Factors associated with different results of allergy tests in children with dust mite-induced atopic dermatitis

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Atopic dermatitis; Pathophysiology; Genetics; Children; Parents; Family atopy; House dust mite; Skin prick test; Atopy patch test

Abstract
Background: Atopic dermatitis (AD) is a public health problem, with an increasing prevalence worldwide. AD is a chronic inflammatory disease characterised by skin lesions and severe itching. Immunologically, AD has two forms, IgE-mediated and cell-mediated, but it may also be idiopathic. In the pathogenesis of AD, the gene mutations for filaggrin, a filament-aggregating protein present in the epidermis, are of pivotal importance, but other genetic factors are also operating, including those linked to family atopy.

Methods: We evaluated the role of family atopy, and of the results of the atopy patch test (APT) in parents, in children with mite-induced AD.

64 children, 38 males and 26 females, mean age 4.97 years, were included for the diagnosis of AD and underwent APT and skin prick test (SPT) with dust mite extracts, with evaluation of atopy and result of APT also in parents.

Results: A positive family history of atopy was shown for children with positivity to both APT and SPT compared to those with negative or only one positive result to APT or SPT (p = 0.08). Significant associations were found concerning APT results in children and parents. In particular, children of a positive-APT parent had an 18-fold higher risk of APT-positivity in comparison with children of negative-APT parents, while the risk was 6.6-fold higher if APT was positive in father.

Conclusion: Family atopy and a positive APT in fathers are risk factors to develop cell-mediated AD, as assessed by the APT, in children.

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Introduction

Atopic dermatitis (AD) is an important issue in public health with a rising prevalence worldwide. AD is a chronic inflammatory disease, with periods of flares and remission, characterised by skin lesions of eczematous type and severe itching. It normally begins early in life and often occurs in people with a personal or family history of asthma and allergic rhinitis. The prevalence of AD is estimated to be 15–30% in children and 2–10% in adults, with a mean value of 17%. Of note, the incidence of AD has increased by 2–3-fold during the past three decades in industrialised countries, and particularly in Northern Europe. The disease is sustained by a complex interaction between genetic and environmental factors. AD skin is characterised by immune dysregulation and epidermal barrier defects such as abnormal terminal differentiation of keratinocytes and increased cornification resulting in epidermal damage and altered permeability to allergens and microbes. The role of genetic factors in AD is clearly demonstrated by studies on twins, showing that the concordance rate for AD is higher among monozygotic twins (77%) than among dizygotic twins (15%).

The importance of genetic factors in AD is further underlined by the finding that a positive parental history is the strongest risk factor for AD; the incidence rate is doubled if AD is present in one parent, and tripled if both parents are affected. Allergic asthma or allergic rhinitis in a parent appears to be a minor factor in the development of AD in their offspring, suggesting AD-specific genes. Genomewide scans have highlighted several possible AD-related loci on chromosomes 3q21, 1q21, 16q, 17q25, 20p9 and 3p26. Some issues were suggested as possibly being involved in AD development. The role of caesarean delivery was analysed in a systematic revision of the literature from 1966 to 2007, the conclusion was that caesarean delivery is associated with a slightly higher risk of allergic rhinitis, asthma and possibly food allergy, but was not associated with AD.

Furthermore, the influence of caesarean delivery on the microbiota colonisation in the newborn and its relationship with the developing immune system do not have any clear evidence to support it.

As regards breastfeeding, no apparent effect on the development of AD was demonstrated, while the introduction of foods such as cow’s milk, hen’s egg, wheat, hazelnut, and others may be connected to an increased risk of food allergy and AD.

Theories to explain the rise in AD include an overall improved awareness of AD, an increased exposure to air pollution, and an increased exposure to allergens. Extensive research has demonstrated that a combination of food allergy, defects in the gut mucosal barrier, and increased intestinal permeability is implicated in the pathogenesis of AD. Moreover, the factors grouped in the so-called “hygiene hypothesis”, such as living in non-affluent countries or in rural areas, the number of siblings, the exposure to endotoxins as occurs in the presence of numerous animals, the kind of feeding, and others are advocated as protective from atopy, but this theory is not universally accepted. Indeed, up to 70% of children with AD have a spontaneous remission before adolescence (with a better prognosis when the AD onset occurs in the first year of life). However, the disease can also start in adults, and in a substantial number of these patients there is no sign of IgE-mediated sensitisation. In the position paper “A revised nomenclature for allergy” it is stated that, “allergic AD would be dominated by the IgE-associated subgroup”, in which the clinical selection is based on Hanifin and Rajka’s criterion, family history of or simultaneous occurrence of symptoms of atopy. Since this is the only immunologically well-defined subgroup, one should always, when appropriate, use the term IgE-associated AD. Another subgroup seems to include cell-mediated forms. It is characterised by positive atopy patch tests to aero- and food allergens or allergen-specific T cells in the peripheral blood or in skin biopsies, but in the absence of IgE sensitisation. The term allergic, T-cell-associated AD might be appropriate. The term nonallergic AD should replace the term “intrinsic/cryptogenic variants”. In the future, all these subgroups may be better defined by immunologic characteristics. The IgE-associated form and the cell-mediated form are clinically similar but show some differences regarding the histology, the kind of cells involved, and the cytokine pattern as well as their response to different allergy tests.

We aimed this study at evaluating the factors possibly involved in the different results to allergy tests in children with house dust mite-induced AD.

Materials and methods

Patients and tests

From subjects referring to the Pediatric Allergy Service in Torremaggiore, Italy, 64 children, 38 males and 26 females (M:F ratio 1.5), mean age 4.97 ± 3.5 years, median age 4.25 years (range: 0.7–15.5 years), were consecutively included by diagnosis of AD according to Hanifin and Rajka criteria. A detailed clinical history was obtained from parents to highlight the relationship between AD exacerbation and dust mite exposure as well as to exclude a causative role of food allergy. Subjects with current respiratory symptoms (rhinosinusitis, asthma) at recruitment were also excluded. Twenty-six children with rhinitis but a negative history for current or past AD served as control group.

All children were investigated using the skin prick test (SPT) and the atopy patch test (APT) with Dermatophagoides extracts. The SPT positivity was evaluated, using extracts from Stallergènes (Antony, France) according to the guidelines from the European Academy of Allergy and Clinical Immunology. The APT was performed using material composed of inert dust mite bodies purified to 20% (Dermatophagoides pteronyssinus and Dermatophagoides farinae, 1:1, Merck, Milan, Italy), with white petroleum jelly (Vaseline), 20%, and white mineral oil used as excipients. The substance to be tested was applied onto intact skin of the lower back and was held firmly in position using adhesive patch tests. These patch tests were made up of aluminium Finn chambers of approximately 20 μL. The application period was 48 h. The test was read no less than 30 min after removal to avoid margin effect. Results were interpreted according to the American Academy of Dermatology using a scale ranging from 1+ (weak reaction) to 3+ (very strong reaction). Only reactions of 2 and 3+ were considered positive for the purpose of this study.
All the parents of the children were asked about their family history of atopy. Parental atopy was defined as a positive response to the question "has the father or the mother ever suffered from allergic asthma, rhinitis or eczema?" The parents also underwent APT with Dermatophagoides spp at the same time, conducted by the same operator (N.F.). An informed consent was obtained from children’s parents, in accordance with the World Medical Association and the Helsinki Declaration.

Statistical analysis

Descriptive statistics were expressed as median and range values. Comparisons among groups were performed by the Mann-Whitney U test. Two-tailed \( \chi^2 \) or Fisher exact test was used to evaluate differences of prevalence, as appropriate. Crude odds ratios (OR) with 95% confidence intervals (95%CI) were calculated as the measure of effect. The statistical analysis was performed using STATISTICA version 6.1 (Stat Soft, Inc., Tulsa, OK, USA).

Results

Table 1 shows the main characteristics of the 64 children included in the study and the 26 control subjects. Family history of atopy was positive in 46 children (71.9%), in eight of them (12.5%) positivity was found in both parents. In particular, the family history was positive for past or current AD and past or current rhinitis.

Seven children (10.9%) had a positive result to SPT, 41 (64.1%) had a positive result to APT, nine (14.1%) had a negative result to both tests, and 7 (10.9%) had a positive result to both tests. No difference was found according to sex and age in the prevalence of APT or SPT positivity in children.

Regarding the results to APT in parents, in 14 cases (21.8%) APT was negative in both parents, in 21 (32.8%) was positive only in father, in 13 (20.3%) only in mother and in 16 (25%) was positive in both parents. Concerning the history, when only one parent was concerned, the 11 mothers with a positive APT reported past AD (1), past AD and rhinitis (2), current AD and rhinitis (1), current rhinitis (4), and current contact dermatitis (3); the 22 fathers with a positive APT reported past AD and current rhinitis (6), current AD and current rhinitis (4), and current rhinitis (12). When both parents were concerned, mothers reported past AD and current contact dermatitis (2), current AD and current rhinitis (1), current rhinitis (4), and current contact dermatitis (1); fathers reported past AD and current rhinitis (2), current AD and current rhinitis (1), and current rhinitis (3). The three mothers with contact dermatitis had a positive patch test for nickel (two subjects) and p-phenylenediamine (one subject).

A positive family history of atopy was shown for all seven children with a positive result to both APT and SPT, but for only 39/57 children (68.4%, \( p = 0.08 \)) with negative or only one positive result to APT or SPT. Instead, significant associations were found concerning APT results in children and parents (Table 2). In particular, children of a positive-APT parent had an 18-fold higher risk of APT-positivity in comparison with children of negative-APT parents (OR: 18.3, 95%CI: 4.3–77.3), while the risk was 6.6-fold higher if APT was positive in the father. No association

<table>
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<tr>
<th>Table 1</th>
<th>Clinical characteristics of study and control subjects.</th>
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<tr>
<td></td>
<td>Study group</td>
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<tr>
<td>Number</td>
<td>64</td>
</tr>
<tr>
<td>M:F ratio</td>
<td>1:5</td>
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<tr>
<td>Age range</td>
<td>0.7–15.5 years</td>
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<tr>
<td>Mean age</td>
<td>4.97 years ± 3.5 years</td>
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<tr>
<td>Monoparental family atopy</td>
<td>38 (59.4%)</td>
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<td>Biparental family atopy</td>
<td>8 (12.5%)</td>
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<tr>
<td>Negative family atopy</td>
<td>18 (28.1%)</td>
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<tr>
<td>Positive SPT</td>
<td>14 (22%)</td>
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<td>Positive APT</td>
<td>48 (75%)</td>
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<th>Table 2</th>
<th>Association between APT results and risk factors.</th>
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<tr>
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<td>Children with negative APT (( n = 16 ))</td>
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<tr>
<td>Age (years)</td>
<td>5.8 (0.9–12.6)</td>
</tr>
<tr>
<td>Positive family history of atopy</td>
<td>10 (62.5%)</td>
</tr>
<tr>
<td>APT positivity in at least one parent</td>
<td>6 (37.5%)</td>
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<tr>
<td>APT positivity in both parents</td>
<td>2 (12.5%)</td>
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<td>APT positivity in father</td>
<td>4 (25%)</td>
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<tr>
<td>APT positivity in mother</td>
<td>4 (25%)</td>
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Factors associated to the response to allergy test

was present between APT positivity in children and in the mother.

In the control group, 15 subjects had a positive SPT while only four had a positive APT. Nine fathers and four mothers had a positive history for rhinitis and/or asthma. APT was negative in both parents in 23 cases, positive in both parents in one case, positive only in the father in one case and positive only in the mother in one case.

Discussion

AD is basically caused by trans-epidermal water loss, although its pathophysiology is, as explained above, very complex. AD may be idiopathic but more often the altered permeability (related to skin barrier defects and especially in mutations of the filament aggregating protein filagrin) may facilitate sensitisation of the skin to environmental allergens. This in turn may elicit immune responses in the skin itself, as well as in other target organs such as the respiratory tree and lungs. Recent research highlighted the important role played by house dust mites (HDM) as a cause of AD, which is sustained by the ability of their allergens to penetrate into the epidermis and worsen AD severity through three mechanisms: inherent proteolytic enzyme activity of the major allergens Der p 1 and Der f 1, activation of protease-activated receptors-2 (PAR-2) and immunoglobulin E (IgE) binding, which leads to inflammation. Concerning the latter mechanism, the airborne proteins from mites bind to specific IgE antibodies and elicit the release of histamine and other inflammatory mediators from mast cells and basophils, which result in tissue damage and exacerbation of the itch-scratch cycle, which can further aggravate AD. As far as allergy testing is concerned, the SPT or the measurement of specific IgE antibodies to HDM in serum is used to indicate sensitisation. However, they show only type-I IgE-mediated allergic responses to a protein, without assessing the ability of the antigen to induce inflammation, which is instead demonstrated by the APT. When biopsy is performed from allergen-induced eczema
tous APT site, a sequence of immunological events occur, including: the generation of allergen-specific T cells, with an initial TH2 cytokine pattern and a subsequent TH1 pattern; an early influx of dendritic epidermal cells that capture the allergen through the IgE receptor and present it to specific T cells; the T cel-mediated inflammatory reaction in the skin site of testing, with macroscopic and microscopic similarities with the lesional skin in AD.

We have previously found in a group of 297 children with different clinical expression (current AD, current AD and respiratory symptoms, past AD and respiratory symptoms, and respiratory symptoms with neither current nor past AD) and tested by APT and SPT with mite extracts, that in all subjects with past or current AD the rate of positivity was significantly higher for APT. At the same time, in subjects with exclusive respiratory symptoms the most frequently positive test was the SPT. Indeed, the patients with AD showed two different patterns of allergic response to allergens, one IgE-mediated, as evaluated by positive SPT, and the other cell-mediated, as evaluated by positive APT, this suggesting reconsideration of the significance of APT.

We aimed the present study at evaluating AD in its IgE-mediated, cell-mediated, or not immunologically mediated (i.e. not associated to any test positivity), presentations by the relationship between positivity to SPT and APT and gender, age, family atopy. In addition, we looked at the association between APT positivity in the study subjects and in their parents, and the relationship between APT positivity and age of father and mother. The results confirm that in children with AD, the APT is the test most frequently positive; in fact, 11% of subjects had a positive result to SPT, while 64% had a positive result to APT, and 11% had a positive result to both tests. The novel aspect of the study is the finding that there is a relationship between the positive result of the APT in children and their parents. In fact, a positive family history of atopy, which is quite common in allergic children, was not significantly associated to positive or negative results of the APT. However, the association between a positive APT in children and a positive APT in one parent was highly significant (<0.0001). In particular, the most significant association appeared to be between children and fathers. The APT positivity in mothers, though it was double in mothers of children with positive APT compared with mothers of children with negative APT, did not reach statistical significance. Of interest, in the control group formed by children with rhinitis but a negative history for AD, the rate of positive APT was significantly lower (15% vs. 75% in the study group). These observations highlight the importance of the previously described genetic background in AD and warrants further investigation on the factors related to skin response to Aeroallergens underlying the development of a positive APT.

In conclusion, this study confirms the important role of the APT with dust mites in the diagnosis of AD in children. It also adds a new observation as regards the positive results of APT in parents, particularly in fathers, as being a factor in the development of a cell-mediated response to mites, as assessed by a positive APT, in children.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

Confidentiality of data. The authors declare that they have followed the protocols of their work centre on the publication of patient data and that all the patients included in the study have received sufficient information and have given their informed consent in writing to participate in that study.

Right to privacy and informed consent. The authors have obtained the informed consent of the patients and/or subjects mentioned in the article. The author for correspondence is in possession of this document.

Conflict of interest

The authors declare they have no conflict of interest.
Acknowledgement

The authors thank Mrs. Laura Shearer for language revision.

References

Dos vacunas en una para un doble beneficio del paciente polisensibilizado
DENOMINACIÓN DEL MEDICAMENTO: Depigoid®DUO. COMPOSICIÓN CUALITATIVA Y CUANTITATIVA: Extractos alérgicos despigmentados, polimerizados con glutaraldehído y adsorbidos en hidróxido de aluminio, estandarizados biológicamente y cuya concentración se expresa en unidades DPP/ml. Formulaciones terapéuticas individualizadas de acuerdo a la composición determinada en la prescripción médica. FORMA FARMACÉUTICA: Suspensión inyectable. DATOS CLÍNICOS: Indicaciones terapéuticas: Tratamiento hiposensibilizante (inmunoterapia específica) de las enfermedades alérgicas respiratorias mediadas por IgE (hipersensibilidad tipo I o inmediata), como rinitis alérgica, conjuntivitis alérgica y/o rinonconjuntivitis alérgica, con o sin asma bronquial alérgico. Posología y forma de administración: Depigoid®DUO debe ser administrado por vía subcutánea. El laboratorio sólo puede facilitar las normas generales de tratamiento obtenidas de la experiencia clínica. El médico responsable del tratamiento debe adaptar estas normas generales a cada enfermo y a su curso clínico. Contraindicaciones: Se consideran contraindicaciones las generales de la inmunoterapia específica con alérgenos, fundamentalmente: coexistencia de enfermedad respiratoria (p.ej. asma grave no controlado, enfisema, bronquiectasias), cardiovascular, renal, hepática, o hematológica, procesos infecciosos agudos, fiebre, enfermedades infecciosas graves, neoplasias malignas o enfermedades del sistema inmunológico, por ejemplo enfermedades autoinmunes, por inmunocomplejos o inmunodeficiencias, síntomas graves de alergia, procesos patológicos en los que el paciente recibe betabloqueantes, antidepresivos tricíclicos o inhibidores de la monoamina-oxidasa, o en los que la adrenalina esté contraindicada, e hipersensibilidad a cualquiera de los excipientes. Advertencias y precauciones especiales de empleo: - Iniciar el tratamiento en periodo asintomático, asegurando que el paciente está clínicamente estable y no existe ninguna situación que contraindique su administración. Comprobar la fecha de administración de la última dosis y la tolerancia a la misma, así como el vial que corresponde administrar, su fecha de caducidad y la dosis. Agitar suavemente el vial antes de extraer la dosis. - Inyectar lentamente por vía subcutánea, en la cara posterolateral del brazo. Se debe evitar estrictamente la administración intravenosa, intracutánea o intramuscular. Alternar los brazos. - Después de la administración del extracto, el paciente debe permanecer en observación, al menos, 30 minutos. No realizar ejercicios o trabajos físicos exhaustivos, ni aplicar baños calientes ni sauna el día de la inyección. Se aconseja también ese día evitar comidas copiosas y la ingesta de alcohol. No frotar ni aplicar ningún masaje o calor sobre la zona inyectada. Instruir al paciente para que busque asistencia médica en caso de aparición síntomas tardíos de reacción sistémica. - Disponer de un equipo de reanimación de emergencia. - En caso de requerirse la administración de dos vacunas Depigoid®DUO de extractos diferentes por separado, se recomienda esperar un intervalo de 2-3 días entre ambas. En caso de no ser posible, se recomienda su inyección en brazos distintos con un intervalo de, al menos, 30 minutos. Interacción con otros medicamentos y otras formas de interacción: No se han realizado estudios de interacción. No debe mezclarse este preparado con otra vacuna antialérgica, deben administrarse en inyecciones separadas. Los medicamentos que modifican la respuesta alérgica (antihistamínicos, corticoides, broncodilatadores, cromonas, antagonistas de los leucotrienos, etc) aumentan el umbral de tolerancia del paciente a la inmunoterapia, si se administran antes de la misma. Pueden surgir reacciones adversas si el paciente olvida tomar su medicación antialérgica habitual antes de la administración de la inmunoterapia. La exposición adicional a alérgenos (oxígena o iatrogénica) puede disminuir la tolerancia a la inmunoterapia. No debe administrarse inmunoterapia específica durante el tratamiento con inmunosupresores. La administración de cualquier otro tipo de vacuna (polio, trivalente, etc.) debe realizarse con un intervalo entre una semana, anterior o posterior a la administración de este tratamiento. Fertilidad, embarazo y lactancia: No hay datos clínicos sobre un posible efecto de Depigoid®DUO sobre la fertilidad. No hay datos clínicos sobre el uso de Depigoid®DUO en el embarazo ni durante el periodo de lactancia. No se recomienda iniciar tratamiento con Depigoid®DUO en una mujer embarazada. Si se produce el embarazo durante el tratamiento con Depigoid®DUO y la paciente presenta buena tolerancia y respuesta clínica, la inmunoterapia podría continuarse previa consulta con el médico especialista. Este realizará una valoración clínica de la paciente para decidir la continuidad o la interrupción de la inmunoterapia. Efectos sobre la capacidad para conducir y utilizar máquinas: En casos muy raros puede aparecer un ligero cansancio. Reacciones adversas: En caso de sobredosis accidental y/o pacientes muy sensibles, pueden presentarse reacciones adversas locales y/o sistémicas, tanto inmediatas (en los primeros 30 minutos siguientes a la inyección), como tardías (pasados los 30 primeros minutos tras la inyección). Ante la aparición de cualquier reacción adversa se deberá consultar con el médico prescriptor antes de proseguir el tratamiento. Puede ser necesario disminuir la dosis o aumentar los intervalos entre las dosis. Reacción local: Eritema, picor, tumefacción y/o calor en el lugar de la inyección. De manera tardía pueden aparecer nódulos subcutáneos en el lugar de la inyección. Estos nódulos son causados generalmente por el hidróxido de aluminio. Suelen desaparecer con el tiempo, pero pueden persistir semanas o meses. Reacción sistémica: Púrpura generalizado, calor generalizado, urticaria, angioedema, tos, rininitis, conjuntivitis, asma leve/moderada, sabor metálico, debilidad, cefalea, disnea, sibilancias, broncoespasmo, estridor, trastornos gastrointestinales con hinchazón, vómitos, dolor abdominal, diarrea, malestar general, mareo, palpitaciones, sudoración, arritmias, hipotensión, sensación de muerte inminente, sincope, pérdida de control de esfínteres (incontinencia), colapso circulatorio, convulsiones y/o perdida de conciencia. Las reacciones adversas sistémicas pueden poner en peligro la vida del paciente, por lo que ante cualquier indicio de reacción sistémica inmediatamente después de la inyección, incluso aunque sea leve, se debe administrar sin demora tratamiento sintomático antialérgico. En el caso de reacciones sistémicas graves (reacción anafiláctica), es fundamental administrar lo antes posible adrenalina, preferentemente por vía intramuscular, antihistamínicos, oxígeno, broncodilatadores inhalados, corticoesteroides y fluidoterapia. Se aconseja también en estos casos el traslado del paciente al servicio de urgencias hospitalario para posterior observación. Pauta para la correcta administración de la adrenalina: La adrenalina se administrará preferentemente por vía intramuscular, a una concentración de 1/1.000 y a una dosis de 0,01 ml/kg de peso. Pauta orientativa en caso de ser necesario disminuir la dosis o aumentar los intervalos entre las dosis: Sobredosis: Un error en la administración del preparado que implique la inyección de una dosis inadecuada, y/o la utilización de una vía de administración distinta a la subcutánea, pueden conducir a la aparición de reacciones adversas. PROPRIEDADES FARMACOLÓGICAS. Grupo farmacoterapéutico: Extractos alérgicos. Código ATC V01AA. DATOS FARMACÉUTICOS: Lista de excipientes: Cloruro sódico, fenol, hidróxido de aluminio, agua para inyectables. Incompatibilidades: En ausencia de estudios de compatibilidad, este producto no debe ser mezclado con otros medicamentos. Periodo de validez: Observar la fecha de caducidad que consta en la etiqueta. Precauciones especiales de conservación: Almacenar en nevera (entre 2ºC y 8ºC). No congelar. Naturaleza y contenido del envase: Suspensión en viales de vidrio tipo I, con tapón de bromobutilo (sin látex) y cápsula de aluminio. FECHA DE REVISIÓN DEL TEXTO: Junio 2013.