the patient presumably containing the suspected allergen and/or product extracts
- Perform repeated open application test, provocative use test, open or semi-open tests

from cells obtained from patients with a positive patch test to Ni and nonallergic individuals [135]. Loftenius *et al.* analyzed blood lymphocytes from 20 patients with oral contact lesions from dental amalgam and ten healthy individuals for HgCl₂-induced proliferation *in vitro* [136]. They found that IFN- γ release was detectable in the cell supernatants from some of the patients but also in those from some of the controls, therefore concluding that the test was not indicative of mercury allergy.

A proposed solution for the low specificity of the test is determining both the antigen-specific proliferation and the release of cytokines. Cederbrant *et al.* measured the production of IL-10 in primary peripheral blood mononuclear cell cultures and found a significantly higher release of IL-10 in Ni²⁺-treated cultures from Ni-allergic as compared with non-Ni-allergic females, providing a better method for discrimination between individuals in the two groups than lymphocyte proliferation [137]. In the same way, Cavani *et al.* observed a high frequency of Ni-specific CD4⁺ T cells in both allergic and non-Ni-allergic individuals, whilst Ni-specific CD8⁺ T cells were found only in allergic subjects compared with nonallergic patients [138]. They also showed a predominance of so-called T-regulatory 1 (Tr1) cells with high IL-10 production in nonallergic subjects. Thus, an IFN- γ /IL-10 quotient combined with classical LTT was considered the promising end point to

distinguish between sensitized and nonsensitized individuals [139]. However, an important caveat for *in vitro* testing using cytokine determination is that the immune response systems may react differently to different antigens, making it difficult to take cytokine end points as predictive values for allergic sensitization. It is possible that a specific ratio of cytokine secretions may be predictive for one allergen but not for another, making it necessary to use different specific tests or combinatory end points for different allergens [140].

Expert commentary

Appropriate management of patients with CD involves both symptomatic treatment and avoidance of further exposure to contactants. Accurate identification of the culprit agent and implementation of effective avoidance measures is the only significant approach in ACD treatment. However, this goal is sometimes unattainable. Therefore, efforts should be made to prevent individuals from becoming sensitized. Furthermore, following the recent advances in the pathophysiology of chemical-induced skin inflammation, we know that ICD and ACD are closely linked; therefore, developing strategies to avoid ICD is also a rational approach to prevent ACD. Strategies of avoidance must be comprehensive and should start early in life.

General measures in the prevention of CD

Educating patients about their everyday practices and how to avoid irritants or allergens is very important. Often, work practices can be improved to reduce contact with irritants such as soap, solvents, oils, alkalis, acids or abrasive materials. However, this may not always be possible, as the characteristics of certain jobs make contact with potentially harmful agents inevitable. When the contactant cannot be avoided, creating protective barriers is the next alternative. Protective measures, such as appropriate gloves and clothing, or barrier creams, can be significant for reducing contact with both irritants and allergens. There is evidence from high-quality studies that certain barrier creams [141,142], high-lipid-content moisturizers [143–146], protective clothing and the use of cotton liners when occlusive gloves are worn [147], are effective in preventing the development of ICD.

Prevention of CD in children

Nowadays, we have to be aware that children are already exposed at an early age to several well-known allergens. The most important allergens are mostly the same as those described in adults (i.e., metals, rubber chemicals and fragrances) [148]. The influence of fashion trends and lifestyle such as piercing, decorative skin paintings, or the use of cosmetic products with fragrances or herbal ingredients, play an important role in the development of ACD in children. Parents should be informed about appropriate skin care in children and should be comprehensively educated about common allergens [149].

Some useful recommendations are as follows:

- Topical products for children should be fragrance-free as well as free of the most well-known cosmetic allergens;
- The list of ingredients for topical products should be complete, including those substances labeled as 'natural';
- Temporary tattoos that include the use of black henna mixtures – containing indigo, henna and chemical coloring agents such as *p*-phenylenediamine and/or diaminotoluens – should be avoided;
- Natural remedies that include herbal preparations and aromatic therapies should be used with great caution;
- Ear piercing should be avoided in children;
- Topical antibiotics should be prescribed only when strictly indicated, and the most common allergens, such as neomycin or bacitracin, should be avoided;
- Pediatric cleansers should be very gentle to avoid ICD and chemically simple to avoid ACD.

Educational strategies in the prevention of CD

Educational strategies are very important to prevent CD, especially in those occupations involving special risks. Implementation of educational measures may increase the awareness of workers for workplace hazards and motivate them

to adopt appropriate measures of skin protection and avoidance. These strategies should preferably be accompanied by well-designed studies to evaluate their impact in the prevention of ACD.

Legislation in the prevention of CD

Legislation is an important instrument to be used in the primary and secondary prevention of CD. Several countries have implemented legislations in an attempt to reduce the exposure of chemicals leading to ACD, and studies examining trends in patch test reactivity have been conducted to determine whether these legislations have been effective. It has been well documented that restriction of skin exposure to allergens is a feasible way of preventing ACD, as illustrated by the effect of reducing hexavalent chromate in cement, or Ni release from metallic items intended to be in close skin contact. Regulations in Ni content and exposures have demonstrated to be effective for primary and secondary prevention of Ni allergy. The efficacy of these regulations is demonstrated in the study by Jensen *et al.*, which strongly suggests that the implementation of the Ni-exposure regulation in 1992 in Denmark has had the intended effect of protecting the female population from becoming allergic to Ni [150]. In 1994, the European Parliament and the Council of the European Union passed a directive on Ni, which recognized that "the presence of Ni in certain objects coming into direct and prolonged contact with the skin may cause sensitization of humans to Ni and may lead to allergic reactions." The Directive prohibits trade with products that released more than 0.5 mg/cm²/week of Ni [151]. For more than 10 years now, Sweden and other Nordic countries have had legislation limiting hexavalent chromium in cement to below 2 ppm [152]. Formaldehyde in clothes is limited in Finland [153] and the German legislation limits exposure to wet work in workplaces [154]. The Cosmetics Directive 76/768/EEC [155] and its amendments aim to guarantee the safety of cosmetic products for human use. Parts of the Directive focus on the prevention of CD, containing data on authorized maximum concentrations and restricted substances. A well-known example of a restriction due to the risk of sensitization refers to methylchlorisothiazolone/methylisothiazolone (MCI/MI or Kathon® CG), which is not allowed in cosmetic products in a concentration above 15 ppm [156]. Defining safe levels of exposure for allergens is becoming even more important in view of the forthcoming prohibition of animal experiments for the evaluation of the safety of cosmetic ingredients. Other improvements include the requirement for the identification of 26 top fragrance allergens since 2005 and the Detergents Regulations of the European Union of 2005 requiring the listing of preservatives in detergents and household products [157].

Wesley and Maibach reviewed several studies examining trends in patch test reactivity before and after the implementation of legislations in different countries to determine whether these legislations have been effective in reducing the incidence of ACD [158]. They concluded that, overall, the evidence suggests a decreasing trend of ACD with appropriate formulation

changes following toxicologic and epidemiologic information. It would be essential also to carry out more well-designed epidemiological studies to extend the scientific basis for legislation and standardization. We absolutely need more population-based data to provide the correct perspective for those generated by clinic patch test studies [159,160]. Patch test frequency generated in testing dermatitis patients only represents the tip of the iceberg, while population-based incidence data on contact allergy indicate a much wider problem [160–162]. It would be of the utmost importance that the aforementioned directives and regulations could be adopted internationally in a global effort to reduce the incidence of sensitization and ACD.

Recommendations for improvements of patch testing materials & methodology

Further standardization and refinement in patch testing materials and methodology are undoubtedly required. More studies are needed to improve the stability of patch test allergens. Consistency is also required concerning the allergen dosage. In the TRUE Test system, the major patch test variables were standardized but, unfortunately, only some allergens are available nowadays. It is expected that more allergens can be added to this system. The problem of false-positive and false-negative patch test reactions needs special consideration. To address this problem, we should always start with a careful and comprehensive clinical history and physical examination in order to produce clear-cut pretest probabilities of fulfilling the case definition for ACD before performing the patch test. In addition, in order to improve the patch testing strategy, we should practice serial dilution testing more frequently. Patch test concentrations are selected as the highest concentration that can be used without inducing an irritant reaction but is still capable of evoking an allergic response in sensitized persons. However, the threshold for irritancy shows huge variance among individuals. Similarly, delayed sensitivity should not be considered an ‘all or none’ phenomenon, since there are large interindividual variations in the amount of allergen required to elicit an allergic reaction. Therefore, using a range of concentrations rather than a single concentration would be more discriminating. Judicious use of dilution testing will help us to discern whether a patch test reaction is false positive due to irritancy, as well as establishing the sensitization threshold in allergic reactions. Ascertaining the patient’s degree of sensitization may have important practical implications *vis à vis* the implementation of rational avoidance measures. Other procedures that may be useful in special situations include testing with different occlusion times, testing with different vehicles or changing the chamber size.

Appropriate negative and positive controls should be used routinely in patch testing. The negative control would be a chamber containing only petrolatum, while the positive control should be a mild irritant which has been demonstrated to induce an erythematous reaction in most people. Different substances have been proposed, such as 75% aqueous propylene glycol, 20% nonanoic acid or sodium lauryl sulphate at 0.25 or 0.5% [163–166].

Improvements in training programs

It is unquestionable that the more background knowledge and experience the investigator has, the more accurately he or she can make the correct diagnosis. More fellowships on CD at a national and international level should produce more dermatologists capable of facing the multifarious challenges of ACD and ICD. Furthermore, the knowledge of dermatologists needs to grow deeper by keeping up with the literature, attending courses and meetings, and using relevant websites.

Five-year view

Contact dermatitis is an area under continuous evolution owing to environmental changes over time due to the introduction of new agents in consumer and industrial products and the withdrawal of other substances. Diagnostic procedures and treatments are also under constant improvement following the advances in the comprehension of the pathogenic mechanisms behind ACD and ICD.

Future treatments

Better understanding of the pathogenesis of CD will provide novel therapeutic targets for immune-modulating therapy. Future therapy might be directed to inhibit the functions of target immune cell populations, influence the cytokine expression, or regulate the balance between effector and suppressor T cells, suppressing effector pathways or augmenting suppressor pathways.

Cytokines play important roles in producing CD. Suppression of activating cytokines and/or activation of inhibitory cytokines are potential therapeutic modalities. IL-1, IFN- α and TNF- α may be targeted locally by inhibiting secretion, preventing binding to their receptors, or blocking pathways downstream of their direct cellular effect. Recent experiments demonstrated the ability of IL-17 to drastically upregulate the expression of inflammatory factors in cutaneous models of ACD. Elevated levels of IL-17 mRNA have been discovered in skin biopsies of patients who have undergone Ni patch testing. IL-17 is able to stimulate T-cell proliferation and plays important roles in inflammatory diseases and autoimmune diseases. IL-17 can promote IL-6, IL-8, GM-CSF, stem cell factor and ICAM-1 expression/production by keratinocytes and chemokine CCL20 (MIP-3 α) expression by dermal fibroblasts, dermal microvascular endothelial and dendritic cells, and keratinocytes [167–169].

Recently developed therapeutic strategies directed to the inhibition of Th17 cells have been demonstrated to be valuable [170,171]. Several regulatory CD4⁺ T-cell subsets (Treg), especially CD4⁺CD25⁺ natural Treg cells, are involved in immunological tolerance and regulation to haptens through the production of the immunosuppressive cytokines IL-10 and TGF- β . Strategies to re-induce immune tolerance to haptens in patients with ACD may include development of new agents able to activate IL-10-producing Treg cells *in vivo*. Inhibition or destruction of effector T cells in affected skin sites may also suppress the reaction. Since these T cells are antigen-specific, the responsible allergen may serve as a delivery system for directing inhibitory or death signals to the relevant T-cell subpopulation.

Targets for inhibiting cell chemotaxis include adhesion molecules such as ICAM-1, VCAM-1 and very late antigen-4 (VLA-4), among others. Tissue-localized reduction in the expression of these adhesion molecules may be a method of treatment.

Future diagnostic methods

Further research in *in vitro* diagnostic methods for delayed sensitivity testing may allow their introduction in the clinical arena, leading to a less invasive and safer diagnosis of ACD [172,173]. Some molecular biology methods, including RNA assays for cytokines, which can be carried out in skin washes obtained from tape stripping, need further investigation [174].

Other future advances

Further development of national and international allergen banks, providing special test materials for aimed patch testing of CD patients, would allow for better collaboration between CD

groups, societies and researchers [126,175]. Development of more fully equipped analytic chemistry laboratories would allow for a better knowledge of the allergens' molecular chemistry, which is essential not only to make the correct diagnosis but also to understand cross-reaction patterns.

Expansion of the current allergen databases [176], as well as the development of new ones, with a focus on allergen alternatives, would be of the utmost importance, both for clinicians and patients.

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Key issues

- Making a correct diagnosis of allergic contact dermatitis (ACD) or irritant contact dermatitis (ICD) and identifying the causative agent(s) is of the utmost importance for the institution of appropriate therapeutic and preventive measures. The identification and avoidance of triggers will help to avert the distress and suffering of chronic contact dermatitis.
- ACD and ICD are similar in many aspects, such as clinical aspects and histopathology, and no pathognomonic clinical signs and symptoms can unambiguously discriminate between ACD and ICD.
- The mechanisms at the origin of the clinical lesions are dissimilar in the two types of dermatitis, at least in which concerns the early stages of the inflammatory response. ICD follows the activation of innate immunity, while in ACD both the innate and acquired immunity are activated and, as a result, antigen-specific effector T cells will drive the inflammatory response. The later stages giving rise to an eczema lesion may, on the other hand, be very similar and involve cytokines, chemokines, phenomena of apoptosis and cellular necrosis, and the recruitment of a polymorphic inflammatory infiltrate. This explains why ACD and ICD lesions can be confused clinically and histologically.
- Irritancy is believed to play a crucial role in the development of ACD. Skin cell damage and cytokine release induced by irritants may represent a 'danger signal' for the immune system, activating antigen-presenting dendritic cells (DCs) and recruiting DC precursors into the skin, thus predisposing to contact sensitization. Hence, early recognition and opportune treatment of ICD will contribute to prevent the development of ACD.
- Patch testing is currently used in clinical practice as the most important investigative and diagnostic method available for studying delayed contact hypersensitivity. Together with a detailed clinical history and a complete physical examination, it constitutes a fundamental step in the diagnostic work-up of ACD.
- Several studies have shown that clinical history and physical examination alone are not adequate to consistently and fully evaluate a patient's contact allergens; therefore, patch testing with the standard series of epicutaneous allergens, as well as additional allergens according to the clinical situation, must be performed. It has been shown to be significant both in confirming contact sensitivities suspected from the clinical history and in unveiling unsuspected sensitivities.
- The validity of patch testing may be considered as good for many allergens – especially those from the standard series – when tested under controlled conditions and in the proper concentration, and when the test is performed and evaluated according to the international guidelines.
- To further investigate the possibility of false-positive or false-negative patch test reactions, additional tests should be carried out, such as testing with a different concentration or performing serial dilutions with a different vehicle, with a different occlusion time or with a different testing method, such as provocative use tests or repeated open application tests. Using a range of concentrations rather than a single concentration would allow us to rule out false-positive irritant reactions more accurately as well as establishing the elicitation threshold in allergic reactions. Ascertaining the patient's degree of sensitization may have important practical implications *vis à vis* the implementation of rational avoidance measures.
- Patch testing results require biological and clinical interpretation. A true positive patch test reaction only indicates that the patient has previously been exposed and sensitized to the substance. It is indispensable to determine the relevance of the patch test reactions to the clinical situation.
- A significant problem with patch testing is the possibility of inducing adverse changes in the patients' immune status. Therefore, development of *in vitro* immunological procedures for delayed contact sensitivity testing, leading to a less invasive diagnostic of contact dermatitis, may be valuable.

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