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Al Halabi Al Attar, Jomana

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JOMANA AL HALABI AL ATTAR

EPIDEMIOLOGICAL AND CLINICAL FEATURES OF CONTACT DERMATITIS. EXPERIENCE OF THE DERMATOVENEROLOGICAL CLINIC OF UNIVERSITY HOSPITAL OF SPLIT FROM 2013 TO JULY 2019.

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Assist. Prof. Deny Anđelinović, MD, PhD

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LIST OF ABBREVIATIONS

APC – Antigen Presenting Cell

CD4 – Cluster of differentiation 4

CD8 – Cluster of differentiation 8

T-Cells – Thymus deriviated lymphocyte

Eg. – for example

IPPD – N-isopropyl-N-phenyl-para-phenylenediamine

ZDC – Zinc bis Diethyldithiocarbamate

DPG – 1,3-diphenylguanidine

ZBC – Zinc bis Dibutyldithiocarbamate

WHO – World Health Organisation

FRP – formaldehyde-releasing preservative

FDA – Food and Drug Administration

T.R.U.E. Test – thin-layer rapid use epicutanous patch test

1. INTRODUCTION

1.1. Allergic and Irritant contact dermatitis

1.1.2. Definition

Allergic contact dermatitis is an eczematous inflammatory skin reaction, in response to an exogenous allergen previously exposed and sensitized to (1). The sensitization process usually lasts 10-15 days and re-exposure to the allergen is needed for the elicitation process to occur. It is mediated by a cell-mediated type IV delayed hypersensitivity reaction (2).

Irritant contact dermatitis is a non-immunological inflammatory skin reaction to a potent irritant substance (3). There is none noted time lapse between the exposure and the onset of irritant contact dermatitis (4). The extent of severity is proportional to the amount of exposure of the irritant substance (5).

1.1.3. Pathogenesis of Allergic contact dermatitis

In the pathogenesis of allergic contact dermatitis, there are two main processes responsible for obtaining an allergic reaction. The primary and mandatory event in developing an allergic reaction is the process of sensitization. The process of sensitization is unique to allergic contact dermatitis and differentiates it from other contact eczemas. It is the process prior to the elicitation of an allergy, which is the secondary event in the cascade of allergic contact dermatitis. The principle is based on a cell-mediated, type four delayed hyper-sensitivity reaction (6). There are four types of hypersensitivity reactions. All four of them have different immunological mediators leading to different clinical pictures. What kind of cell types are actually responsible for allergic reactions is still under investigation and there are new discoveries yearly. What is known till now, is that dermal dendritic cells, also known as Langerhans cells, play a major role in the sensitization of cells by priming naive T-Cells and therefore forming a bridge between innate and adapted immune response (7). Langerhans cells act as so-called antigen-presenting cells (APC's), to collaborate and form a complex with a potent antigen, which wouldn't be recognized and immunological active otherwise (8). The combined complex of Langerhans cell and antigen will travel through the afferent lymphatic system to adjacent lymph nodes and present their attached antigen to attracted naive T-lymphocytes (9). When a match occurs between the APC and the T-lymphocyte, cytokines are released from both cell types leading to an immune reaction (10). The cytokines released are responsible for the proliferation of antigen specific cytotoxic CD4+ and CD8+ lymphocytes, also called memory T-Cells. The antigen specific lymphocytes will migrate through the efferent lymphatic system back to the skin and other tissues, where they interact with residual antigens

and Langerhans cells, to form newly sensitized T-lymphocytes (11). The sensitization process usually takes up to 10-15 days and is in most cases asymptomatic (2). If the skin is re-exposed to the allergen, an elicitation process is started. The re-exposure to the allergen is mandatory to fully develop a clinical active allergy. Underneath the skin are not only Langerhans cells but also IL-1 secreting keratinocytes, which will combine with the antigen to act as an APC for the sensitized T-lymphocytes (11). The cytotoxic CD8+ and CD4+ T-lymphocytes will release cytokines and chemokines upon activation by the APCs. These inflammatory mediators attract leukocytes from the blood to the skin, leading to an inflammatory reaction in the dermis, hence an allergy is presented to the outside (12).

1.1.4. Differences between Allergic and Irritant Contact dermatitis

Overall, irritant contact dermatitis is more common than allergic contact dermatitis. The ratio is approximately 80 to 20 percent, respectively. In irritant contact dermatitis, the chemical substance itself is responsible for the induced inflammation of the skin, in contrary to the immune modulated sensitization and elicitation process of allergic contact dermatitis (13). There is no time lapse between exposure and onset of inflammation in irritant contact dermatitis, in contrast to allergic contact dermatitis, in which sensitization lasts for approximately 10-15 days and re-exposure is needed for elicitation of an allergy (14). Therefore, irritant contact dermatitis often has a more acute onset than allergic contact dermatitis, which can be acute, subacute and chronic in onset. In practice that generally means, irritant contact dermatitis will appear very early and also heal very promptly, we can speak of a fast onset and fast recovery phenomena. In allergic contact dermatitis, there is a rather slow onset and slow recovery phenomena, which differentiates it from irritant contact dermatitis. Another differentiating factor is the nature of discomfort. Allergic contact dermatitis has more of a pruritic nature rather than burning, whereas in irritant contact dermatitis patients usually complain of burning rather than itching (15). Furthermore, in irritant contact dermatitis the first exposure to a substance will lead to inflammation, whereas in allergic contact dermatitis the first contact to the allergen will mostly be asymptomatic and re-exposure is necessary for inflammation to occur. In irritant contact dermatitis, the inflammation is usually limited to the place of exposure and the amount and time of the substance applied to the skin seems to be proportional to the severity of reaction. In allergic contact dermatitis the inflammation can be more disseminated and generalized and only a small fraction of substance can lead to an immense reaction (16).

Even for experienced and trained professionals it is sometimes hard to differentiate between irritant and allergic contact dermatitis (15). The character and distribution of inflammatory lesions can give important clues about the underlying cause of dermatitis.

1.1.5. Character and distribution of dermatitis

The character and distribution of contact dermatitis can tell you a lot in the investigation of a potent allergen or irritant. For a precise analysis of the lesions, it is important to note some important facts about the distribution and character of the inflammatory lesions. First, the inflammatory skin reactions do not have to be bilateral, even though the exposure of the allergen was bilaterally, an example would be allergic contact dermatitis to gloves (17). Second, it is important to note that it can involve the palms and soles as well (18). Third, the character of the inflammatory lesions can be patchy despite of a uniformly distributed exposure, for example in contact dermatitis of face or hand lotions (19). It is also important to note, that allergic and irritant contact dermatitis can present in a variety of possibilities, including non-eczematous skin lesions or lichen planus like lesions. It is not always the typically explained clinical picture.

However, it appears most of the time at the actual area of exposure in an eczematous inflammatory pattern. In allergic contact dermatitis, the skin will show signs of diffuse erythema, including papules and it can present with vesicular lesions in severe reactions (20). In addition, the skin will be infiltrated with inflammatory cells, due to the attraction of leukocytes to the dermis, as explained above. In irritant contact dermatitis, the severity of lesions will depend on the amount and time of exposure to the irritant substance. When interpreting the Patch Test results, it will appear as a discrete patchy erythematous lesion and there will be no infiltration of inflammatory cells (16). Usually the margins are well demarcated and there are no signs of edematous skin lesions. As a guidance to help differentiating between allergic and irritant reaction, one should keep in mind that the onset and recovery in irritant dermatitis has more acute pattern, than in allergic contact dermatitis.

In general, it is always important to take a detailed history including the occupation of the patient (19). Questions to ask the patient, should always include changes of habits, for example if they changed any products lately, like detergents, creams, new jewelry or fragrances. You should also ask the patient if they recently encountered any known potent natural allergen or irritant, like poison ivy or nickel. In experienced professionals, it is justified to follow the "frequent is frequent" approach to establish a diagnosis (5). At the end, clues in the anamnesis of the patient and the character and distribution of the dermatological lesions will lead you to a

suspicion of a potent differential diagnosis, but ultimately only the Patch Test can give you the guarantee of a certain diagnosis (21).

1.1.6. Cross Reactions

Cross reactivity, also called cross-sensitization, is the phenomena in which one primary allergen sensitizes the cells to itself plus another secondary allergen, which shares structural similarity. The sensitized cells react to both allergens, as if they were the same, causing contact dermatitis (10).

1.2 Patch Test

1.2.1. Definition

The Patch Test is the universal and only tool of dermatologists worldwide to evaluate and diagnose allergic contact dermatitis to a specific allergen (20). The procedure is identical all over the world and therefore applicable everywhere. Nevertheless, it is important to note that the reading and interpretation of the results can only be performed by specialists and trained individuals (21).

In most cases, there is more than one allergen tested at the same time, usually clustered on the back of the patient or on the inner parts of the arm. There is a commercial FDA approved ready-to-us Patch Test available, which simplifies the utilization and practice of testing (22). It is called the T.R.U.E. Patch Test (Thin-layer Rapid Use Epicutaneous Patch Test for topical use only). The T.R.U.E. Test originally includes 23 allergens, plus one control field. Today there is a test available, including up to 35 allergens, plus one control field (23). There is one allergen uniformly distributed on each patch. Besides the commercial Patch Test, there is also the possibility to use individual selected allergens adjusted to each patient, which is more time consuming and expensive, however, it will not allow to miss an allergen otherwise not tested in the commercial standard constitution.

Collection and surveillance of Patch Test data over a period of time is an important tool in recognizing epidemics and trends of contact dermatitis which can be of great significance (1).

1.2.2. Specific Allergens – Natural appearance and explanation

We included twenty-three allergens in our study, based on the patch test model in the dermatovenerological department of the University Hospital Split. The tested allergens have

subjective properties and different natural appearances, leading to less or more severe immunological reactions in the human body. It is important to know the natural occurrence of the allergens as well as their chemical consistencies.

Potassium dichromate 0.5% whose active allergenic compound is chromium, is found in cement, cement adhesives, cement hardeners, gum, glass, bleaching agents, surgical tools made of chrome, cordial for tanning skin, shoes, leather, fur, yellow and orange color (eg. in ceramics), wood preservatives, detergents containing phosphate, matches, inks, paper goods, photographic chemicals, tv antennas, degreasing agents for grill and ovens, cooling oils and linoleum (24).

Cobalt Chloride 1% whose active allergenic component is cobalt, is an ingredient in cobalt pigments, and it is used in a variety of colors such as textile, rubber, synthetic resins, glass, porcelain and ceramic glazes, enamel, watercolor, printing and masonry paints, fluorescent color, tattoo color and color in chalk. Cobalt is also used as a catalyst in the chemical and pharmaceutical industries, used as a drying agent in varnishes and paints for synthetic resins, used as a supplement to animal food and used in vitamin preparations (25).

Balsam of Peru 25% is a resin found in a specific tree in South America, Myroxylon balsamum pereirae. It is a mixture of different compounds, but the exact composition is still not completely known. Its known components are benzyl benzoate, benzyl cinnamate, cinnamic acid alcohol, benzoic acid, vanillin and nerolidol. It is found in various local medicines, earplugs, cosmetics, lipstick, perfumes, hair lotions, childcare products, insect repellents, sunscreens, suntan lotions, fragrances for shampoos and soaps, dental materials and toothpaste. The mixture is also used to accelerate granulation and the healing of wounds. It is also used in flavoring foods such as citrus fruits, marmalade, pastries, chewing gum, flavored tea, tobacco, cough syrups, ice cream, coca cola, pickled vegetables, root vegetables, vanilla, chocolate, curry, ketchup, chili sauce, vermouth and beverages with root extract (26).

Epoxy Resin 1% consists of diglycidylether of bisphenol A, which is the active allergenic compound of the substance. It is used to make vacuum tubes, sheaths for electrical installations, floor covering and wall covering. Epoxy resin is also used as a constituent of plaster, it is found in cement, adhesives for glass metals and ceramics, used as anti-rust agents, wood and metal vanishes, glass wool fastener, dental prosthetics and used in production of pesticides (27).

Rosin is prepared for the Patch Test as 20% Rosin prepared in petroleum jelly. Rosin is, by the process of distillation, the solid form of Resin. It is composed of a series of organic acids,

mostly resin acids, which are related to terpenes. The main active ingredient of rosin is abietic acid. Pine is the main source of rosin. It is inflammable but has a low toxicity.
It is used as an ingredient in high-pressure adhesives, adhesive tapes and plasters, isolation materials, floor tiles, linoleum lining, cement and corrosion inhibitors. It is also used in the cosmetic industry as an ingredient for eye shadow, mascara, lip gloss, nail polish and depilatory wax (28).

Ammoniated Mercury 10% is a metal used in instruments, tooth fillings, disinfectants, skin-lightening creams, fungicides, herbicides, insecticides, detonators, emulsion paints, jewelry, as well as in the production of soda and chlorine. In medicine, ammoniated mercury is used in the topical treatment of psoriasis (29).

Benzocaine 5% is a composition of five percent benzocaine, an ethyl ester of p-aminobenzoic acid (PABA), prepared in petroleum jelly for the Patch Test. Benzocaine 5% is mostly used as a local anesthetic in medical practice. It is also used in treating burns to the relief pain and itching. Furthermore, it is found in cough dragees and lozenges for the throat, depilatory creams, tanning preparations, shaving cream and soap (30).

Rubber Antioxidant IPPD 0.1%, N-isopropyl-N-phenyl-para-phenylenediamine (IPPD), is an antioxidant, or stabilizer, used in rubber. It is a black-greyish compound, non-soluble in water, and highly inflammable. Contact and irritation of the skin with IPPD, leads to a very distinct pattern and distinct reddish color. It is used as an antiozonant in rubbers, particular tires, to protect them from drying and cracking. People allergic to IPPD, can also react on hair dyes and derivatives of paraphenyldiamine (31).

Rubber- Mercapto 2% the mixture consists of four compounds, which are Mercaptobenzothiazol, Dibenzothiazol disulfide, N-Cyclohexylbenzothiazol Sulfonamide and Morpholinylmercaptobenzothiazol. Rubber-Mercapto is used to vulcanize rubber, which improves its elasticity and stability. The World Health Organization (WHO) identified Rubber-Mercapto as a possible carcinogen to humans (31).

Rubber - Thiuram 1% the mixture consists of three compounds, which are tetramethyl thiuram monosulfide, tetramethyl thiuram disulfide, and tetraethyl thiuram disulfide. All three compounds are moderately toxic and are irritative to the skin and eye. Thiuram is used in the vulcanization of rubber which improves its elasticity and strength. Therefore, Thiuram is for instance found in heat-resistant tires, mattresses, rubber boots, raingear, shoes, swimming caps, rubber bands in underwear, stockings and socks, flexible rubber tubes and even condoms. In medicine Thiuram can be found in catheters, stethoscopes, elastic bandages, antifungal and anti-Scabies preparations, as well as preparations used to treat chronic alcoholism (31).

Rubber - Carbamate 3% consists of three compounds, including 1,3-diphenylguanidine (DPG), Zinc bis Diethyldithiocarbamate (ZDC) and Zinc bis Dibutyldithiocarbamate (ZBC). All three are whitey, scaly substances. ZDC is a very strong irritant of the eye lenses. The components of this mixture are used as accelerators in the vulcanization of rubber and are especially found in latex dispersions and foams (31).

Coal Tar 12% is the composition of twelve percent Coal tar prepared in petroleum jelly. Coal Tar is used in the cosmetic industry as a base for perfumes, in the chemical industry it is used in the production of turpentine, in medicine it is found in cough syrup and it also serves as a supplier of fat in many preparations, including ointments for various skin diseases as psoriasis and anti-fungal topical preparations. Coal tar is also used as a softener in the plastic and rubber industries, it can be found in paints, varnishes, isolation material, cement and asphalt (32).

Mixture of Parabens 15% is found in various topical eye, nose and ears drops, vaginal pessaries, ampules drugs, powders, bandage material, cosmetics, electrode traps, cleaning wipes, food articles for example ketchup, puree, vegetables, syrup, marmalade, jelly, preserved fish and sausage (33).

Neomycin sulfate 20% is an aminoglycoside antibiotic and usually prepared as an ointment or cream. It is used to treat superficial skin, eye and ear infections. It is also found in various cremes, powders, lotions, sprays, vaccines, cosmetics, dental preparations, soaps, deodorants, food article and preparations in veterinary medicine (30).

Petroleum jelly, colloquial known as Vaseline, is a semi-solid colorless compound, consisting of hydrocarbons. It is most known for its lubricating and coating properties in lip and skin care. It is also used to improve the preparation of various agents for Patch testing, including Coal tar, Benzocaine and Rosin (34).

Mixed Fragrances usually contains a variety of different components that can be obtained from natural or synthetic sources and from animal or vegetable sources. In our study the mixture contains hexyl cinnamic aldehyde, coumarin, farnesol, liral, citral, and citronela. The mixture is used to add flavor or aroma to various products, including the following toiletries and cosmetics, bathing oils, tube paste, detergent, hair care, lotion, cremes, shampoo, mouth wash. They are an integral part of some food and spices to add aroma to the product (35).

Lanolin is a natural product derived from sheep wool containing a mixture of fatty alcohols, sterols, and fatty acids. Fatty alcohols are considered to be the allergenic component responsible for sensitization to lanolin. As lanolin is a biological product, the number of allergens may be different in preparations from different sources. Hypersensitivity to lanolin

can be significantly increased in people with eczema-type skin diseases. In addition to natural lanolin, there are modified preparations, such as e.g. acetylated, hydrogenated and dewaxed lanolin, which are less sensitive than the natural product. The cosmetic industry is increasingly using hydrogenated lanolin, which can cause sensitization in people who have not been found sensitive to natural lanolin. Lanolin is present in skin care products, it is also used in industrial processes in processing of fur, leather and paper. It is also present in wore shoe creams, parquet waxes and some corticosteroid fats may contain lanolin for topical use (36).

Thiomersal is an organic compound of Mercury and mainly used as a preservative. It is also known for its antifungal and antiseptic properties. In medicine, it is especially used as a preservative for vaccines but also for immunoglobin preparations and eye and ear preparations. Thiomersal is very toxic when inhaled, ingested or contact with the skin (30).

Quaternium-15 is a white crystalline formaldehyde-releasing preservative (FRP), commonly used in cosmetics. Quaternium is an antimicrobial agent and one of the most commonly used preservatives. In cosmetics it is added to cosmetics to provide protection against fungus, mold and pseudomonas. It is found in various hair products, eye shadow, make-up bases, lotions, cremes, shaving preparations, bath salts, liquid soaps, various powders, moisturizers and cleaning supplies. Since quaternium releases formaldehyde, most people having a reaction to quaternium will also show a reaction to formaldehyde (37).

Phenylmercury acetate 0.01% (in water) appears as an odorless white crystalline powder when not prepared in purified water. It is toxic when inhaled, ingested or absorbed by the skin. Contact with the eyes and skin can cause burns. Phenylmercury acetate can be present in contraceptives, cosmetics, ophthalmologic solutions, shampoo, colors and nasal agents. They are added to eye solutions for their effective action on bacterial infections. Mostly it is used for its antiseptic properties in humans, but it can also be used as a herbicide and fungicide (38).

Nickel Sulfate 5% is an ubiquitous metal in crystalline form with six to seven water molecules. Nickel is found in many products including jewelry, suspenders, zippers, button snaps, belt buckles, eye- glasses frames, cell phones, nickel-containing coins, keys, musical instruments and implantable medical devices. It is also used in the production of insecticides and fungicides. It is important to note, that many nickel-containing materials contain both chromium and/or cobalt, leading to a small amount of hypersensitivity to those products as well (39).

Formaldehyde 1% (in water) is a colorless gas, with preservative and disinfectant properties, often used for histological and anatomical preparations. In medicine it is used for vaccines, bandages, ampoules, dialysis solutions, dental materials, local contraceptives and

preservation of pathological samples. Besides its medical uses, formaldehyde is also found in ointments, creams, cosmetics, deodorants, shampoos, soaps, bath foams, hair and body care products, nail polish and toothpaste, to name a few. It is also used for textile items to achieve additional qualities by means of waterproofing or as an antistatic. Interestingly, it can also be found in all types of smoke, including cigarette smoke (40).

Paraphenylene-diamine (Ursol 5%) is a crystalline compound, easily absorbed through the skin and toxic by inhalation. Paraphenyl-diamine is found in hair and fur dyes, which leads to an increasing number of contact dermatitis in hairdressers and furriers. It is also used in the leather industry and vulcanization of tires. It is an ingredient in printing inks and lithography (41).

1.2.3. Interpretation of the Patch Test

The interpretation of the Patch Test is a very important feature and potential source of bias in the field of patch testing (42). Medical professionals might interpret the same reactions differently. However, there is a universal guide for the analysis of patch test results from T.R.U.E. TEST (Thin-layer Rapid Use Epicutanous Patch Test), a global FDA approved Patch Test used since the nineties (23). There are five different options for the interpretation. The first option is a weak positive reaction, meaning erythema, infiltration and possible papules. It is indicated by one plus (+). The second option, is a strong positive reaction, meaning erythema, infiltration and possible vesicles, indicated by two pluses (++). The third option for interpretation, is an extreme positive reaction, meaning intense erythema, intense infiltration and possible coalescing vesicles, indicated by three pluses (+++) (43). The fourth option is a doubtful reaction, indicated by faint erythema and no infiltration. Professionals will tag this with a question mark (?). The last and fifth option indicates an irritant reaction, meaning discrete patchy erythema and no infiltration. The results are reviewed once after 48 hours and once after 72 hours (44). The interpretation of the time lapse is of great significance to differentiate between possible allergic or irritant contact dermatitis. This is also a potential source of bias, if two different professionals review the results at different times and interpret them differently. It is important to note that some reactions will occur within 48 hours and vanish within 72 hours, as well as some reactions will not appear within 48 hours but will within 72 hours. There is also the possibility that a reaction will appear and remain positive for the whole testing time. Some reactions will start off less severely and progress to a more severe reaction in time, as well as the other way around. The test results should always be collected precisely and afterwards saved in a data pool for further analysis (1).

2. OBJECTIVES

2.1. Aims of the study

The aim of this study is to evaluate the epidemiological and clinical features of contact dermatitis. We used the experience of the dermatovenerological clinic of the University Hospital Split, from 2013 to July 2019. It is of great significance and importance to collect and analyze Patch Test data over a period of time, in order to recognize epidemics and trends of contact dermatitis. If a specific trend or epidemic is recognized by the analysis, it is important to ask and search for possible causes. This in turn can lead to future disease prevention and health benefits for the whole population.

2.2. Hypothesis

1. There is a specific trend of contact dermatitis in the population of patients recorded at the allergological ambulance in University Hospital Split, from 2013 till July 2019.
2. There is a noted difference between the testing after 48 hours and 72 hours according to irritant or allergic contact reactions.
3. There are different results for the female and male population in our epidemiological study.
4. Some allergens have the tendency to appear more commonly together.
5. Some allergens have the tendency to cause a more severe reaction than others.
6. We can observe cross reactions in our study population

3. MATERIALS AND METHODS

3.1. Study Population

An epidemiological study was conducted with the data from Patch test results of the allergological ambulance from 2013 till July 2019 at the department of dermatology and allergology, University Hospital Split. After applying inclusion and exclusion criteria, the data collection included 3228 patients in total, whereof 2279 patients were of female gender and 949 were of male gender. Inclusion criteria for this study are all standardized patch test data recorded at the University Hospital Split from 2013 till July 2019. Exclusion criteria for this study are specific hair dye and medication testings.

As explained above, we included twenty-three allergens in our study, based on the Patch Test model in the dermatovenerological department of the University Hospital Split. The results were interpreted from a weak positive reaction (+), strong positive reaction (++) up to an extreme positive reaction (+++/++++). We also included a time-lapse factor, including the reaction after 48 hours and the reaction after 72 hours.

3.2. Methods of data collection and processing

The data was collected from record books in the allergological ambulance of University Hospital Split and inserted into the data base program Microsoft Access. From Microsoft Access the data was transferred to Microsoft Excel, forming a table including and combining all factors considered. In the epidemiological analysis we used a filter system to refine different factors and interpret the results.

3.3. Bias

The study has an intermediate risk of bias. There are a few steps in the data collection which are predisposed to bias. First, we already explained before that even experienced and educated professionals sometimes have difficulties interpreting the results of the Patch Test. The differentiation of irritant and allergic contact dermatitis can be very difficult, it demands more than just the interpretation of the skin after Patch testing. A detailed history can be of great significance in the differentiation of possible causes. Second, there can be more than one professional involved in the interpretation of the Patch Test. One professional could interpret the results after 48 hours and a different one could interpret the results after 72 hours, leading to different results. Third, there is always the possibility that an individual is not trained enough to interpret the results correctly, leading to a biased result.

4. RESULTS

4.1. Baseline Characteristics after 48 hours and 72 hours

In the epidemiologic study of Patch Test data from 3228 patients in total, we had the following baseline characteristics. After 48 hours our patients had the following results which can be observed in (Table 1) and after 72 hours we had following results which can be observed in (Table 2).

The most common allergen causing a reaction in our population, is Cobalt Chloride 1% with 29.70% of all patients reacting to it after 48 hours. After 72 hours, Cobalt Chloride 1% is still the most common allergen causing reaction, with a slight decrease to 26.60% of the whole study group. The second most common allergen is Nickel Sulfate 5%, with 19.80% of all patients reacting to it after 48 hours and slightly more reacting to it after 72 hours with 20.50%. Interestingly, Potassium Dichromate 0.5% is the third most common allergen after 48 hours (14.53%) but not after 72 hours. After 72 hours the third most common allergen is Thimerosal with 11.03% and Potassium Dichromate 0.5% is fourth most common, with 9.57%. It can already be noted for the first four allergens, that some reactions appear within 48 hours and become less apparent after 72 hours or the other way around. In the following, we emphasize on similar cases to point out the importance of the time lapse between result interpretation. Balsam of Peru 25% almost halves their cases with 10.60% after 48 hours and 5.89% after 72 hours (Table 1 and Table 2). Similar phenomena are seen in other cases, to give another example, Ursol 5% decreases its numbers from 4.65% (48 hours) to 1.70% (72 hours). Nickel Sulfate 5%, Thimerosal and Ammoniated Mercury 10% are the only cases to point out the reverse phenomena, by increasing their numbers of reactions after 72 hours. Thimerosal increases from 9.94% after 48 hours to 11.02% after 72 hours. Nickel Sulfate 5%, as noted above already, increases from 19.80% after 48 hours to slightly more after 72 hours with 20.50%. Ammoniated Mercury 10% slightly increases their numbers from 7.06% after 48 hours to 7.68% after 72 hours.

Overall, the differences in time of the appearance of a reaction, indicates if the reaction is irritant or allergic in origin. Based on that character we can decide if the causative allergen is causing an allergic contact reaction or irritant contact reaction.

Table 1. Percentage of the whole population after 48 hours

Patch Test Allergens	Percentage after 48 hours
Cobalt Chloride 1%	29.70%
Nickel Sulfate 5%	19.80%
Potassium dichromate 0.5%	14.53%
Coal Tar 12%	13.51%
Balsam of Peru 25%	10.60%
Thimerosal	9.94%
Ammoniated Mercury 10%	7.06%
Formaldehyde 1% (in water)	6.13%
Phenylmercury acetate 0.01% (in water)	4.80%
Paraphenylene-diamine (Ursol 5%)	4.65%
Mixed Fragrances	4.55%
Rubber - Carbamate 3%	3.90%
Rosin	3.75%
Mixture of Parabens 15%	3.16%
Rubber - Thiuram 1%	2.97%
Benzocaine 5%	2.85%
Vaseline	2.45%
Epoxy Resin 1%	1.95%
Lanolin	1.90%
Rubber- Mercapto 2%	1.86%
Neomycin sulfate 20%	1.83%
Rubber Antioxidant IPPD 0.1%	1.18%
Quaternium 15	1.18%

Table 2. Percentage of the whole population after 72 hours

Patch Test Allergens	Percentage after 72 hours
Cobalt Chloride 1%	26.60%
Nickel Sulfate 5%	20.50%
Thimerosal	11.03%
Potassium dichromate 0.5%	9.57%
Coal Tar 12%	8.80%
Ammoniated Mercury 10%	7.68%
Balsam of Peru 25%	5.89%
Mixed Fragrances	3.97%
Rubber - Carbamate 3%	2.48%
Formaldehyde 1% (in water)	2.26%
Rosin	2.14%
Rubber - Thiuram 1%	2.14%
Benzocaine 5%	1.95%
Neomycin sulfate 20%	1.74%
Paraphenylene-diamine (Ursol 5%)	1.70%
Phenylmercury acetate 0.01% (in water)	1.67%
Mixture of Parabens 15%	1.24%
Vaseline	1.05%
Lanolin	1.05%
Rubber- Mercapto 2%	1.02%
Epoxy Resin 1%	0.99%
Rubber Antioxidant IPPD 0.1%	0.80%
Quaternium 15	0.71%

4.2. Gender characteristics

In the following, emphasis is drawn to the Patch Test results of each gender and possible differences of them. Our study includes 2279 females and 949 males, which already concludes that there is a potential positive tendency of female gender to contact dermatitis. An overview of the Patch Test results after 48 hours and after 72 hours for each gender individually is summarized in Table 3 and Table 4 respectively.

After 48 hours, the most common allergen causing a reaction is Cobalt Chloride 1%, for both equally, the male and female study population. There is a slightly higher number in females than in males with 30.72% and 27.19% respectively. For the male population, the second most common allergen is Potassium Dichromate 0.5% with 17.80% of the population positively tested after 48 hours. In the female population, the second most common allergen is Nickel Sulfate 5% with 22.99% of the population positively tested after 48 hours. Interestingly, only 12,12% (48 hours) of the male population were positive for Nickel Sulfate 5%, thus only half of the female population positive to Nickel Sulfate 5%. Based on the results of Table 3 and Table 4, there are significant more males than females positive to Thimerosal, Balsam of Peru 25%, Coal Tar 12% and Potassium Dichromate 0.5%. On the other hand, significant more females are positive to Nickel Sulfate 5% and Cobalt Chloride 1%. Ammoniated Mercury 10% has an equal distribution in both gender groups, with exact 7.06% (48 hours) for the female and the male population. Similar equal distribution is seen for Rosin with 3.68% and 3.77% (48 hours) for the male and female population respectively. Interestingly, in both groups the amount of positive reactions to Rosin declines after 72 hours. In the female population the numbers decrease to 2.37%, whereas in the male population a steeper decrease to 1.58% can be noted, indicating an irritant reaction. In the case of Rubber Thiuram 1%, the male population has overall 3.05% positive reactions to it after 48 hours. In contrast, after 72 hours the percentage approximately halves to 1.47%, indicating an irritant contact reaction. In the female population on the other hand, there are overall 2.94% positive reactions to Rubber Thiuram 1% after 48 hours, and still overall 2.41% positive reactions after 72 hours, which concludes to an allergic contact reaction.

Overall, we can observe based on the results of Table 3 and Table 4, that there are differences in the results of the male and female population.

Table 3. Total Percentage of male and female population after 48 hours

Total Percentage of Male and Female population	**Male 48 Hours**	**Female 48 Hours**
Potassium dichromate 0.5%	17.80%	13.16%
Cobalt Chloride 1%	27.19%	30.72%
Balsam of Peru 25%	12.96%	9.60%
Epoxy Resin 1%	2.42%	1.75%
Rosin	3.68%	3.77%
Ammoniated Mercury 10%	7.06%	7.06%
Benzocaine 5%	3.58%	2.54%
Rubber Antioxidant IPPD 0.1%	1.69%	0.95%
Rubber- Mercapto 2%	2.95%	1.40%
Rubber - Thiuram 1%	3.05%	2.94%
Rubber - Carbamate 3%	4.95%	3.46%
Coal Tar 12%	15.81%	12.51%
Mixture of Parabens 15%	4.85%	2.46%
Neomycin sulfate 20%	2.10%	1.71%
Vaseline	2.74%	2.34%
Mixed Fragrances	4.32%	4.65%
Lanolin	2.95%	1.45%
Thimerosal	13.70%	8.38%
Quaternium 15	1.26%	1.14%
Phenylmercury acetate 0.01% (in water)	6.06%	4.30%
Nickel Sulfate 5%	12.12%	22.99%
Formaldehyde 1% (in water)	6.53%	5.97%
Paraphenylene-diamine (Ursol 5%)	5.90%	4.13%

Table 4. Total Percentage of Male and female population after 72 hours

Total Percentage of Male and Female population	**Male 72 Hours**	**Female 72 Hours**
Potassium dichromate 0.5%	11.80%	8.64%
Cobalt Chloride 1%	22.55%	28.30%
Balsam of Peru 25%	7.16%	5.35%
Epoxy Resin 1%	1.37%	0.83%
Rosin	1.58%	2.37%
Ammoniated Mercury 10%	7.16%	7.90%
Benzocaine 5%	2.21%	1.84%
Rubber Antioxidant IPPD 0.1%	1.16%	0.66%
Rubber- Mercapto 2%	0.94%	1.05%
Rubber - Thiuram 1%	1.47%	2.41%
Rubber - Carbamate 3%	2.74%	2.36%
Coal Tar 12%	9.06%	8.68%
Mixture of Parabens 15%	1.16%	1.27%
Neomycin sulfate 20%	1.58%	1.80%
Vaseline	0.84%	1.41%
Mixed Fragrances	3.26%	4.25%
Lanolin	1.26%	0.96%
Thimerosal	13.07%	10.18%
Quaternium 15	0.73%	0.70%
Phenylmercury acetate 0.01% (in water)	2%	1.53%
Nickel Sulfate 5%	9.59%	25.01%
Formaldehyde 1% (in water)	2.21%	2.28%
Paraphenylene-diamine (Ursol 5%)	1.37%	1.84%

4.3. Characteristic of severity

The characteristics of severity for each allergen are summarized in Table 5 (after 48 hours) and Table 6 (after 72 hours). The severity grades start from one, a mild reaction, up to four, a severe reaction. The explanation of interpreting the results is explained above.

The highest percentage can be noted in severity grade one. Cobalt Chloride 1% is the allergen with the highest percentage in that severity grade, with 19.39% after 48 hours and 10.53% after 72 hours. Second, Potassium Dichromate 0.5% and Coal Tar 12% show relatively high numbers with approximate 11% after 48 hours and declining percentage after 72 hours. Similar phenomenon is seen in Balsam of Peru 25%, with 8.92% after 48 hours and 3.47% after 72 hours. It can be noted that for severity grade one, the numbers decrease significantly after 72 hours for each allergen.

Severity grade three is less common than the severity grades one and two. In our study population, the allergen with the highest numbers of this severity grade is Nickel Sulfate 5%. The numbers approximately double after 72 hours, with 4.86% after 48 hours and 10.84% after 72 hours. It is the opposite phenomenon of what we noted in severity grade one and two. Cobalt Chloride 1 % shows a similar phenomenon, with 1.86% after 48 hours and 3.78% after 72 hours, it is the allergen with the second highest numbers in severity grade three. Another example is Thimerosal, which has the third highest numbers for severity grade three. The numbers increase from 0.65% after 48 hours up to 2.14% after 72 hours. It can be noted that most of the allergens increase their numbers after 72 hours for severity grade three, in contrast to severity grade one and two, where numbers rather decrease after 72 hours.

Severity grade four is less than one percent for each allergen, in both results, after 48 hours and 72 hours. Some of the allergens do not even have a severe grade four reaction. However, Nickel Sulfate 5% is the allergen with the highest numbers in our study population with a reaction of severity grade four, 0.40% after 48 hours and 0.65% after 72 hours. These numbers are significantly lower than in severity grade three reactions. We can also note, that for grade four severity, the numbers rather increase after 72 hours rather than decrease.

In summary, we can conclude that in our study population mild reactions are by far more common than severe reactions. More severe reactions occur more often after 72 hours rather than 48 hours, whereas mild reactions occur early rather than late.

Table 5. Characteristic of severity (Numbers in percentage) after 48 hours

Severity Grades	1	2	3	4
Potassium Dichromate 0.5%	11.25	2.51	0.71	0.06
Cobalt Chloride 1%	19.39	8.40	1.86	0.06
Balsam of Peru 25%	8.92	1.27	0.40	-
Epoxy Resin 1%	1.67	0.22	0.06	-
Rosin	2.63	0.53	0.43	0.15
Ammoniated Mercury 10%	4.52	2.08	0.43	0.03
Benzocaine 5%	1.98	0.68	0.15	0.03
Rubber Antioxidant IPPD 0.1%	1.05	0.12	-	-
Rubber- Mercapto 2%	1.55	0.19	0.09	0.03
Rubber- Thiuram 1%	2.01	0.65	0.28	0.03
Rubber- Carbamate 3%	3.00	0.68	0.22	-
Coal Tar 12%	11.12	2.11	0.28	-
Mixture of Parabens 15%	2.85	0.15	0.15	-
Neomycin sulfate 20%	1.64	0.19	-	-
Vaseline	2.04	0.28	0.12	-
Mixed Fragrances	3.13	1.05	0.31	0.06
Lanolin	1.70	0.15	0.03	-
Thimerosal	7.19	2.11	0.65	-
Quaternium 15	1.12	0.06	-	-
Phenylmercury acetate 0.01% (in water)	3.87	0.81	0.12	-
Nickel Sulfate 5%	8.27	6.26	4.86	0.40
Formaldehyde 1% (in water)	5.36	0.71	0.03	0.03
Paraphenylene-diamine (Ursol 5%)	3.90	0.59	0.09	0.06

Table 6. Characteristic of severity (Numbers in percentage) after 72 hours

Grades of Severity	1	2	3	4
Potassium Dichromate 0.5%	4.86	2.94	1.70	0.06
Cobalt Chloride 1%	10.53	12.14	3.78	0.15
Balsam of Peru 25%	3.47	1.80	0.56	0.06
Epoxy Resin 1%	0.71	0.09	0.19	-
Rosin	0.74	0.50	0.71	0.19
Ammoniated Mercury 10%	4.21	2.45	0.87	0.15
Benzocaine 5%	0.87	0.65	0.40	0.03
Rubber Antioxidant IPPD 0.1%	0.62	0.15	-	0.03
Rubber- Mercapto 2%	0.46	0.40	0.12	0.03
Rubber- Thiuram 1%	0.87	0.81	0.46	-
Rubber- Carbamate 3%	1.55	0.71	0.22	-
Coal Tar 12%	5.76	2.39	0.65	-
Mixture of Parabens 15%	0.71	0.43	0.09	-
Neomycin sulfate 20%	0.77	0.59	0.37	-
Vaseline	0.56	0.28	0.09	0.12
Mixed Fragrances	1.83	1.24	0.74	0.15
Lanolin	0.71	0.25	0.09	-
Thimerosal	4.86	3.97	2.14	0.06
Quaternium 15	0.50	0.19	0.03	-
Phenylmercury acetate 0.01% (in water)	1.02	0.53	0.12	-
Nickel Sulfate 5%	3.38	5.61	10.84	0.65
Formaldehyde 1% (in water)	1.27	0.68	0.25	0.06
Paraphenylene-diamine (Ursol 5%)	0.71	0.56	0.40	0.03

4.4. Frequent combinations of allergens

In the following tables we can observe the most common combinations of allergens occurring together.

In Table 7, we can observe the most common positive allergens in combination with Potassium Dichromate 0.5% after 72 hours in a descending order. The allergen with the most frequent combination to Potassium Dichromate 0.5% is Cobalt Chloride 1% with 73.95%. Second most frequent combination is seen for Nickel Sulfate 5% with 38.60%. High percentage are also seen for Thimerosal (14.88%), Coal Tar 12% (12.56%) and Balsam of Peru 25% (12.09%). Lowest numbers (1.40%) are seen for Epoxy Resin 1% and Rubber Antioxidant IPPD 0.1% (see Table 7).

Table 7. The most common positive allergens in combination with **Potassium Dichromate 0.5%** of the whole population in percentage

Combinations to Potassium Dichromate 0.5%	**72 hours**
Cobalt Chloride 1%	73.95
Nickel Sulfate 5%	38.60
Thimerosal	14.88
Coal Tar 12%	12.56
Balsam of Peru 25%	12.09
Ammoniated Mercury 10%	9.30
Rosin	5.58
Rubber - Carbamate 3%	5.58
Mixed Fragrances	5.12
Rubber - Thiuram 1%	4.65
Neomycin sulfate 20%	4.65
Mixture of Parabens 15%	3.72
Formaldehyde 1% (in water)	3.26
Phenylmercury acetate 0.01% (in water)	3.26
Paraphenylene-diamine (Ursol 5%)	2.79
Benzocaine 5%	2.33
Vaseline	2.33
Lanolin	2.33
Rubber- Mercapto 2%	1.86
Quaternium 15	1.86
Epoxy Resin 1%	1.40
Rubber Antioxidant IPPD 0.1%	1.40

In Table 8, we can observe the most common positive allergens in combination with Cobalt Chloride 1% after 72 hours in a descending order. The allergen with the most frequent combination to Cobalt Chloride 1% is Nickel Sulfate 5% with almost halve of the population being also positive to it (48.13%). Second most frequent combination is Potassium Dichromate 0.5% with 26.48%. We can also observe high numbers for Coal Tar 12% and Thimerosal (see Table 8).

Table 8. The most common positive allergens in combination with **Cobalt Chloride 1%** of the whole population in percentage

Combinations to Cobalt Chloride 1%	**72 hours**
Nickel Sulfate 5%	48.13
Potassium Dichromate 0.5%	26.48
Thimerosal	12.31
Coal Tar 12%	9.03
Ammoniated Mercury 10%	6.07
Balsam of Peru 25%	5.30
Mixed Fragrances	4.36
Rubber - Carbamate 3%	4.21
Rosin	3.12
Formaldehyde 1% (in water)	2.65
Phenylmercury acetate 0.01% (in water)	2.49
Neomycin sulfate 20%	2.49
Rubber - Thiuram 1%	2.34
Paraphenylene-diamine (Ursol 5%)	1.71
Epoxy Resin 1%	1.71
Benzocaine 5%	1.56
Rubber- Mercapto 2%	1.56
Quaternium 15	1.56
Lanolin	1.40
Mixture of Parabens 15%	1.25
Vaseline	1.25
Rubber Antioxidant IPPD 0.1%	0.93

In Table 9, we can observe the most common positive allergens in combination with Balsam of Peru 25% after 72 hours in a descending order. The allergen with the most frequent combination to Balsam of Peru 25% is Coal Tar 12% with 33.04%, followed by Cobalt Chloride 1% with 22.32%. Quiet high numbers of combination are also seen for Mixed Fragrances (17.86%) and Nickel Sulfate 5% (15.18%). Lowest numbers (0.89%) of combinations are seen for Mixture of Parabens 15% and Paraphenylene-diamine (Ursol 5%).

Table 9. The most common positive allergens in combination with **Balsam of Peru 25%** of the whole population in percentage

Combinations to Balsam of Peru 25%	**72 hours**
Coal Tar 12%	33.04
Cobalt Chloride 1%	22.32
Mixed Fragrances	17.86
Nickel Sulfate 5%	15.18
Potassium Dichromate 0.5%	14.29
Thimerosal	13.39
Ammoniated Mercury 10%	10.71
Rosin	8.04
Vaseline	7.14
Rubber - Carbamate 3%	4.46
Rubber - Thiuram 1%	3.57
Benzocaine 5%	3.57
Epoxy Resin 1%	3.57
Phenylmercury acetate 0.01% (in water)	2.68
Rubber- Mercapto 2%	2.68
Rubber Antioxidant IPPD 0.1%	2.68
Quaternium 15	2.68
Formaldehyde 1% (in water)	1.79
Lanolin	1.79
Mixture of Parabens 15%	0.89
Paraphenylene-diamine (Ursol 5%)	0.89
Neomycin sulfate 20%	-

In Table 10, we can observe the most common positive allergens in combination with Epoxy Resin 1% after 72 hours in a descending order. The allergen with the most common combination to Epoxy Resin 1% is Thimerosal with 50%, followed by Cobalt Chloride 1% and Ammoniated Mercury 10% with 30% for both allergens. Factual no combination is seen for Rubber-Mercapto 2%, Rubber-Carbamate 3%, Neomycin Sulfate 20%, Lanolin, Quaternium-15 and further (see Table 10).

Table 10. The most common positive allergens in combination with **Epoxy Resin 1%** of the whole population in percentage

Combinations to Epoxy resin 1%	**72 hours**
Thimerosal	50.00
Cobalt Chloride 1%	30.00
Ammoniated Mercury 10%	30.00
Coal Tar 12%	20.00
Mixed Fragrances	20.00
Rosin	20.00
Rubber Antioxidant IPPD 0.1%	20.00
Balsam of Peru 25%	10.00
Potassium Dichromate 0.5%	10.00
Nickel Sulfate 5%	10.00
Vaseline	10.00
Mixture of Parabens 15%	-
Paraphenylene-diamine (Ursol 5%)	-
Benzocaine 5%	-
Rubber - Thiuram 1%	-
Phenylmercury acetate 0.01% (in water)	-
Formaldehyde 1% (in water)	-
Rubber- Mercapto 2%	-
Rubber - Carbamate 3%	-
Neomycin sulfate 20%	-
Lanolin	-
Quaternium 15	-

In Table 11, we can observe the most common positive allergens in combination with Rosin after 72 hours in a descending order. The allergens with the most frequent combination to Rosin are, Cobalt Chloride 1% and Potassium Dichromate 0.5% with 42.50% and 32.50% respectively. High numbers are also seen for Nickel Sulfate 5% (25%), Balsam of Peru 25% (20%) and Ammoniated Mercury 10% (17.50%). None combinations are seen for Vaseline, Rubber Antioxidant IPPD 0.5%, Lanolin and further (see Table 11).

Table 11. The most common positive allergens in combination with **Rosin** of the whole population in percentage

Combinations to Rosin	**72 hours**
Cobalt Chloride 1%	42.50
Potassium Dichromate 0.5%	32.50
Nickel Sulfate 5%	25.00
Balsam of Peru 25%	20.00
Ammoniated Mercury 10%	17.50
Coal Tar 12%	15.00
Mixed Fragrances	12.50
Rubber - Thiuram 1%	10.00
Thimerosal	10.00
Rubber - Carbamate 3%	7.50
Epoxy Resin 1%	7.50
Formaldehyde 1% (in water)	5.00
Rubber- Mercapto 2%	5.00
Quaternium 15	5.00
Neomycin sulfate 20%	5.00
Phenylmercury acetate 0.01% (in water)	2.50
Mixture of Parabens 15%	2.50
Benzocaine 5%	-
Lanolin	-
Paraphenylene-diamine (Ursol 5%)	-
Rubber Antioxidant IPPD 0.1%	-
Vaseline	-

In Table 12, we can observe the most common positive allergens in combination with Ammoniated Mercury 10% after 72 hours in a descending order. The allergen with the most frequent combination to Ammoniated Mercury 10%, is Cobalt Chloride 1% with 27.08%. Second most frequent combination is seen for Nickel Sulfate 5% with 25%. High percentage of combination is also seen for Thimerosal (15.63%) and Potassium Dichromate 0.5% (12.50%). Lowest numbers are seen for Mixture of Parabens 15%, Quaternium-15, Epoxy Resin 1% and Formaldehyde 1% with 1.04% for each allergen.

Table 12. The most common positive allergens in combination with **Ammoniated Mercury 10%** of the whole population in percentage

Combinations to Ammoniated Mercury 10%	**72 hours**
Cobalt Chloride 1%	27.08
Nickel Sulfate 5%	25.00
Thimerosal	15.63
Potassium Dichromate 0.5%	12.50
Coal Tar 12%	11.46
Balsam of Peru 25%	10.42
Mixed Fragrances	7.29
Rubber - Carbamate 3%	7.29
Rosin	6.25
Paraphenylene-diamine (Ursol 5%)	5.21
Phenylmercury acetate 0.01% (in water)	4.17
Benzocaine 5%	4.17
Neomycin sulfate 20%	3.13
Lanolin	2.08
Rubber - Thiuram 1%	2.08
Rubber Antioxidant IPPD 0.1%	2.08
Formaldehyde 1% (in water)	1.04
Epoxy Resin 1%	1.04
Mixture of Parabens 15%	1.04
Quaternium 15	1.04
Vaseline	-
Rubber- Mercapto 2%	-

In Table 13, we can observe the most common positive allergens in combination with Benzocaine 5% after 72 hours in a descending order. It shows how much percentage of the Benzocaine 5% positive population also reacts to other allergens. The allergen with the most frequent combination to Benzocaine 5% is Ammoniated Mercury 10% with 22.86%. High percentage of combinations is also seen for Cobalt Chloride 1% (20%), Nickel Sulfate 5% (17.14%) and Thimerosal (17.14%). Lowest percentage of combination or even none, are seen for Neomycin Sulfate 20% and the Rubber allergens (Mercapto, Thiuram, IPPD).

Table 13. The most common positive allergens in combination with **Benzocaine 5%** of the whole population in percentage

Combinations to Benzocaine 5%	**72 hours**
Ammoniated Mercury 10%	22.86
Cobalt Chloride 1%	20.00
Nickel Sulfate 5%	17.14
Thimerosal	17.14
Coal Tar 12%	14.29
Potassium Dichromate 0.5%	11.43
Balsam of Peru 25%	8.57
Lanolin	8.57
Phenylmercury acetate 0.01% (in water)	5.71
Paraphenylene-diamine (Ursol 5%)	5.71
Mixed Fragrances	5.71
Vaseline	2.86
Formaldehyde 1% (in water)	2.86
Epoxy Resin 1%	2.86
Rubber - Carbamate 3%	2.86
Quaternium 15	2.86
Mixture of Parabens 15%	-
Rosin	-
Rubber Antioxidant IPPD 0.1%	-
Rubber- Mercapto 2%	-
Rubber - Thiuram 1%	-
Neomycin sulfate 20%	-

In Table 14, we can observe the most common positive allergens in combination with Rubber Antioxidant IPPD 0.1% after 72 hours in a descending order. The allergen with the most frequent combination to Rubber Antioxidant IPPD 0.1% is Cobalt Chloride 1% with 40% of the population being positive for it as well. High percentage (20%) of combinations are also seen for Balsam of Peru 25%, Nickel Sulfate 5%, Potassium Dichromate 0.5%, Epoxy Resin 1% and Ammoniated Mercury 10%. Virtually none percentage is seen for Phenylmercury acetate 0.01%, Quaternium-15, Rosin, Mixed fragrances and further (see Table 14).

Table 14. The most common positive allergens in combination with **Rubber Antioxidant IPPD 0.1%** of the whole population in percentage

Combinations to Rubber Antioxidant IPPD 0.1%	**72 hours**
Cobalt Chloride 1%	40.00
Balsam of Peru 25%	20.00
Nickel Sulfate 5%	20.00
Potassium Dichromate 0.5%	20.00
Epoxy Resin 1%	20.00
Ammoniated Mercury 10%	20.00
Neomycin sulfate 20%	10.00
Formaldehyde 1% (in water)	10.00
Paraphenylene-diamine (Ursol 5%)	10.00
Mixture of Parabens 15%	10.00
Thimerosal	10.00
Rubber - Thiuram 1%	-
Coal Tar 12%	-
Rosin	-
Benzocaine 5%	-
Rubber- Mercapto 2%	-
Rubber - Carbamate 3%	-
Vaseline	-
Mixed Fragrances	-
Lanolin	-
Quaternium 15	-
Phenylmercury acetate 0.01% (in water)	-

In Table 15, we can observe the most common positive allergens in combination with Rubber – Mercapto 2% after 72 hours in a descending order. The allergen with the most frequent combination to Rubber – Mercapto 2% is Cobalt Chloride 1% with 35.29%. Second most frequent combination is seen for Rubber – Thiuram 1% with 23.53%. High percentage (17.65%) is also seen for Balsam of Peru 25% and Thimerosal. No combination is seen for Epoxy Resin 1%, Benzocaine 5%, Ammoniated Mercury 10%, Quaternium-15 and further (see Table 15).

Table 15. The most common positive allergens in combination with **Rubber – Mercapto 2%** of the whole population in percentage

Combinations to Rubber – Mercapto 2%	**72 hours**
Cobalt Chloride 1%	35.29
Rubber - Thiuram 1%	23.53
Balsam of Peru 25%	17.65
Thimerosal	17.65
Potassium Dichromate 0.5%	11.76
Paraphenylene-diamine (Ursol 5%)	5.88
Rubber - Carbamate 3%	5.88
Coal Tar 12%	5.88
Rosin	5.88
Phenylmercury acetate 0.01% (in water)	5.88
Mixture of Parabens 15%	5.88
Rubber Antioxidant IPPD 0.1%	5.88
Nickel Sulfate 5%	5.88
Formaldehyde 1% (in water)	-
Vaseline	-
Epoxy Resin 1%	-
Ammoniated Mercury 10%	-
Benzocaine 5%	-
Neomycin sulfate 20%	-
Mixed Fragrances	-
Lanolin	-
Quaternium 15	-

In Table 16, we can observe the most common positive allergens in combination with Rubber – Thiuram 1% after 72 hours in a descending order. It shows how much percentage of the Rubber – Thiuram 1% positive population also reacts to other allergens. The allergen with the most frequent combination to Rubber – Thiuram 1% is Rubber – Carbamate 3% with 44.19%. Second most frequent combination is seen for Cobalt Chloride 1% (30.23%) and Nickel Sulfate 5% (27.91%). High percentage is also recognized in Coal Tar 12%, Potassium Dichromate 0.5% and Thimerosal. Low percentage (2.33%) is seen for Formaldehyde 1%, Mixture of Parabens 15%, Lanolin and Vaseline. None percentage of combinations are seen in Epoxy Resin 1%, Neomycin Sulfate 20% and Quaternium-15 and further (see Table 16).

Table 16. The most common positive allergens in combination with **Rubber – Thiuram 1%** of the whole population in percentage

Combinations to Rubber – Thiuram 1%	**72 hours**
Rubber - Carbamate 3%	44.19
Cobalt Chloride 1%	30.23
Nickel Sulfate 5%	27.91
Coal Tar 12%	20.93
Potassium Dichromate 0.5%	18.60
Thimerosal	11.63
Ammoniated Mercury 10%	9.30
Rubber- Mercapto 2%	9.30
Balsam of Peru 25%	6.98
Rosin	6.98
Mixed Fragrances	6.98
Rubber Antioxidant IPPD 0.1%	6.98
Paraphenylene-diamine (Ursol 5%)	4.65
Formaldehyde 1% (in water)	2.33
Mixture of Parabens 15%	2.33
Lanolin	2.33
Vaseline	2.33
Phenylmercury acetate 0.01% (in water)	-
Benzocaine 5%	-
Epoxy Resin 1%	-
Neomycin sulfate 20%	-
Quaternium 15	-

In Table 17, we can observe the most common positive allergens in combination with Rubber – Carbamate 3% after 72 hours in a descending order. The allergens with the most frequent combination are Cobalt Chloride 1% and Rubber – Thiuram 1 % with 40.48%, for both allergens. High percentage of combination is also seen for Nickel Sulfate 5% (33.33%), Coal Tar 12%, Potassium Dichromate 0.5% and Thimerosal (26.19%). Low percentage is seen for Mixed fragrances and Paraphenylene acetate 0.01% (2.38%.). None percentage of combinations is seen in Epoxy Resin 1%, Rubber Antioxidant IPPD 0.1% and Vaseline.

Table 17. The most common positive allergens in combination with **Rubber – Carbamate 3%** of the whole population in percentage

Combinations to Rubber – Carbamate 3%	**72 hours**
Cobalt Chloride 1%	40.48
Rubber - Thiuram 1%	40.48
Nickel Sulfate 5%	33.33
Coal Tar 12%	26.19
Potassium Dichromate 0.5%	26.19
Thimerosal	26.19
Balsam of Peru 25%	9.52
Mixture of Parabens 15%	7.14
Ammoniated Mercury 10%	7.14
Lanolin	7.14
Neomycin sulfate 20%	7.14
Quaternium 15	7.14
Formaldehyde 1% (in water)	4.76
Rosin	4.76
Rubber- Mercapto 2%	4.76
Phenylmercury acetate 0.01% (in water)	4.76
Mixed Fragrances	4.76
Paraphenylene-diamine (Ursol 5%)	2.38
Benzocaine 5%	2.38
Epoxy Resin 1%	-
Rubber Antioxidant IPPD 0.1%	-
Vaseline	-

In Table 18, we can observe the most common positive allergens in combination with Coal Tar 12% after 72 hours in a descending order. The allergen with the most frequent combination to Coal Tar 12% is Cobalt Chloride 1% with 29.82%, followed by Balsam of Peru 25%, with 18.71%. High percentage of combination is also seen for Nickel Sulfate 5% (16.96%) and Potassium Dichromate 0.5% (11.70%). Low percentage of combinations is seen for Rubber Antioxidant IPPD 0.1%, Quaternium-15 and Mixture of Parabens 15% (see Table 18).

Table 18. The most common positive allergens in combination with **Coal Tar 12%** of the whole population in percentage

Combinations to Coal Tar 12%	**72 hours**
Cobalt Chloride 1%	29.82
Balsam of Peru 25%	18.71
Nickel Sulfate 5%	16.96
Potassium Dichromate 0.5%	11.70
Thimerosal	11.70
Ammoniated Mercury 10%	11.11
Rubber - Carbamate 3%	9.36
Mixed Fragrances	9.36
Rubber - Thiuram 1%	7.02
Vaseline	6.43
Rosin	6.43
Phenylmercury acetate 0.01% (in water)	3.51
Epoxy Resin 1%	3.51
Paraphenylene-diamine (Ursol 5%)	2.92
Benzocaine 5%	2.34
Formaldehyde 1% (in water)	2.34
Neomycin sulfate 20%	2.34
Rubber- Mercapto 2%	2.34
Lanolin	1.75
Quaternium 15	1.75
Rubber Antioxidant IPPD 0.1%	1.17
Mixture of Parabens 15%	0.58

In Table 19, we can observe the most common positive allergens in combination with Mixture of Parabens 15% after 72 hours in a descending order. The allergens with the most frequent combination to Mixture of Parabens 15% are Potassium Dichromate 0.5% and Cobalt Chloride 1% with 33.33% for both allergens. High percentage is also seen for Nickel Sulfate 5%, Neomycin Sulfate 20%, Rubber-Carbamate 3% and Mixed fragrances (see Table 19). Low percentage (6.67%) of combination is seen for Rosin, Ammoniated Mercury 10%, Benzocaine 5%, Vaseline and Rubber - Thiuram 1%.

Table 19. The most common positive allergens in combination with **Mixture of Parabens 15%** of the whole population in percentage

Combinations to Mixture of Parabens 15%	**72 hours**
Potassium Dichromate 0.5%	33.33
Cobalt Chloride 1%	33.33
Nickel Sulfate 5%	20.00
Neomycin sulfate 20%	20.00
Rubber - Carbamate 3%	20.00
Mixed Fragrances	20.00
Coal Tar 12%	13.33
Thimerosal	13.33
Balsam of Peru 25%	13.33
Phenylmercury acetate 0.01% (in water)	13.33
Rosin	6.67
Ammoniated Mercury 10%	6.67
Benzocaine 5%	6.67
Vaseline	6.67
Rubber - Thiuram 1%	6.67
Rubber- Mercapto 2%	-
Formaldehyde 1% (in water)	-
Paraphenylene-diamine (Ursol 5%)	-
Epoxy Resin 1%	-
Rubber Antioxidant IPPD 0.1%	-
Lanolin	-
Quaternium 15	-

In Table 20, we can observe the most common positive allergens in combination with Neomycin Sulfate 20% after 72 hours in a descending order. The allergen with the frequent combination to Neomycin Sulfate 20% is Cobalt Chloride 1% with 50%. Followed by Potassium Dichromate 0.5%, Mixture of Parabens 15% and Nickel Sulfate 5% with 25% for each allergen.

Table 20. The most common positive allergens in combination with **Neomycin Sulfate 20%** of the whole population in percentage

Combinations to Neomycin Sulfate 20%	**72 hours**
Cobalt Chloride 1%	50.00
Potassium Dichromate 0.5%	25.00
Mixture of Parabens 15%	25.00
Nickel Sulfate 5%	25.00
Ammoniated Mercury 10%	16.67
Lanolin	16.67
Coal Tar 12%	8.33
Vaseline	8.33
Mixed Fragrances	8.33
Phenylmercury acetate 0.01% (in water)	8.33
Balsam of Peru 25%	8.33
Rubber - Carbamate 3%	8.33
Rubber - Thiuram 1%	8.33
Rosin	-
Thimerosal	-
Epoxy Resin 1%	-
Benzocaine 5%	-
Rubber Antioxidant IPPD 0.1%	-
Rubber- Mercapto 2%	-
Quaternium 15	-
Formaldehyde 1% (in water)	-
Paraphenylene-diamine (Ursol 5%)	-

In Table 21, we can observe the most common positive allergens in combination with Vaseline after 72 hours in a descending order. The allergen with the most frequent combination to Vaseline is Coal Tar 12% with 47.37%, followed by Cobalt Chloride 1%, with 36.84%. High percentage is also seen for Balsam of Peru 25% (31.58%), Mixed Fragrances (31.58%), Potassium Dichromate 0.5% (15.79%), Nickel Sulfate 5% (15.79%) and Ammoniated Mercury 10% (15.79%).

Table 21. The most common positive allergens in combination with **Vaseline** of the whole population in percentage

Combinations to Vaseline	**72 hours**
Coal Tar 12%	47.37
Cobalt Chloride 1%	36.84
Balsam of Peru 25%	31.58
Mixed Fragrances	31.58
Potassium Dichromate 0.5%	15.79
Nickel Sulfate 5%	15.79
Ammoniated Mercury 10%	15.79
Lanolin	10.53
Thimerosal	5.26
Benzocaine 5%	5.26
Rosin	5.26
Neomycin sulfate 20%	5.26
Rubber- Mercapto 2%	-
Rubber - Thiuram 1%	-
Rubber - Carbamate 3%	-
Formaldehyde 1% (in water)	-
Paraphenylene-diamine (Ursol 5%)	-
Epoxy Resin 1%	-
Rubber Antioxidant IPPD 0.1%	-
Mixture of Parabens 15%	-
Quaternium 15	-
Phenylmercury acetate 0.01% (in water)	-

In Table 22, we can observe the most common positive allergens in combination with Mixed Fragrances after 72 hours in a descending order. The allergen with the most frequent combination to Mixed Fragrances is Cobalt Chloride 1% with 30.38% after 72 hours. High percentage of combination is also seen for Nickel Sulfate 5% (27.85%) Coal Tar 12% (26.58%) and Balsam of Peru 25% (24.05%). Low percentage of combination to Mixed fragrances is seen for Rubber – Mercapto 2%, Quaternium-15, Benzocaine 5% and Rubber Antioxidant IPPD 0.1% (see Table 22).

Table 22. The most common positive allergens in combination with **Mixed Fragrances** of the whole population in percentage

Combinations to Mixed fragrances	**72 hours**
Cobalt Chloride 1%	30.38
Nickel Sulfate 5%	27.85
Coal Tar 12%	26.58
Balsam of Peru 25%	24.05
Ammoniated Mercury 10%	20.25
Thimerosal	11.39
Potassium Dichromate 0.5%	10.13
Rosin	10.13
Vaseline	7.59
Lanolin	7.59
Neomycin sulfate 20%	7.59
Paraphenylene-diamine (Ursol 5%)	3.80
Epoxy Resin 1%	3.80
Rubber - Carbamate 3%	3.80
Mixture of Parabens 15%	3.80
Phenylmercury acetate 0.01% (in water)	2.53
Formaldehyde 1% (in water)	2.53
Rubber - Thiuram 1%	2.53
Rubber Antioxidant IPPD 0.1%	2.53
Benzocaine 5%	1.27
Quaternium 15	1.27
Rubber- Mercapto 2%	-

In Table 23, we can observe the most common positive allergens in combination with Lanolin after 72 hours in a descending order. The allergen with the most frequent combination to Lanolin, is Cobalt Chloride 1% with 38.46%. Second most frequent combinations are seen for Potassium Dichromate 0.5% and Nickel Sulfate 5%, with 30.77% for both allergens. High percentage of combinations are also seen for Mixed fragrances and Ammoniated Mercury 10% (see Table 23).

Table 23. The most common positive allergens in combination with **Lanolin** of the whole population in percentage

Combinations to Lanolin	**72 hours**
Cobalt Chloride 1%	38.46
Potassium Dichromate 0.5%	30.77
Nickel Sulfate 5%	30.77
Ammoniated Mercury 10%	23.08
Mixed Fragrances	15.38
Rubber - Carbamate 3%	15.38
Coal Tar 12%	15.38
Mixture of Parabens 15%	15.38
Thimerosal	15.38
Benzocaine 5%	15.38
Neomycin sulfate 20%	15.38
Vaseline	7.69
Quaternium 15	7.69
Balsam of Peru 25%	7.69
Epoxy Resin 1%	7.69
Rosin	7.69
Phenylmercury acetate 0.01% (in water)	7.69
Paraphenylene-diamine (Ursol 5%)	-
Formaldehyde 1% (in water)	-
Rubber Antioxidant IPPD 0.1%	-
Rubber- Mercapto 2%	-
Rubber - Thiuram 1%	-

In Table 24, we can observe the most common positive allergens in combination with Thimerosal after 72 hours in a descending order. The allergen with the most frequent combination to Thimerosal, is Cobalt Chloride 1% with 34.80%, followed by Nickel Sulfate 5% with 20.10%. High percentage is also seen for Potassium Dichromate 0.5% (14.22%) and Ammoniated Mercury 10% (10.78%). Low percentage (0.49%) of combinations are seen for Mixture of Parabens 15%, Rubber Antioxidant IPPD 0.1% and Quaternium-15.

T**able 24.** The most common positive allergens in combination with **Thimerosal** of the whole population in percentage

Combinations to Thimerosal	**72 hours**
Cobalt Chloride 1%	34.80
Nickel Sulfate 5%	20.10
Potassium Dichromate 0.5%	14.22
Ammoniated Mercury 10%	10.78
Coal Tar 12%	9.31
Balsam of Peru 25%	6.86
Rubber - Carbamate 3%	5.88
Mixed Fragrances	4.90
Paraphenylene-diamine (Ursol 5%)	2.45
Rubber - Thiuram 1%	2.45
Formaldehyde 1% (in water)	1.96
Vaseline	1.96
Rosin	1.47
Phenylmercury acetate 0.01% (in water)	1.47
Epoxy Resin 1%	1.47
Rubber- Mercapto 2%	1.47
Benzocaine 5%	1.47
Lanolin	0.98
Neomycin sulfate 20%	0.98
Mixture of Parabens 15%	0.49
Rubber Antioxidant IPPD 0.1%	0.49
Quaternium 15	0.49

In Table 25, we can observe the most common positive allergens in combination with Quaternium-15 after 72 hours in a descending order. The allergens with the most frequent combination to Quaternium-15 are Potassium Dichromate 0.5 and Cobalt Chloride 1% with 60% for each allergen. High percentage (40%) of combination is also seen for Nickel Sulfate 5%, Ammoniated Mercury 10% and Rubber – Carbamate 3%.

Table 25. The most common positive allergens in combination with **Quaternium-15** of the whole population in percentage

Combinations to Quaternium 15	**72 hours**
Potassium Dichromate 0.5%	60.00
Cobalt Chloride 1%	60.00
Nickel Sulfate 5%	40.00
Ammoniated Mercury 10%	40.00
Rubber - Carbamate 3%	40.00
Coal Tar 12%	20.00
Rosin	20.00
Formaldehyde 1% (in water)	20.00
Mixed Fragrances	20.00
Phenylmercury acetate 0.01% (in water)	20.00
Balsam of Peru 25%	20.00
Epoxy Resin 1%	20.00
Neomycin sulfate 20%	20.00
Thimerosal	20.00
Benzocaine 5%	-
Lanolin	-
Rubber Antioxidant IPPD 0.1%	-
Rubber- Mercapto 2%	-
Rubber - Thiuram 1%	-
Mixture of Parabens 15%	-
Vaseline	-
Paraphenylene-diamine (Ursol 5%)	-

In Table 26, we can observe the most common positive allergens in combination with Phenylmercury acetate 0.01% after 72 hours in a descending order. The allergen with the most frequent combination is Cobalt Chloride 1% with 40%, followed by Nickel Sulfate 5% with 36%. High percentage is also seen for Ammoniated Mercury 10% (24%), Coal Tar 12% (20%), Potassium Dichromate 0.5% (20%) and Thimerosal (20%).

Table 26. The most common positive allergens in combination with **Phenylmercury acetate 0.01%** of the whole population in percentage

Combinations to Phenylmercury acetate 0.01%	**72 hours**
Cobalt Chloride 1%	40.00
Nickel Sulfate 5%	36.00
Ammoniated Mercury 10%	24.00
Coal Tar 12%	20.00
Potassium Dichromate 0.5%	20.00
Thimerosal	20.00
Balsam of Peru 25%	16.00
Mixed Fragrances	12.00
Quaternium 15	8.00
Benzocaine 5%	4.00
Rubber - Carbamate 3%	4.00
Vaseline	4.00
Rosin	4.00
Formaldehyde 1% (in water)	4.00
Lanolin	-
Neomycin sulfate 20%	-
Paraphenylene-diamine (Ursol 5%)	-
Epoxy Resin 1%	-
Rubber Antioxidant IPPD 0.1%	-
Rubber- Mercapto 2%	-
Rubber - Thiuram 1%	-
Mixture of Parabens 15%	-

In Table 27, we can observe the most common positive allergens in combination with Nickel Sulfate 5% after 72 hours in a descending order. It shows how much percentage of the Nickel Sulfate 5% positive population also reacts to other allergens. The allergen with the by far most frequent combination is Cobalt Chloride 1% with 62.62%. Second most frequent combination is seen for Potassium Dichromate 0.5%, with 17.30%. Low percentage of combinations to Nickel Sulfate 5% are seen for Epoxy Resin 1% (0.80%), Rubber Antioxidant IPPD 0.1% (0.80%) and Rubber – Mercapto 2% (0.40%).

Table 27. The most common positive allergens in combination with **Nickel Sulfate 5%** of the whole population in percentage

Combinations to Nickel Sulfate 5%	**72 hours**
Cobalt Chloride 1%	62.62
Potassium Dichromate 0.5%	17.30
Thimerosal	8.75
Ammoniated Mercury 10%	7.16
Coal Tar 12%	6.76
Mixed Fragrances	5.37
Balsam of Peru 25%	4.77
Rubber - Carbamate 3%	3.58
Formaldehyde 1% (in water)	3.18
Rubber - Thiuram 1%	3.18
Neomycin sulfate 20%	2.78
Phenylmercury acetate 0.01% (in water)	2.39
Rosin	1.79
Mixture of Parabens 15%	1.59
Paraphenylene-diamine (Ursol 5%)	1.39
Benzocaine 5%	1.19
Quaternium 15	1.19
Lanolin	0.99
Vaseline	0.99
Epoxy Resin 1%	0.80
Rubber Antioxidant IPPD 0.1%	0.80
Rubber- Mercapto 2%	0.40

In Table 28, we can observe the most common positive allergens in combination with Formaldehyde 1% after 72 hours in a descending order. The allergens with the most frequent combination to Formaldehyde 1% are Nickel Sulfate 5% and Cobalt Chloride 1% with 31.43% for both allergens. High percentage of combinations is also seen for Potassium Dichromate 0.5% and Thimerosal (see Table 29). Low percentage (2.86%) of combinations is seen for Mixture of Parabens 15%, Phenylmercury acetate 0.01%, Quaternium-15, Vaseline, Rubber – Thiuram 1% and Paraphenylene acetate 0.01% (see Table 29).

Table 28. The most common positive allergens in combination with **Formaldehyde 1%** of the whole population in percentage

Combinations to Formaldehyde 1%	**72 hours**
Nickel Sulfate 5%	31.43
Cobalt Chloride 1%	31.43
Potassium Dichromate 0.5%	22.86
Thimerosal	14.29
Ammoniated Mercury 10%	11.43
Coal Tar 12%	8.57
Lanolin	8.57
Neomycin sulfate 20%	8.57
Rubber - Carbamate 3%	5.71
Rosin	5.71
Balsam of Peru 25%	5.71
Benzocaine 5%	5.71
Rubber Antioxidant IPPD 0.1%	5.71
Mixed Fragrances	5.71
Paraphenylene-diamine (Ursol 5%)	2.86
Rubber - Thiuram 1%	2.86
Vaseline	2.86
Quaternium 15	2.86
Phenylmercury acetate 0.01% (in water)	2.86
Mixture of Parabens 15%	2.86
Epoxy Resin 1%	-
Rubber- Mercapto 2%	-

In Table 29, we can observe the most common positive allergens in combination with Paraphenylene-diamine (Ursol 5%) after 72 hours in a descending order. The allergen with the most frequent combination is Cobalt Chloride 1% with 24.14%. Followed by Coal Tar 12% with 20.69%. High percentage is also seen for Potassium Dichromate 0.5% (17.24%), Ammoniated Mercury 10% (13.79%) and Nickel Sulfate 5% (13.79%).

Table 29. The most common positive allergens in combination with **Paraphenylene-diamine (Ursol 5%)** of the whole population in percentage

Combinations to Paraphenylene-diamine (Ursol 5%)	**72 hours**
Cobalt Chloride 1%	24.14
Coal Tar 12%	20.69
Potassium Dichromate 0.5%	17.24
Ammoniated Mercury 10%	13.79
Nickel Sulfate 5%	13.79
Thimerosal	10.34
Benzocaine 5%	10.34
Mixed Fragrances	10.34
Rubber - Thiuram 1%	6.90
Rubber - Carbamate 3%	6.90
Rubber Antioxidant IPPD 0.1%	6.90
Formaldehyde 1% (in water)	3.45
Rubber- Mercapto 2%	3.45
Mixture of Parabens 15%	3.45
Rosin	3.45
Balsam of Peru 25%	-
Vaseline	-
Epoxy Resin 1%	-
Neomycin sulfate 20%	-
Lanolin	-
Quaternium 15	-
Phenylmercury acetate 0.01% (in water)	-

4.5. Cross reactions

There are already known cross sensitizations of specific allergens. We used the data from specific papers in the allergological ambulance of the University Hospital Centre Split and compared them to our data.

In our study population, 18.71% of the positive Coal Tar 12% population was also positive for Balsam of Peru 25%. Furthermore, 9.36% of the population was also positive for Mixed fragrances and 6.34% were positive for Rosin.

Table 30. Cross reactions to Coal Tar 12%

Cross reactions to Coal Tar 12%	**72 Hours**
Balsam of Peru 25%	18.71%
Mixed fragrances	9.36%
Rosin	6.34%

In our study population, 20% of the positive Rosin population was also positive for Balsam of Peru 25%.

Table 31. Cross reactions to Rosin

Cross reactions to Rosin	**72 Hours**
Balsam of Peru 25%	20%

There are 62.62% of the Nickel Sulfate 5% positive population, being positive for Cobalt Chloride 1% as well. The other way around, there are 48.13% of the positive Cobalt Chloride 1% population also positive for Nickel Sulfate 5%.

Table 32. Cross reactivity between Nickel Sulfate 5% and Cobalt Chloride 1%

Cross reactivity	**72 Hours**
Nickel Sulfate 5% positive population	62.62%
Cobalt Chloride 1% positive population	48.13%

5. DISCUSSION

Contact dermatitis is a very common diagnosis in the field of dermatology and affects a large percentage of the population. Therefore, contact dermatitis is of considerable interest. This study not only investigated specific trends of contact allergens, but also studied the differences between genders, and the unique characteristics of the allergens related to severity and time. We also included the frequency of combination in between the allergens.

The results identified that Cobalt Chloride 1% had the highest incidence in our study group overall. In contrast to data from Boonchai and Chaiwanon, who identified Nickel Sulfate 5% as the most common allergen (45). Furthermore, the North American Contact Dermatitis Group (NACDG), also identified Nickel Sulfate 5% as the most common allergen with 21.1% (46). In our study, there were no differences in between genders for Cobalt Chloride 1%. The severity of skin reaction was rather mild, with 19.39% of the Cobalt Chloride 1% positive reactions being only mild after 48 hours.

The results also identified that Nickel Sulfate 5% had the second most common incidence in our study group. Revealing a total of 19.80% after 48 hours and 20.5% after 72 hours. We did not identify a significant difference between the time measurements. However, we identified that there is a female predominance for this allergen, with 22.99% of females but only 12.12% of males being positive after 48 hours. Similar effect is seen after 72 hours, with 25.01% of females but only 9.59% of males being positive for Nickel Sulfate 5%. Comparing to the data of Garg and Thyssen, where they also identified that woman had an overall increased prevalence for Nickel Sulfate 5% (47). However, a new trend has been mentioned by Ahlström and Thyssen, where the gender difference becomes less apparent, due to changing trends in body piercing (48).

The overall incidence of occurrence was also high for Potassium Dichromate 0.5% (14.53%) and Coal Tar 12% (13.51%) in our population.

The results demonstrated that most of the allergens had no significant increase or decrease in incidence after 72 hours. When a reaction is positive at 48 hours and remains positive at 72 hours, the causative allergen is causing an allergic contact reaction. If a reaction is positive after 48 hours but becomes negative after 72 hours, it indicates an irritant contact reaction. In our population Balsam of Peru 25% halved their cases at 72 hours, with total 10.60% of reactions after 48 hours and only 5.89% after 72 hours, indicating that most of the people express an irritant contact reaction to the allergen. Ursol 5% also decreased their incidence to halve their cases, revealing an irritant contact reaction. However, most of the allergens in our study expressed an allergic contact reaction pattern. In most of the cases there was a slight decline in incidence after 72 hours. In only a few cases, we observed a slight

increase in incidence after 72 hours. Nickel Sulfate 5%, Thimerosal and Ammoniated Mercury 10% are the allergens which revealed an increase in incidence after 72 hours. Thimerosal's incidence was 9.94% for the whole population after 48 hours and after 72 hours the incidence increased to 11.03%. Less obvious seen for Ammoniated Mercury 10%, with an incidence of 7.06% after 48 hours and 7.68% after 72 hours.

Our analysis demonstrated that there are allergens causing more severe reactions than others. Nickel Sulfate 5% demonstrated a much more severe reaction than the other allergens. The severity increased after 72 hours with 10.84% having a severe reaction, compared to 4.84% after 48 hours.

Furthermore, our study identified frequent combinations between allergens. Especially high percentage of combinations is seen for Cobalt Chloride 1%. It occurs together with almost all allergens in a high percentage. Interestingly, all (100%) of the Quaternium-15 positive patients showed also a positive reaction to Nickel Sulfate 5%. High percentage of combination was also seen for Epoxy Resin 1% and Balsam of Peru 25%, as well as for Rubber-Thiuram 1% and Rubber-Carbamate 3%.

Our study also identified cross reactions in between allergens. Most importantly, 62.62% of the positive Nickel Sulfate 5% population was also positive for Cobalt Chloride 1% and on the other way around 48.16% of the Cobalt Chloride 1% positive population was also positive for Nickel Sulfate 5%. The two allergens are known to show cross reactivity (49).

Limitations to this study must be considered when interpreting and applying the conclusion. Firstly, our study population included overall more female patients, with 2279 female compared to 949 male patients. These disproportionate numbers in male and female records, already conclude to a gender difference for contact dermatitis. Additionally, another limiting factor to this study is, the fact that it included only patients and record data from the dermatological clinic of the University Hospital Split and not from other outpatient and private dermatological clinics in Split. That simply means that conclusions drawn are specifically related to this subpopulation and not the whole population of Split.

6. CONCLUSION

The evidence provided by this study leads to the proof of some of our hypotheses from the beginning.

Our first hypothesis was, that there is specific trend of allergens causing contact dermatitis in our study population. The hypothesis was proofed correct, since Cobalt Chloride 1% and Nickel Sulfate 5% showed an increased incidence in our study group. The incidence was also high for Potassium Dichromate 0.5% and Coal Tar 12%. This specific trend can only be applied to our subpopulation and not the whole population of Split.

Second, we proofed the importance of interpreting the results after 48 hours and 72 hours in regards of making a diagnosis of irritant or allergic contact reaction. Our analysis showed that most of the allergens had a higher incidence after 48 hours and slightly declining incidence after 72 hours, indicating an allergic contact reaction. A few allergens showed to be positive after 48 hours and negative after 72 hours, indicating an irritant contact reaction. Some allergens however, revealed an increasing incidence after 72 hours. We can conclude, that there is indeed an importance in the time lapse for interpreting the results.

Additionally, we assumed that there will be a difference in between the female and male population. The analysis showed that there are differences between the male and female population. A positive reaction to Nickel Sulfate 5% was more common in the female population when compared to the male. On the other hand, a positive reaction to Balsam of Peru 25% was more common in the male population when compared to the female. Nonetheless, we also analyzed that there is an even gender distribution for many other allergens. Limitations must be considered, since our study group included much more female (2279) than male (949) patients.

Furthermore, we assumed that there are frequent combinations of some allergens. In our analysis we precisely showed that there are many combinations of allergens. Cobalt Chloride 1% occurred together with almost each allergen with a relatively high percentage. We also noted that there are many other combinations of allergens.

Another hypothesis was that some allergens have the tendency to cause a more severe reaction than others. The hypothesis was proofed correct. Nickel Sulfate 5% showed severe skin reactions rather than mild. It also showed, that the severity increased after 72 hours.
Overall, we can conclude that in our study population mild reactions are by far more common than severe reactions. More severe reactions occur more often after 72 hours rather than 48 hours, whereas mild reactions occur rather early than late.

We proofed the cross reactivity of some allergens with each other.

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8. SUMMARY

Objectives: The aim of this study is to evaluate the epidemiological and clinical features of contact dermatitis. We used the experience of the dermatovenerological clinic of the University Hospital Split, from 2013 to July 2019. It is of great significance and importance to collect and analyze Patch Test data over a period of time, in order to recognize epidemics and trends of contact dermatitis. If a specific trend or epidemic is recognized by the analysis, it is important to ask and search for possible causes. This in turn can lead to future disease prevention and health benefits for the whole population.

Materials and methods: An epidemiological study was conducted with the data from patch test results of the allergological ambulance from 2013 till July 2019 at the department of dermatology and allergology, University Hospital Split. The data collection included 3228 patients in total, whereof 2279 patients were of female gender and 949 were of male gender. As explained above, we included twenty-three allergens in our study, based on the Patch Test model in the Dermatovenerological Department of the University Hospital Split. The results were interpreted from a weak positive reaction (+), strong positive reaction (++) up to an extreme positive reaction (+++/++++). We also included a time-lapse factor, including the reaction after 48 hours and the reaction after 72 hours.

Results: There is a high incidence of Cobalt Chloride 1% associated contact dermatitis in our study population. Overall, the allergens revealed a higher incidence of reaction after 48 hours and declining incidence after 72 hours. Nickel Sulfate 5% exhibited a more severe reaction than other allergens. The frequency of combination between the allergens was especially high for Cobalt Chloride 1%, which occurred together with almost all allergens.

Conclusion: We can observe a specific trend of contact dermatitis in our study population. There are differences in the results when comparing the female and male population. Furthermore, we studied that there is a difference in the reaction after 48 hours and 72 hours in our study population. There is a difference in the severity of reaction for each allergen. Many combinations are observed between the different allergens.

9. CROATIAN SUMMARY

Naslov: Epidemiološke i kliničke značajke kontaktnog dermatitisa. Iskustvo Klinike za kožne i spolne bolesti Kliničkog bolničkog centra u Splitu od 2013. do srpnja 2019.

Ciljevi: Cilj ovog rada je evaluirati epidemiološke i kliničke značajke kontaktnog dermatitisa. Korišteni su podaci iz kliničke prakse Klinike za kožne i spolne bolesti Kliničkog bolničkog centra u Splitu, od 2013 do srpnja 2019. Iznimno je važno skupljati i analizirati rezultate patch testova u određenom vremenskom intervalu kako bi se mogli pratiti epidemiološki trendovi. Ako se određeni trend ili epidemiološki čimbenik uoči tijekom analize, u tom slučaju važno je potražiti moguće uzroke. Pomoću
ovih zaključaka mogla bi se poboljšati prevencija bolesti za dobrobit cjelokupne populacije.

Materijali i metode: Studija je bazirana na rezultatima patch testiranja provođenih od 2013. do srpnja 2019. na Odjelu za alergologiju Kliničkog bolničkog centra u Splitu. Uključeno je 3228 bolesnika, od kojih je 2279 bilo ženskog spola, a 949 muškog. U studiji su proučavani rezultati za 23 alergena prisutna na standardnom setu koji se koristi na Klinici za dermatovenerologiju KBC-a Split. Rezultati su opisani kao slaba pozitivna reakcija (+), jaka pozitivna reakcija (++) i vrlo jaka pozitivna reakcija (+++/++++). Također je u obradu podataka uključen vremenski čimbenik te su obrađeni rezultati testiranja nakon 48 sati i 72 sata.

Rezultati: Uočena je visoka incidencija kontaktnog dermatitisa povezanog s 1% kobalt kloridom unutar ispitivane populacije. Generalno se uočava veća incidencija reakcija nakon 48 sati, a manja incidencija reakcija nakon 72 sata. Niklov sulfat u koncentraciji od 5% izaziva jače reakcije od ostalih alergena. Uz reakciju na 1% kobalt klorid najčešće su prisutne kombinirane reakcije s gotovo svim ostalim alergenima.

Zaključci: Može se uočiti specifični trend kontaktnog dermatitisa unutar ispitivane populacije. Postoje razlike u rezultatima testa između ženske i muške populacije. Također je uočeno da postoji razlika u reakcijama nakon 48 i 72 sata. Postoji razlika u jačini reakcije kod svakog alergena. Unutar ispitivane populacije uočene su kombinirane reakcije preosjetljivosti na više različitih alergena.

10. CURRICULUM VITAE

PERSONAL DATA:

NAME AND SURNAME: Jomana Al Halabi Al Attar
DATE AND PLACE OF BIRTH: 12.07.1994; Stadtlohn
NATIONALITY: German
ADDRESS: Bernhard-Letterhausstraße 9, 48691 Vreden, Germany
E-MAIL: j.al-attar@gmx.de

EDUCATION:

2014 - 2020: University of Split – School of medicine
2004 - 2013: Gymnasium Georgianum Vreden
2000 - 2004: St. Marien Schule - Elementary School

PRACTICAL EXPERIENCES:

30.01.20 – 29.02.20 – Clinical Rotations – Internal Medicine; St. Marien Hospital Ahaus
01.01.20 – 29.01.20 – Clinical Rotations – General Surgery; St. Marien Hospital Ahaus
09.09.19 – 27.09.19 – Clinical Traineeship – Practice for Dermatology, Allergology and Phlebology; Dr.med. Heisterkamp
14.08.17 – 24.08.17 – Clinical Traineeship – Department of Dermatology – Klinikum Vest Recklinghausen
02.09.13 – 30.06.13 – Federal Volunteer Service – St. Vinzenz Hospital Rhede