UNIVERSIDADE DA BEIRA INTERIOR
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Study of atopic markers in allergic rhinitis in the elderly

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Dedicatória

À minha família: ao meu pai, às minha irmãs, aos meus avós e, apesar de já não se encontrar entre nós, à minha mãe.

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Resumo Alargado

A Rinite Alérgica (RA) é uma doença com elevada prevalência a nível mundial, afectando cerca de 10 a 20% da população. A sua prevalência tem vindo a aumentar durante as últimas décadas. A rinite é definida como sendo uma inflamação da mucosa nasal e é caracterizada por um ou mais dos seguintes sintomas: congestão nasal, rinorreia, comichão nasal, e espirros. A rinite pode ser classificada etiologicamente como alérgica ou não alérgica. A RA é induzida pela exposição a alergénios que desencadeiam uma inflamação das vias nasais mediada pela imunoglobulina E (IgE) e que dá origem aos sintomas. A RA está muitas vezes acompanhada de conjuntivite, denominando-se rinoconjuntivite. As normas ARIA classificaram a RA como "intermitente" se os sintomas estão presentes durante menos de 4 dias por semana ou menos de 4 semanas consecutivas e como "persistente" se os sintomas estão presentes mais de quatro dias por semana e durante mais de 4 semanas consecutivas. A severidade dos sintomas é classificada como "moderada" se eles estão presentes mas não causam problemas e como "moderada/severa" se existirem distúrbios de sono, prejuízo das actividades diárias ou dias perdidos de escola ou trabalho. A atopia é conhecida como um factor de risco no desenvolvimento de patologias alérgicas como a RA; esta é a tendência em produzir anticorpos IgE, em resposta a baixas doses de aeroalergénios. O aumento dos níveis de IgE sérica é observado em doentes atópicos. A determinação da IgE total sérica é um dos marcadores de inflamação alérgica e é usada frequentemente. O Phadiatop é um teste de IgE específica para uma mistura de alergénios. Vários estudos concluíram que a atopia diminui com o aumento da idade e que há uma maior prevalência nas mulheres do que nos homens.

O objectivo deste estudo foi investigar a relação entre os três marcadores atópicos (Phadiatop, IgE total e testes cutâneos de alergia por picada, TCA) com a rinite alérgica numa população de idosos e jovens adultos da Cova da Beira. Este estudo realizou-se na Faculdade de Ciências da Saúde e no Centro de Saúde da Covilhã. A população deste estudo consistiu em dois grupos de indivíduos da Beira Interior: um grupo de adultos jovens (nascidos entre Janeiro de 1976 e Dezembro de 1993) e outro de idosos (nascidos antes de 1944). Um questionário validado foi aplicado a todos os voluntários. Para além disso estes foram avaliados através de três marcadores atópicos de uso comum: TCA, IgE sérica total e Phadiatop. A amostra da população em estudo incluiu 403 voluntários. Embora todos os voluntários tenham respondido ao questionário, apenas 381 foram avaliados face à resposta a 5 aeroalergénicos regionais comuns através TCA e apenas 356 aceitaram proceder à colheita de sangue para determinação da IgE total e do Phadiatop. A análise dos dados foi baseada nos resultados do Phadiatop. De entre os 356 indivíduos escolhidos aleatoriamente, 239 eram idosos (média das idades = 73; 141 indivíduos do sexo feminino) e 117 adultos jovens (média das idades = 28; 70 indivíduos do sexo feminino). Destes, 96 voluntários (38 idosos e 58 jovens) tiveram Phadiatop positivo e 48 (21 idosos e 27 jovens) apresentaram

testes cutâneos positivos. Para além disso, encontraram-se diferenças estatisticamente significativas nos dois grupos, no que diz respeito ao grau académico, classe social e tipo de residência. Este estudo sugere que os idosos são menos atópicos em relação aos jovens; em ambos os grupos a prevalência de atopia, de acordo com o Phadiatop, revelou ser maior nos homens; obteve-se uma correlação significativa na relação entre a positividade do Phadiatop e a sensibilização demonstrada por TCA. No que diz respeito à IgE total, não se verificou uma diferença significativa dos valores da sua concentração com a idade dos voluntários nem se conseguiu relacionar com a concentração do Phadiatop. A RA foi definida com o Phadiatop positivo e presença de sintomas da doença. 69 voluntários tinham RA (42 jovens e 27 idosos). Destes, os idosos são significativamente mais sensibilizados aos alergénios outdoor do que os jovens e menos sensibilizados aos alergénios indoor, mas não significativamente. No geral, a população que tem resposta ao TCA para alergénios outdoor sente que a época polínica agrava os seus sintomas. Com este estudo foi possível concluir que a RA tem maior prevalência nos adultos jovens e afecta mais os homens de ambos os grupos; para além disso, a valor médio da concentração da IgE total da população é mais elevado nos adultos jovens. Foi ainda possível concluir que os idosos são mais sensibilizados para os alergénios outdoor, enquanto que os jovens são mais sensibilizados aos alergénios indoor.

Abstract

Allergic rhinitis (AR) is a prevalent disease worldwide, affecting 10% to 25% of the world population. AR's prevalence has increased during the past few decades. Rhinitis is defined as an inflammation of the nasal mucosa and is characterized by one or more of the following symptoms: congestion, rhinorrhea, itching of the nose, postnasal drip, and sneezing. Atopy is known to increase the risk of developing allergic disorders such as AR; this is the tendency to produce the IgE antibody in response to low doses of aeroallergens. The aim of this study was to evaluate atopy through different methods in an elderly population in Covilhã. The population of this study consisted in two groups of individuals of Beira Interior: one of young adults (born between January 1976 and December 1993) and another of elderly (born before 1944). A standardised questionnaire was carried out in all volunteers. In addition, they were evaluated by three commonly used approaches: Skin Prick Tests (SPT), total serum IgE and Phadiatop. The study sample included 403 volunteers. Although all patients answered the questionnaire, only 381 patients were evaluated for SPT reaction to 5 common regional aeroallergens and only 356 accepted to collect blood for total IgE and Phadiatop determination. Data analysis was based on Phadiatop results. Among the 356 random subjects involved, 239 were elderly (mean age = 73; 141 females) and 117 were young adults (mean age= 28; 70 females). Of these, 96 volunteers (38 elderly and 58 young adults) had positive Phadiatop and 48 (21 elderly and 27 young adults) had positive SPT. Furthermore, significant differences were found on both groups, concerning to academic degree, social class and residence. This study suggests that elderly subjects are less atopic than younger subjects in the population under study. In both groups atopy prevalence according to Phadiatop, was higher in men; a significant correlation was found between Phadiatop positivity and positive SPT. In what concerns total IgE, no significant relation exists between was found on its concentration values and volunteers' age. AR was defined with positive Phadiatop and presence of disease symptoms. 69 volunteers had AR (42 young adults and 27 elderly). Of those, elderly are more sensitized to outdoor allergens than young adults and less sensitized to indoor allergens, but not significantly. Overall, the population that has positive TCA for outdoor allergens feels like pollen season increases their symptoms. With this study was possible to conclude that AR has a higher prevalence on young adults and affects more men in both groups.

Keywords

Allergic rhinitis, elderly, atopy, IgE, Phadiatop.

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List of Abbreviations

AR	Allergic Rhinitis
ARIA	Allergic Rhinitis and its Impact on Asthma
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL-2	Interleukin 2
ISSAC	International Study of Asthma and Allergies in Childhood
PAQUID	Personnes Agées Quid
QRESERCH	Quantum Marquet Research
SAPALDIA	Swiss Study on Air Pollution and Lung diseases in Adults
SPT	Skin Prick Test
TH1	Helper T 1
TH2	Helper T 2
IFN	Interferon

Chapter 1

Introduction

1.1 Allergic Rhinitis

1.1.1 Classification

Rhinitis is defined as an inflammation of the nasal mucosa and is characterized by one or more of the following symptoms: congestion, rhinorrhea, itching of the nose, postnasal drip, and sneezing[1,2]. In the geriatric population, a broad interpretation of this symptom complex may also include crusting within the nose, cough, excessive drainage, olfactory loss, and nasal dryness.

Rhinitis can be classified by etiology as allergic or nonallergic and differentiated from conditions that mimic symptoms of rhinitis[3].

Nonallergic rhinitis is characterized by non-immunoglobulin E (IgE)-mediated symptoms typical of rhinitis, such as congestion and clear rhinorrhea, with less proeminence of sneezing and ocular/nasal pruritus. The associated symptoms may be perennial or sporadic, lacking a clear seasonality, and may be exacerbated by nonspecific triggers such as odors, food, emotions, or change in atmospheric conditions[1].

Approximately 50% of all cases of rhinitis are caused by allergy[4]. The condition originates when individuals are exposured to allergens they are sensitized to, like airborne agents such as pollens, mold spores and dust-born mites[5,6]. Allergic rhinitis (AR) is induced by exposure to allergens that trigger an IgE-mediated inflammation of the nasal passageways that can result in chronic or recurrent symptoms of rhinorrhea, congestion and sneezing[1,5]. Itching of the ears and throat can also be associated with allergic rhinitis[7]. AR is often accompanied by allergic rhinoconjuctivitis (a complex sometimes referred to as allergic rhinoconjuctivitis) that results in conjunctival injection and chemosis and symptoms of itchy eyes and tearing[7].

Symptoms of AR may be classified as seasonal or perennial. An international working group modified this classification scheme due to potential difficulties in differentiating between seasonal and perennial symptoms and created the Allergic Rhinitis and its Impact on Asthma (ARIA) Report. The ARIA guidelines temporally classify AR as 'intermittent' if symptoms are present less than four days per week or less than four consecutive weeks, or as 'persistent' if symptoms are present more than four days per week and for more than four consecutive weeks. Severity of symptoms is graded as 'mild' if they are present but not troublesome, and as 'moderate/severe' if they lead to sleep disturbance, impairment of daily activities, or impairment of school or work[1,8].

1.1.2 Physiological changes

Rhinitis is an inflammatory disease, as such, mechanisms and presentation of the condition are altered as immune function changes with age, a concept entitled immunosenescence. A critical component of the immune system is the thymus, which rapidly involutes from adolescence to near middle age, followed by an approximate 1% cellular loss per year thereafter. The decline in functional mass causes depressed production of naïve T-cells leading to impaired cell-mediated immunity. Despite thymic involution, the total T-cell pool remains constant due to an increase in production of memory T-cells. With the aging process T-cell responsiveness to growth factors decreases, lymphocyte response to specific antigens is altered, and IL-2 production and receptor expression are diminished. B-cells also change with age; although the peripheral B-cell population remains constant, there is less IgG isotype class switching, and the number of antigen-specific antibodies decreases while the number of autoantibodies and circulating immune complexes increase. These changes might also contribute to the milder symptoms as well as the decreased incidence of allergic rhinitis in the geriatric population. Furthermore, as individuals age, several changes in nasal anatomy and physiology occur which may affect the development and expression of rhinitis[1].

1.1.3 Pathophysiology and Clinical Presentation of Rhinitis

AR is the result of type I hypersensitivity reactions whereby exposure to allergens in susceptible individuals leads to sensitization by production of specific IgE antibodies directed against these extrinsic proteins. This antibody then binds to the surface of mast cells, and when the allergen is reintroduced, IgE cross-binding to the antigen leads to mast cell degranulation. Within seconds of contact, inflammatory mediators such as histamine, leukotrienes, and prostaglandin D2 are released causing vascular endothelial dilation, which subsequently causes leakage and mucosal edema. This leads to nasal obstruction and symptoms of congestion, redness, tearing, swelling, ear pressure, and postnasal drip. Irritant receptors are stimulated by the allergen causing itching and sneezing[1].

Within four to eight hours of initial exposure, cytokines attracted by previously released mediators lead to recruitment of other inflammatory cells to the mucosa, such as neutrophils, eosinophils, lymphocytes, and macrophages (see figure 1.1). The inflammation persists and this stage is termed the late-phase response. The late-phase response presents similarly to the early phase, however, sneezing and itching are less proeminent, whereas congestion and mucus production are more severe. The late phase may persist for hours or days[1].

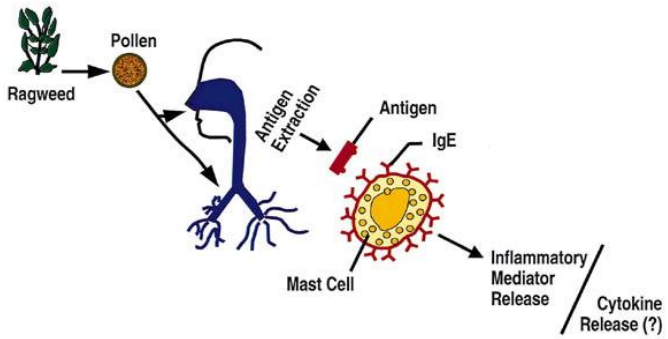

Figure 1.1- Example of an allergic response. Adapted from Togias A. et al [27]

When allergen challenges are given repeatedly, the amount of allergen required to induce an immediate response decreases. This priming effect is thought to be a result of the release of inflammatory mediators from effector cells during ongoing, prolonged allergen exposure and repeated late-phase responses. Consequently, at the end of a pollen season, symptoms may decline at a slower rate than the pollen count. Therefore, it is important to know the full spectrum of aeroallergens to which the patient responds as well as seasonal variations in symptoms[3].

Individual host sensitivity to an aeroallergen influences the intensity of symptoms; for example, the pollen counts that cause symptoms may vary on the basis of an individual's degree of sensitivity and may be different for different pollens. Studies have not been consistently able to demonstrate symptom and/or medication reduction with any of the commonly used environmental control measures in patients with rhinitis[3].

Patients with AR caused by pollens may be exposed to allergen from nonpollen plant fragments, allergenic bioaerosols without intact pollen grains, and even high pollen concentrations of insect-pollinated plants. Pollen counts are generally highest on sunny, windy days with low humidity. Because the interplay of different weather factors (eg, wind, temperature, rain, and humidity) is complex, it may not be possible to reliably predict levels of outdoor aeroallergens on the basis of the influence of a single weather factor[3].

1.1.4 Atopy and IgE

Atopy is a predisposition to develop an IgE-mediated immune response to environmental allergens that do not sensitize nonatopic individuals. The expression of an atopic phenotype requires the interaction of a partly genetic predisposition with environmental allergen exposure[9].

Atopy is known to increase the risk of developing allergic disorders such as AR. Atopy, as measured by allergen skin prick testing, is an important attribute of allergic disease. Not all atopic individuals are symptomatic and not all those with allergic symptoms are atopic[10].

According to Gold and Kemp, the distinction between atopy and atopic disease is important. A child with atopy produces specific IgE antibodies after exposure to common environmental allergens and is said to be sensitized to that allergen. Eczema, asthma and

rhinoconjunctivitis are clinical syndromes each defined by a collection of symptoms and signs and are commonly referred to as the atopic diseases. While most children with these conditions are atopic, some are not, and, conversely, some children with atopy may not manifest atopic disease[11].

IgE concentration in the serum is the lowest of the 5 immunoglobulin subtypes, has the shortest half-life (approximately 2 days) and its expression is tightly regulated in the absence of disease. IgE shows no transplacental transfer. In the absence of disease, IgE levels in cord blood are low (<2 kIU/L; < 4.8 mg/L), gradually increase throughout childhood with a peak at 10 to 15 years of age, and then decrease throughout adulthood. Total IgE levels are also influenced by genetic makeup, race, immune status, and environmental factors (eg, pollen exposure)[9].

Increased IgE levels are seen in patients with atopic diseases, with the highest levels generally being seen in patients with atopic dermatitis, followed by those with atopic asthma, perennial allergic rhinitis, and seasonal allergic rhinitis. For seasonal allergens, peak IgE levels occur 4 to 6 weeks after the peak of the pollen season. Increased IgE levels are also seen in other disorders, including parasitic infections (eg, strongyloidiasis, ascariasis, and schistosomiasis), nonparasitic infections (eg, EBV, cytomegalovirus, HIV, and *Mycobacterium tuberculosis*), inflammatory diseases (eg, Kimura disease, Churg-Strauss vasculitis, and Kawasaki disease), hematologic malignancies (eg, Hodgkin´s lymphoma and IgE myeloma), cutaneous diseases (eg, Netherton syndrome and bullous pemphigoid), cystic fibrosis, nephrotic syndrome, and primary immunodeficiency diseases. Increased IgE levels are also detected after hematopoietic stem cell transplantation, in smokers (particularly male smokers), and in those with alcoholism[9].

The presence of one allergic disorder significantly increases the risk of developing other allergic disorders, affecting different organ systems[12].

Patients with AR have allergen-specific IgE demonstrable both systemically (e.g., positive skin tests) as well as local IgE produced in the nasal mucosa[3].

Serum total IgE measurement was one of the first allergic inflammation marker tests. It has been extensively used for the diagnostic of allergy[23]. The Phadioatop test is a specific IgE test for multiple allergens being its main use as a screening test[6].

For evaluation and diagnosis a thorough allergy history remains the best diagnostic tool available. The history will include the patient's chief concerns and symptoms and often includes the pattern, chronicity, seasonality, and triggers of nasal and related symptoms, family history, current medications, occupational exposure, and a detailed environmental history. Questions relating symptoms to pollen and animal exposure have been shown to have positive predictive value for diagnosing allergic rhinitis[3].

Determination of specific IgE, preferably by skin testing, is indicated to provide evidence of an allergic basis for the patient's symptoms, confirm suspected causes of the patient's symptoms, or assess the sensitivity to a specific allergen for avoidance measures

and/or allergen immunotherapy[3]. Blood eosinophil count and total serum IgE level tend to be elevated in allergic rhinitis[13].

The prevalence of allergic sensitization is lower in the most advanced ages[14]. Many studies concluded that atopy decreases with increasing age either in the general population samples or in population samples of healthy individuals (i.e. with no allergy-related symptoms). There is also a higher prevalence in females than in males, in spite of that, lifetime prevalence of AR is higher in males before the teenage prevalence peak[15,1,16]. The observation of lower IgE in older subjects compared with younger subjects has been reported in cross-sectional studies. Morais-Almeida and colleagues estimated a prevalence of rhinitis of 26% in a large population sample selected in the primary care centres of Mainland Portugal including 6859 questionnaire responses corresponding to a mean age of 48.3 years[16].

According to results from SAPALDIA Study, which studied the influence of sex, age and smoking habits on total serum IgE and allergen-specific IgE antibody concentrations (assessed by means of the Phadiatop test) on the prevalence of hay fever; Phadiatop tests, positive skin tests and atopy decreased significantly with age. This study also demonstrated that tobacco smoking is associated with increased IgE levels and negatively related to atopy and hay fever[29]. Another study analyzed results from 8329 randomized adults from SAPALDIA Study and revealed that the prevalence of positive Phadiatop, positive SPT (at least, one out of eight SPT to common aeroallergens with a wheal of > or = 3 mm), and positive total IgE (IgE > or = 100 kU/L) were 29, 23, and 23%, respectively [17]. Some studies have shown a significant age-related decline of IgE for both genders, with levels in females being significantly lower than in males[18]. A study conducted in France (PAQUID cohort) with subjects aged 65 years and over also found an association between smoking and IgE level independent of allergic reactivity to common allergens in the elderly. Questionnaires were applied by telephone or letter and total serum IgE and Phadiatop were determined. A positive Phadiatop test was not related to gender and smoking but significantly associated with total IgE and rhinitis. The study demonstrates persistence of respiratory allergies after age 65 years and confirms an association between smoking and IgE level independent of allergic reactivity to common allergens for elderly people. Furthermore, IgE level was significantly higher in males than in females[18].

Another study performed in Germany analyzed questionnaires, total IgE and specific IgE and concluded that the total IgE had a negative correlation with age in all patients and also allergen specific IgE was significantly decreased in the elderly suffering from AR[19].

1.1.5 Epidemiology

Allergic rhinitis (AR) is a prevalent disease worldwide, affecting 10% to 25% of the world population[1,2,5]. AR prevalence has increased during the past few decades. Though its peak incidence is during young adulthood, AR is prevalent among older people. In fact, the 2005 National Center for Health Statistics report stated that 10.7% of individuals between 45-

64 years of age, 7.8% of patients 65-75 years of age, and 5.4% of patients older than 75 are affected by AR. Along with the anatomic and physiologic changes of the nose, non-specific immune changes such as decreased mucus production and ineffective cough mechanisms are all thought to contribute to persistent or late-onset allergic disease in older people, as these processes are necessary for clearance of allergens and irritants.

A cross-sectional study, based on questionnaires and SPT, made in East Germany showed that the prevalence of hay fever and atopic sensitization increased significantly between 1991 and 1996[20]. In England, Ghouri and co-workers analyzed the incidence rate per 1000 person-years of AR for each of five years from the QRESEARCH database. These data showed an overall 33.0% increase during the period 2001-2005[15]. In Belgium, the prevalence of AR was 29.8%[21].

Phase 1 of the ISAAC study reported worldwide rates of rhinoconjunctivitis in the range of 1.4-39.7% in adolescents of 13-14 years of age, and between 0.8-14.9% in children aged 6-7 years[22].

1.1.6 Risk factors

Several studies have shown that the frequency of AR increases with age and positive allergy skin tests are significant risk factors for the development of new symptoms of hay fever[4]. Risk factors for AR include family history of atopy, serum IgE>100 IU/mL before age 6 years and the presence of a positive allergy SPT[3]. There appears to be a higher prevalence of rhinitis in higher socioeconomic classes, in nonwhites, in some polluted areas and in individuals born during the pollen season. Additionally, studies in children in the first year of life have shown that the risk of rhinitis was higher in those youngsters with early introduction of foods or formula, heavy maternal cigarette smoking in the first year of life, exposure to indoor allergens such as animal dander and dust mites and parental disorders[4]. Children with a bilateral family history of atopy may develop symptoms more frequently and at a younger age than those with an unilateral family history. Aeroallergen sensitization rarely begins before 6 months of age but may start between 6 months and 2 years of life. Infants born to atopic families are sensitized to pollen aeroallergens more frequently than to indoor aeroallergens in the first year of life. Seasonal allergic rhinitis symptoms generally do not develop until 2 to 7 years of age. The prevalence of seasonal AR is higher in children and adolescents, whereas perennial allergic rhinitis has a higher prevalence in adults[3].

1.1.7 Effects of rhinitis on quality of life

AR can be a considerable source of morbidity in poorly managed patients. It impairs social and work functions, and can significantly affect the patient's quality of life[13]. Several studies have shown the deleterious effects of rhinitis on the quality of life in symptomatic patients[1]. Nasal obstruction can cause sleep disturbances that reduce a patient's daytime concentration and lead to daytime sleepiness. Complaints of poor sleep are already common

among older individuals due to various sleep disorders as well as the normal aging process, thus AR may exacerbate these problems[23]. Lack of sleep can alter physiological processes such as glucose metabolism, cognition, appetite control, and endocrine function, all critical physiologic processes in older people[1].

AR is also capable of markedly altering the patient's performance, learning and productivity. In addition, AR is commonly associated with other respiratory diseases, like asthma (ARIA), and the cost resulting from these comorbidities increases even more the socioeconomic impact of the disease [24].

1.2 Objective of the study

Although AR has a high prevalence and greatly impacts on daily life, there are only few studies if we compare them with those on other allergic diseases. Therefore the aim of this study was to investigate the relationship between Phadiatop, serum total IgE concentration and SPT and allergic rhinitis symptoms in a population of elderly and young adults of Cova da Beira.

Chapter 2

Methods

2.1 Study Design and Selection of Subjects

This study was carried out at the Faculty of Health Sciences of the University of Beira Interior and at the Covilhã Primary Health Care Centre.

This was a cross-sectional study using a random sample. The population consisted of 2 groups of individuals living in Cova da Beira: one of young adults (born between January 1976 and December 1993) and another of elderly individuals (born before 1944).

Participants were randomly selected using a stratified strategy from individuals who were registered in the list of all General Practitioners (GP) at Covilhã Primary Health Care Centre. Stratification implied selecting the patients according to the 2 age groups.

The study was approved by the Ethics Committee of Sub-Regional Health Administration of Castelo Branco and all volunteers signed a written informed consent, in accordance with the Declaration of Helsinki (Annex I).

2.2 Subject Recruitment

Volunteers' recruitment started on June 2008 through 2011. Data analysis includes all information collected on that period (questionnaires, skin prick tests, Total IgE and Phadiatop).

Initially, volunteers were contacted by post mail. If they didn't reply to the letters they lately were contacted by phone. Those who were not currently living in Cova da Beira, who had died or that we were unable to contact were excluded from the study.

Patients who refused to participate in the study or were not able to go to the Primary Health Care Centre were asked to answer the questionnaire by phone.

2.3 Questionnaires

A standardized questionnaire was given to all selected individuals (Annex II). It contained validated questions regarding signs and symptoms of allergic rhinitis and atopy-related risk factors like animal ownership, housing conditions or smoking exposure. We also collected data about demographic, clinical situation, personal and familiar history.

Furthermore, the questionnaires included relevant data about age, gender, place of residence, smoking habits, education and current and past professions. The completion of the questionnaires was made by the investigators.

2.4 Skin Prick Tests

SPT are employed for screening allergic sensitisation in patients with suspected allergic diseases. SPTs were performed on the volar aspects of both forearms, using a battery containing 3 single and 2 mixtures of the most prevalent aeroallergens in Cova da Beira (Annex III). Allergens used in this study were chosen according to a previous study that revealed the most prevalent allergens in Cova da Beira and include house dust mites (*Dermatophagoides pteronyssinus*), olive tree (*Olea europea*), grass pollen, weed pollen and *Parietaria judaica*. Histamine (10mg/ml) and allergen dilluent were used as positive and negative controls, respectively. Allergen extracts were manufactured by Leti (Barcelona, Spain) and all belonged to the same batch.

The skin was disinfected with ethanol and numbers were written on it to indicate where the allergen extracts would be located. A drop of each allergen extract was placed upon the epidermis and then pricked through using a 1.5 mm-long lancet tip (Stallergenes, France).

The mean wheal size was recorded after 15 minutes with a wheal size reader. SPT was regarded as positive with a wheal size minimum of 3 mm and if the response was less than 2 mm diameter, the SPT was regarded as negative. A skin test panel was considered valid if the correct outcomes for the controls were verified, including a histamine wheal greater than 3mm in diameter and an absence of wheal at the negative control site. Otherwise tests were considered inconclusive and if possible were repeated at least one week afterwards.

In the case volunteers had taken tricyclic antidepressants or antihistamines or if they had applied any product on the skin containing corticosteroids within the previous 7 days, skin prick tests would be postponed.

2.5 Definition of rhinitis and AR

Rhinitis was defined by a positive response to the question number 2.1 and current rhinitis by a positive response to the question number 2.2 of the questionnaire, or if the volunteer was under medication for the treatment of allergic rhinitis.

When rhinitis symptoms were reported by volunteers (positive response to the 2.2 question of the questionnaire) with both negative SPTs and Phadiatop they were regarded as

having non-allergic rhinitis. If patients had symptoms of rhinitis and atopy (positive response to the 2.2 question of the questionnaire) and they were confirmed by positive SPTs and/or positive Phadiatop, they were regarded as having AR.

2.6 Sample Processing for Total IgE and Phadiatop

A sample of 10 ml of peripheral blood was collected from patients by venopuncture, into a biochemistry tube containing coagulation accelerator (SARSTEDT, Germany) and two hemogram tubes containing EDTA (SARSTEDT, Germany).

Venous blood from the biochemistry tube was centrifuged at 4000 rpm for 10 minutes. Serum samples were stored at -20ºC until tested. Both Total IgE and Phadiatop tests were made in Hospital Sousa Martins, Guarda. Leucocytes were isolated from the hemogram tubes, after red blood cells lysis, washing and subsequent frizzing in liquid nitrogen for posterior analysis.

2.7 Phadiatop

ImmunoCAP Phadiatop (Phadia, Sweden) is a blood test designed to differentiate between atopic and non-atopic patients. Results indicate high or low probability for atopy. A negative result indicates that the symptoms are not caused by common environmental allergens, and the physician may explore other possibilities. The manufacturer has not revealed the precise formulation of the test.

Phadiatop assay is graded for the determination of atopy with semiquantitative or qualitative results.

Semiquantitative Phadiatop results are expressed as Phadia Arbitrary Units/L (PAU/L) indicating the degree of sensitization. A Phadiatop PAU/L value above the limit of quantification indicates that the patient is atopic (positive), i.e. measurable levels of specific IgE antibodies to common inhalant allergens have been detected. A Phadiatop PAU/L value below the limit of quantification indicates that the patient is non-atopic (negative), i.e. the level of specific IgE antibodies is undetectable. Higher Phadiatop PAU/L values indicate a higher degree of sensitization, i.e. higher levels of specific IgE antibodies to common inhalant allergens.

Qualitative Phadiatop results are expressed as positive or negative. A positive Phadiatop result indicates that the patient is atopic, a negative result indicates that the patient is non-atopic, i.e. not sensitized to inhalant allergens.

The technology of the test is based on an extremely high total binding capacity, achieved through a high binding capacity per mg cellulose in combination with an optimal

amount of cellulose in each solid phase. This ensures binding of all relevant antibodies, regardless of antibody affinity, still giving low non-specific binding.

The ImmunoCAP solid phase consists of a cellulose derivative enclosed in a capsule. The hydrophilic, highly branched polymer provides an ideal microenvironment for allergens, binding them irreversibly while maintaining their native structure.

The test is designed as a sandwich immunoassay. A balanced mixture of relevant inhalant allergens, covalently coupled to the solid phase, reacts with the specific IgE in the patient serum sample. After washing away non-specific IgE, enzyme-labelled antibodies against IgE are added to form a complex. After incubation, unbound enzyme-labelled anti-IgE is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate is measured. The higher the flouresence, the more specific IgE is present in the sample.

2.8 Total IgE

ImmunoCAP Total IgE is an *in-vitro* test for quantitative measurement of the total amount of circulating IgE in human serum or plasma samples. IgE antibodies appear as a result of sensitisation to allergens and measurement of circulating total IgE provides an aid in the clinical diagnosis of IgE-mediated allergic disorders. Elevated levels of circulating total IgE are usually seen in patients suffering from extrinsic asthma, hay fever or atopic eczema.

The serum concentration of total IgE is age-related. It increases during childhood and, at about 10 years of age, serum total IgE reaches values that are maintained during adult life.

The technology is based on a similar system as that of Phadiatop.

Serum total IgE determination was made automatically in ADVIA Centaur® Bayer analytic system and consisted in an sandwich immunoassay in two steps, using direct chemiluminescence technology that uses constant amounts of two human anti-IgE antibodies. The first one is a caprine anti-human IgE antibody labeled with acridine ester; the second one is in the solid phase (covalently binded to paramagnetic particles) and is an anti-human IgE mouse antibody. Volunteers' samples were incubated with both antibodies and after a washing step, equal amounts of acid and basic reagent were added to initiate the chemiluminescent reaction. Light relative unities amount (RLUs) detected by the system is proportional to total IgE quantity on the sample. Calibration curve values were introduced in the system through a code bar reader and after that, an adjustment to the curve was made using two calibrators standardized by the preparation of 75/502 World Health Organization reference.

2.9 Statistical analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS).

Data obtained were studied based on the relative and absolute frequencies of each studied variable (descriptive statistics). Chi square tests were used to compare the distribution of the factors between both groups (young adults and elderly adults). A p value of less than 0.05 was considered statistically significant.

Chapter 3

Results

3.1 Characterization of the Study Population

There were 1835 patients randomized into this study (926 young adults and 909 elderly). From those, 416 accepted to participate in this study. Of the 416 volunteers 403 patients answered to a validated questionnaire and 381 patients were evaluated for their skin prick test response to 5 common regional aeroallergens. In addition, 356 were analyzed for their serum total IgE and Phadiatop. Following analysis were carried out in this subgroup of volunteers.

Table 3.1- Number of completed questionnaires, skin prick tests, Phadiatop and total IgE analysis

	Elderly adults	*Young Adults*	*Total*
Questionnaires	270	132	402
Skin Prick Tests	252	128	380
Phadiatop	239	117	356
Total IgE	239	111	350

Among the 357 random subjects involved, there were 239 elderly subjects (mean age = 73; 141 females) and 117 young adult subjects (mean age= 28; 70 females).

Both groups were paired regarding gender. Data on academic degree, jobs and residence can be found on the following tables.

Table 3.2- **Academic degree of the study population**

		Elderly		Young Adults	
		%	n	%	n
Academic degree	No studies	14,23	34	0%	0
	Less than 4 years	35,15	84	0%	0
	4-9 years	37,66	90	14,5%	16

	9-12 years	9,21	22	40,9%	45
	More than 12 years	3,77	9	44,5%	49
	Total	100%	239	100%	110
	Didn't answered				7

There is a significant difference between academic degree of both groups (Chi-square test, p<0,000). All young adults studied more than 4 years and 45% studied more than 12 years. In contrast, 14% of the elderly didn't study at all and only 4% studied more than 12 years.

Table 3.3- Jobs classification, stratified according to the National Reader Survey Scale, United Kingdom[26]

		Elderly		Young Adults	
		%	n	%	n
Jobs	A	7,4	17	19,3	18
	B	11,7	27	15,0	14
	C1	10,0	23	19,3	18
	C2	23,0	53	10,7	10
	D	29,0	67	12,9	12
	E	16,5	38	22,5	21
	Total	100,0	231	100,0	93

A- Upper middle class; B-middle class; C1- lower middle class: C2-skilled working class; D- working Class; E- those at the lower level of subsistence

There is a significant difference between jobs stratification of both groups (Chi-square test, p<0,001). Young adults have a major percentile at "A" (19%) and "E" (23%) classification and the elderly have a major percentile to the "C2" (23%) and "D" (29%) classification.

Table 3.4: Residence of the study population

		Elderly		Young Adults	
		%	n	%	n
Residence	Urban	81,5	194	82,9	97
	Rural village	6,7	16	10,2	12
	rural farm	10,9	26	1,7	2
	Rural and urban	0,8	2	5,1	6
	Total	100,0	238	100,0	117

The majority of the volunteers have urban residence (83% and 82%, for young adults and elderly, respectively). However there are statistically significant differences between young adults and elderly regarding to residence, probably due to the different distribution on the rural residence (Chi-square, p= 0,005).

3.2 Phadiatop results and SPT

We next analyzed the data regarding Phadiatop test**.** Of the 239 elderly volunteers, 38 had positive Phadiatop (16%). On the young adults group, 58 of the 117 had positive Phadiatop (50%), which means that the prevalence of atopy measured by Phadiatop was significantly higher in the young adults group (Chi-Square test, p=0,000).

Table 3.5 - Distribution of Phadiatop in the two groups

	Young Adults		Elderly	
	n	%	n	%
Positive Phadiatop	58	49,6	38	15,9
Negative Phadiatop	59	50,4	201	84,1
Total	117	100,0	239	100,0

Significant difference was found in the distribution of Phadiatop with gender in young adults (Chi-Square test, p=0,02) and elderly (Chi-Square test, p=0,05); with higher frequencies being found in males (53,2% and 21,2 %) (Table 3.6).

Table 3.6- Phadiatop distribution by gender in young and elderly.

		Young Adults				Elderly			
		Male		Female		Male		Female	
		n	%	n	%	n	%	n	%
Phadiatop	Positive	25	53,2	33	47,1	21	21,2	17	12,1
	Negative	22	46,8	37	52,9	78	78,8	123	87,9
	Total	47	100	70	100	99	100	140	100

Assuming Phadiatop results as the gold standasr we found the existence of 83 false positives and 28 false negatives (see table 3.7) when evaluating SPT.

Table 3.7- Comparison between Phadiatop and SPT results.

		SPT	
		Positive	Negative
Phadiatop	Positive	63	28
	Negative	83	165

Comparing sensitization to the SPTs and a positive Phadiatop, we obtained 62,5 % of the positive Phadiatop young adults with also positive SPT (at least one positive SPT with a wheal size minimum 3 mm). In regard to the elderly group, we obtained 80% of the Phadiatop positive patients with positive SPTs.

The relation between Phadiatop and SPT is organized by gender for young adults and elderly on tables 3.8 and 3.9, respectively.

Table 3.8- Comparison between Phadiatop and SPT results in young adults.

Phadiatop	SPT	Gender			
		Female		Male	
		n	%	n	%
Positive	Positive	19	55,9	12	54,5
	Negative	15	44,1	10	45,5
Negative	Positive	14	42,4	7	30,4
	Negative	19	57,6	16	69,6

Table 3.9 - Comparison between Phadiatop and SPT results in elderly.

Phadiatop	SPT	Gender			
		Female		Male	
		n	%	n	%
Positive	Positive	19	57,6	16	69,6
	Negative	14	42,4	7	30,4
Negative	Positive	15	44,1	10	45,5
	Negative	19	55,9	12	54,5

The percentage of positive Phadiatop and SPTs was higher in females in young adults (55,9% in comparison with 54,5% for males) but lower in the elderly (57,6 % versus 69,9% in males).

In order to see if there is a correlation between the Phadiatop concentration and SPT wheal diameters a graphic representation was made relating the wheal size of each allergen with Phadiatop concentration for young adults (figure 3.1) and for elderly (figure 3. 2)

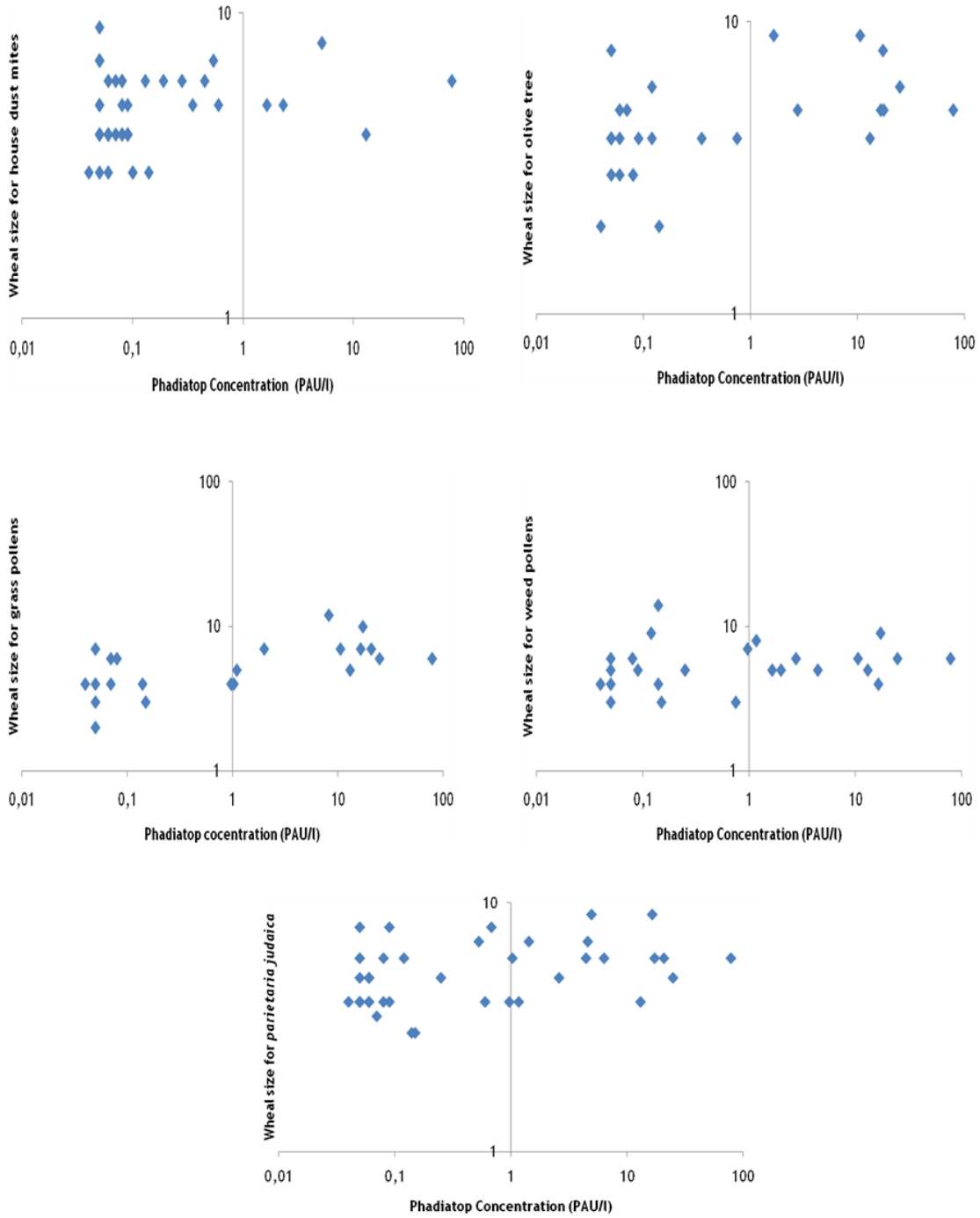

Figure 3.1- Graphic representation for Phadiatop concentration and the wheal diameter for the aeroallergens tested in elderly.

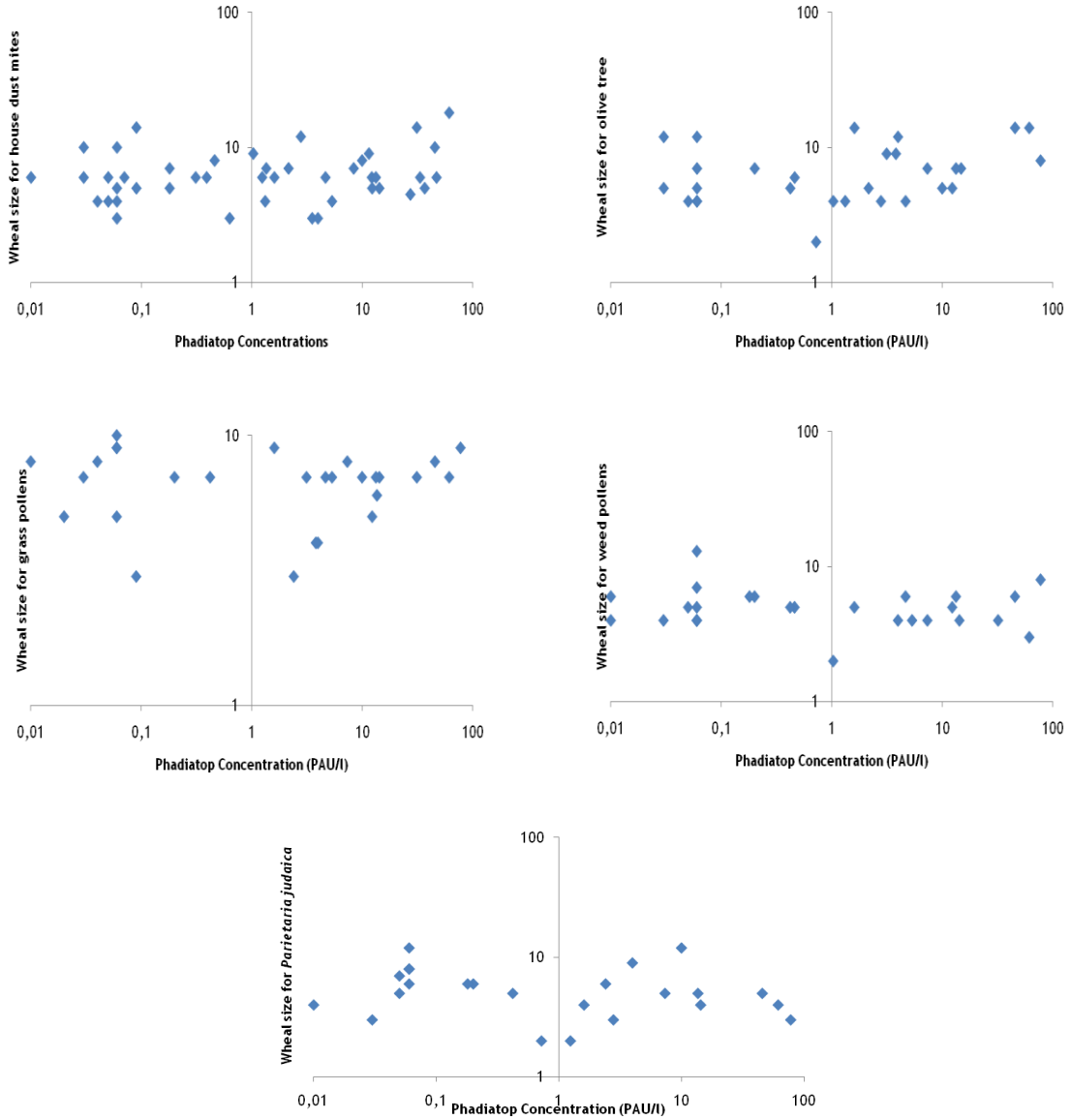

Figure 3.2- Graphic representation for Phadiatop concentration and the wheal diameter for tested aeroallergens in young adults.

Using Spearman's test no correlation is observed for Phadiatop concentration and the wheal diameter for aeroallergens in elderly or in young adults.

3.3 Phadiatop and total IgE concentration

Some studies state that there is not a clear correlation between Phadiatop positivity and total IgE values. To access if this was also true in our population we performed correlations between Phadiatop and total IgE values.

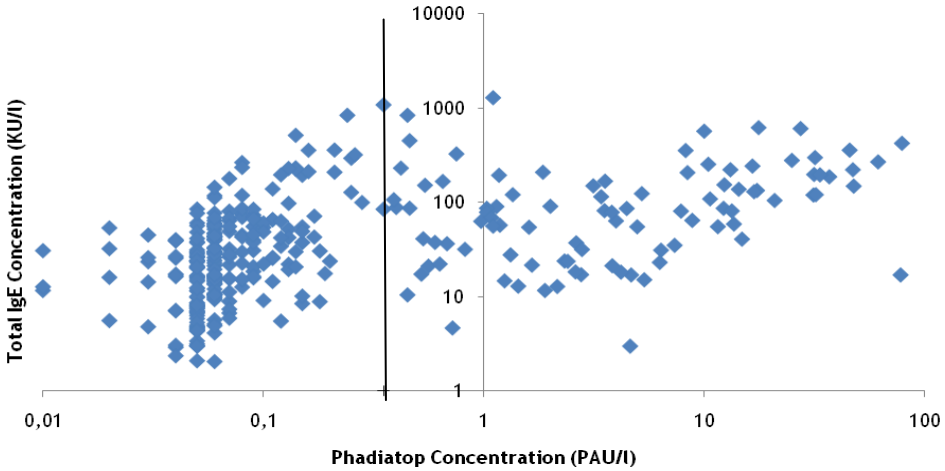

Figure 3.3- Graphic representation of the distribution of the Phadiatop and total IgE concentrations in all volunteers.

Using Spearman's test no correlation is observed between Phadiatop and IgE concentration in the total sample (young adults plus elderly). 0,35 is the value from which Phadiatop was considered positive.

The distribution of the Phadiatop and total IgE concentrations in young adults and elderly patients separately is represented on the following graphics.

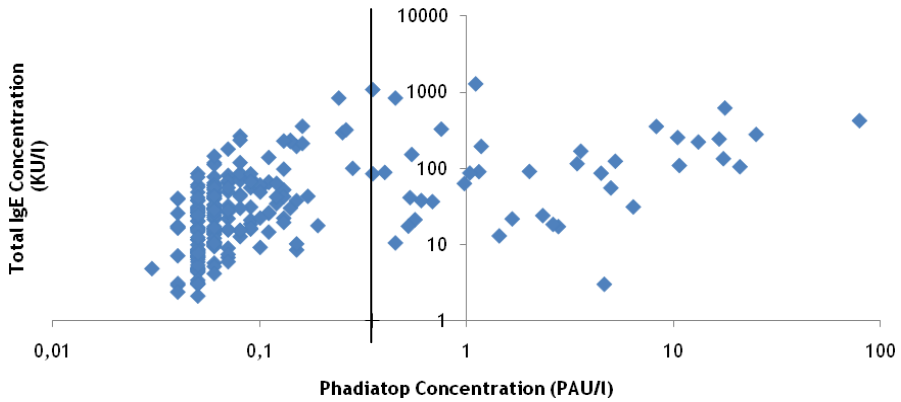

Figure 3.4- Graphic representation of the distribution of Phadiatop and total IgE concentrations in the young adults.

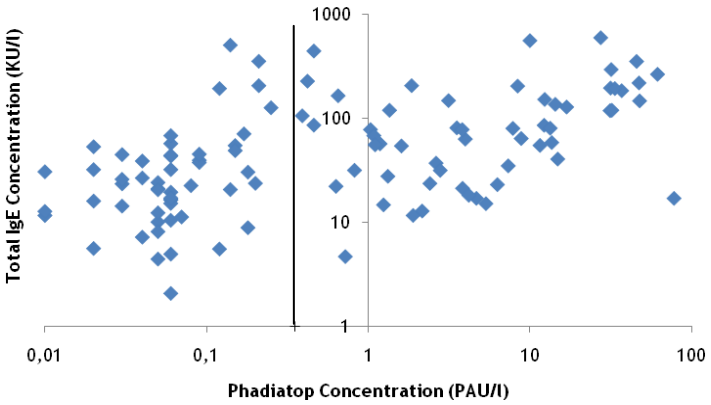

Figure 3.5 Graphic representation of the distribution of Phadiatop and total IgE concentrations in the elderly.

Applying the Spearman test to the stratified population didn't show any correlation between Phadiatop and total IgE values (figures 3.4 and 3.5 respectively).

Figure 3.6 shows the values of total serum IgE and volunteers' age.

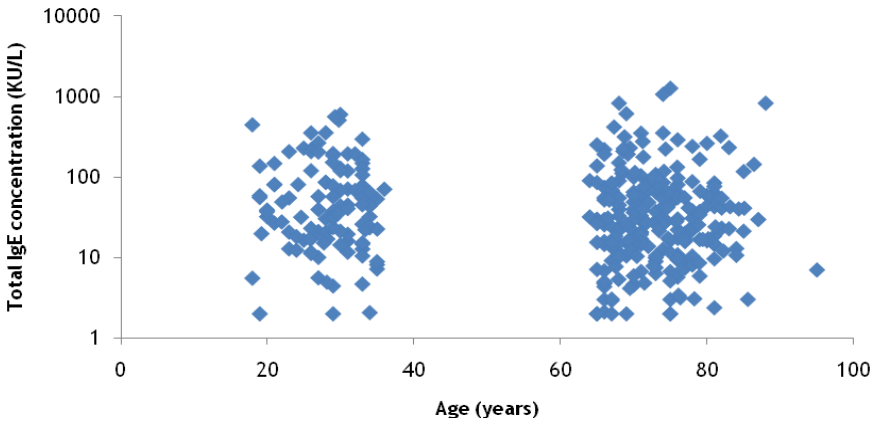

Figure 3.6 – Graphic representation of volunteers' age versus total IgE.

Applying Spearman test no correlation was found for the relation between total IgE concentration and the age of volunteers. However, the mean total IgE concentration in young adults is higher than in the elderly (81,6 and 71,7 respectively).

3.4 Clinical characteristics

As stated in the methods section, AR was defined as a positive answer to question 2.2 or use of anti-allergic medication and a positive Phadiatop value. Thus 69 volunteers (27 elderly and 42 young adults) had AR (see table 3.10).

Table 3.10- Volunteers with AR

	Young Adults		Elderly	
	n	%	n	%
Have AR	42	48,9	27	16,6
Have not AR	44	51,1	136	83,4
Total	86	100,0	163	100,0

There is a significant difference between both groups (Qui-Square test, p=0,000). Young adults have a higher prevalence of AR than the elderly (48,9% versus 16,6%).

Comparing positive SPT of the volunteers with rhinitis of the two groups, we obtained 65,9 % of young adults and 87,5 % of elderly with positive SPT (see table 3.11)

Table 3.11- Distribution of SPT in the two groups

	Young Adults		Elderly	
	n	%	n	%
Positive SPT	27	65,9	21	87,5
Negative SPT	14	34,1	3	12,5
Total	41	100	24	100

There is a significant difference between both groups regarding the result of SPT in volunteers with AR (Qui-Square test, p=0,05).

In order to access triggering factors for AR, positive SPTs were compared to reported symptoms. Allergens were classified as indoor (mites) and outdoor (pollens). The distribution of these allergens is on table 3.12 and 3.13, respectively.

Table 3.12- Distribution of the sensitization to indoor allergens.

Sensitization to indoor allergens	Young Adults		Elderly	
	n	%	n	%
Positive	22	53,7	5	20,0
Negative	19	46,3	20	80,0
Total	41	100,0	25	100,0

53,7 % of the young volunteers and 20,0 % of the elderly were sensitized to indoor allergens, however, no difference in the sensitization profile regarding indoor allergens is observed between both groups (Chi-Square test, p>0,05).

Table 3.13- Distribution of the sensitization to outdoor allergens.

Sensitization to outdoor allergens	Young Adults		Elderly	
	n	%	n	%
Positive	17	41,5	18	72,0
Negative	24	58,5	7	28,0
Total	41	100,0	25	100,0

There is a significant difference between both groups in sensitization to outdoor allergens (Qui-Square test, p=0,01) with the elderly being more sensitized (72,0% versus 41,5%).

18,1% of the volunteers were sensitized to both indoor and outdoor allergens.

Mites' sensitization (indoor allergen) was compared to factors that could exacerbate AR symptoms, specifically house dust and the presence of carpets at home. Furthermore, pollens' sensitization (outdoor allergens) accessed by SPT was compared with the exacerbation or not of the symptoms with the pollen season. Results are on table 3.14.

Table 3.14- Relation between positive SPT for mites and symptoms worsened by house dust and pollens.

			Symptoms worsened by triggering factors			
Triggering factors			Yes		No	
			n	%	n	%
House dust	Positive SPT for house dust mites	Yes	6	66,7	2	33,3
		No	3	33,3	4	66,7
Carpets at home	Positive SPT for house dust mites	Yes	3	33,3	24	42,1
		No	6	66,7	33	57,9
Pollens	Positive SPT	Yes	13	86,7	0	0
		No	2	13,3	0	0

Symptoms worsened by triggering factors were assessed by a positive answer to question 2.4 (which includes house dust and pollens) and 5.2 (carpets at home) of the questionnaire.

Mites' sensitization (indoor allergen) was not related to factors that could exacerbate AR symptoms, specifically house dust and the presence of carpets at home (Chi-Square test, $p>0,05$). Similarly, AR symptoms were not related to the presence of carpets or house dust (Chi-Square test, $p>0,05$). However, a correlation was found for pollen sensitization (outdoor allergens) as all the volunteers sensitized to pollens complained of worsening of symptoms in the pollen season.

Chapter 4

Discussion and conclusions

In comparison with asthma, allergic rhinitis have been less extensively investigated, although this does not mean that it should be regarded as a minor disorder but rather as an important pathology that affects the quality of life of the patients and their families, generating considerable direct and indirect costs.

This is a multifactor disorder without a single causal agent, in which the most important component is the genetic predisposition of the patient (atopy), modulated by environmental factors, exposure to allergens, infections and irritants, among others [22]. Rhinitis in the elderly may be caused by types of rhinitis common in other age groups but may also be influenced by age-related physiologic changes such as cholinergic hyperactivity, anatomic changes and medications taken for other medical conditions (usually elderly take many medication). Over the past few years, several studies supporting the hygiene hypothesis have suggested that early exposure to viral and bacterial infections, such as day care attendance or more siblings may reduce the incidence of atopic disease by redirecting the immune system away from the allergic T-helper 2(TH2) pattern to the T-Helper 1(TH1) pattern. One early explanation proposed that the increased incidence of atopy as explained by the hygiene hypothesis is a result of the reduced production of IL-12 and interferons (IFNs) by cells of the innate immune system that are normally stimulated by bacterial products via their Toll-like receptors[30].

From a clinical perspective, supported by epidemiological investigations, there would appear to be a decline with age in both the incidence and severity of atopic diseases, particularly among the elderly population who are 60 years or older. Atopic incidence declines, symptoms severity declines, and there would appear to be a general humoral alteration of the propensity for atopy reflected by age-associated declines in serum total IgE values. The decline of the onset of allergic symptoms observed in ageing might result from a decrease of serum total IgE due to an unbalance of cytokines and soluble factors involved in its production[28].

The natural course of allergy is based on persistent antigen exposure; the repeated stimulation with identical antigenic proteins induces on one hand the expression of new surface molecules and, on the other hand, the expansion of effector and memory cells. It follows that, with aging, the number of naive cells decreases and the sensitized cells increase.

Cross-sectional studies show that the prevalence of IgE sensitization is lower in older age groups than younger age groups. This could reflect either a decrease in sensitization with aging or a higher prevalence of sensitization in more recent birth cohorts.

Although IgE sensitization and total IgE are both associated with an increased risk of allergic disease in individuals, they exhibit very different changes with aging and with cohort.This may reflect their differing association with environmental factors[29]. Evidence that the prevalence of atopic diseases, including asthma and hay fever, has increased over the past 20-30 years comes mainly from questionnaire based surveys; objective measurements being limited. Atopy can be demonstrated by SPT or increase in IgE measured as serum total IgE or specific IgE. We measured SPTs and serological markers of atopic sensitization in stored serum samples that had been collected from the population stated on Results. The serological markers included total IgE and Phadiatop. Both measure IgE, but whilst total IgE provides a measurement for all produced IgE independently of the underlying disorder, Phadiatop gives a sensitisation screening by measuring the concentration of specific serum IgE against common aeroallergens. We also used skin prick tests to assess atopy, but we must consider that they measure not IgE, but skin mast cell degranulation. Skin prick test are the most useful single modality for demonstrating an IgE-mediated underlying mechanism in suspected allergic diseases. The test is reliable, cheap, and easy to perform and they offer a prompt result. However, SPT may be subjected to a number of problems such as choice and storage of allergens, prick test technique and individual interpretation. Advantages of serum specific IgE assays are convenience for the patient, lack of risks and the possibility of testing subjects unable to stop medication that could alter the results of SPT. A major disadvantage of serum specific IgE assays is their high cost, especially in case of assays for multiples allergens. Although the test developed successive variants, all of them have as the common principle to test for serum specific IgE to a mixture of relevant allergens causing common inhalant allergies. Diagnostic accuracy of Phadiatop may vary not only with the prevalence of allergic sensitization in the studied population, but also with the aeroallergen profile of a given area. In this sense is should be noted that the aeroallergen composition of Phadiatop is fixed and not stated by the manufacturer.

In this study we used Phadiatop as our gold standard and results were based on its positivity. The test is well characterized and has been used in other studies. In the PAQUID cohort, Raherison and colleagues collected data by questionnaire and based their study on total IgE concentration and Phadiatop to investigate the specific relationship of serum IgE and Phadiatop with rhinitis and smoking habits using a random sample of 352 elderly subjects[18]. Jarvis and co-workers in the ECRHS study tried to identify environmental risk factors for the development of IgE sensitization in adult life determining serum specific IgE to common allergens and total IgE, measured in 2 occasions about 9 years apart. In an observational, descriptive, cross-sectional study, Navarro and co-workers studied a sample of allergic patients treated in consultations in the Spain health system. The diagnosis of AR was mainly based of clinical history, physical examination and skin prick tests[29]. Mediaty and colleagues

evaluated the effects of age on total and specific IgE in patients with AR across a population of 559 individuals randomly selected[19].

Our study showed that there were significant differences in demographic data of young adults and elderly. All young adults studied more than 4 years whereas 14% of elderly didn´t study at all and only 4 % studied more than 12 years unlike young adults that have a higher percentage (45 %). These results are in agreement with the results achieved for jobs stratification, which also have a significant difference between both groups. A higher academic degree corresponds to a higher social class. Individuals who are better educated are more likely to understand the pathophysiological basis of AR and hence the importance of treatment, avoidance of allergens and prevention of episodes of disease. It has already been proven that lower level of education is an independent predictor of a poor quality of life in individuals with AR[30].

Regarding residence, the majority of our volunteers have urban residence; however there are significant differences in both groups, probably due to the different distribution on the rural residence.

We also found that the Phadiatop positivity is significantly higher in the young adults when compared with the elderly. This result is concordant with other studies in other countries which have also reported that young adults have a higher sensitization than older adults [19].

Furthermore, comparing genders, Phadiatop positivity is significantly higher in men of both groups, 53,2% in young adults and 21,2% in elderly. These results are in conformity with some studies that also found a higher prevalence in men[18]; however other studies found a higher prevalence in women[15, 6] . Regarding the association between the Phadiatop result and the SPT we found the existence of 83 false positives and 28 false negatives. They were classified as false positives if a SPT result was positive but Phadiatop was negative; false negatives were those with a negative SPT but positive Phadiatop (considering Phadiatop our gold standard). This could happen because SPT and Phadiatop don't measure exactly the same (as said before). Criteria for interpreting skin test results differ between younger and older patients and the incorrect interpretation of skin testing may lead to false negative responses. One possible explanation can be found in the age-associated reduction in skin reactivity of the elderly to histamine and allergens.

We also looked for a correlation between the Phadiatop concentration and SPT wheal diameters; graphic representations were made relating the wheal size of each allergen with Phadiatop concentration for young adults and for elderly, but no correlation was observed. Once again, this could be due to the different parameters each test determines.

Another atopic marker is serum total IgE. No significant correlation between Phadiatop concentration and total IgE concentration was found neither for all population either for young adults or elderly. Some studies state that there is not a clear correlation between Phadiatop positivity and total IgE values. One possible explanation is that total IgE is genetically determined while sensitization can be environmentally determined.

Many studies affirmed that total IgE decreases with increasing age [19,29]. We obtained no correlation. However, the mean total IgE concentration in young adults is higher than in elderly (81,6 and 71,7 respectively). This could reflect either a decrease in sensitization with aging or a higher prevalence of sensitization in more recent birth cohorts.

Defining AR with positive Phadiatop and presence of symptoms (question 2.2 of the questionnaire) or use of anti-allergic medication, we found 69 volunteers with AR (42 young adults and 27 elderly). The prevalence of rhinitis is higher in young adult, as reported in other studies[6,16].

Volunteers with rhinitis also showed a significant difference regarding to SPTs. There is a significant difference between both groups regarding the result of SPT in volunteers with AR. 87,5% of the elderly had positive SPTs and young adults had 65,9%. This could mean that in elderly the combination of these two tests is more significant than in the young adults and is an interesting result because is contradictory with less skin sensibilization that is common in elderly.

Genetic factors may influence immunologic development. However current rapid rise in allergic diseases cannot be fully explained only by genetic factors. The complex interplay between immune responses of the host, the level and variety of the environmental exposure, and the interactions between the genetic background and the range of exposures are likely to affect the development of allergic diseases. To assess the involvement of the gene-environment interaction in the onset of allergic disorders, Tanaka and colleagues believed that it would be useful to list candidate environmental factors associated with allergic disorders [30]. In this study we chose three possible triggering factors: house dust and presence of carpets at home (for indoor allergens) and pollens (for outdoor allergens). In what concerns to outdoor allergens, we found a correlation for aggravation of the symptoms in the pollen season when compared to the SPT sensitization for pollens; everyone who had a positive SPT for pollens complained that pollens worsened their symptoms. Indeed, elderly had a major prevalence of sensitization for outdoor allergens than for indoor (72% versus 20%). On reverse, young adults had major sensibilization for indoor allergens (53,7% versus 41,5%). However we found no correlation for indoor allergens. 66,7% of volunteers with positive SPT for house dust mites also reported aggravation of rhinitis symptoms. This difference could be due to the fact that the elderly belong to a decade in which people worked more outdoor and then they were more exposure to outdoor allergens.

This study had some limitations. Participation in the study was volunteer, which may have skewed the results as individuals are more likely to respond if they identify themselves with the problem of the study. Increased awareness of the previously diagnosed patients as well as the presence of symptoms of allergy probably explains thar the majority of responding young adults reported symptoms. The sample selection was limited to individuals registered at the Health Care Centre, and data refering to contact is incomplete or outdated, especially for young adults. Another limitation is that potential cognitive

impairment can lead the subjects to give approximate responses in the questionnaire because of memory problems.

Although there are some weaknesses in the present study, there were also some strong points.Our study compared demographic, clinical and sensitisation characteristic between elderly and young adults. In spite of a potential selection bias, our sample was random and paired regarding gender. Finally, our methodological approach was thorough as is involved not only a validated questionnarie, but also skin prick tests, total IgE and Phadiatop evaluation.

In summary our study showed that young adults have a higher prevalence of allergic rhinitis when compared with the elderly group and it is more prevalent in men in both groups. Furthermore, the mean IgE concentration of the population was also higher in young adults and we found that elderly are more sensitized to outdoor allergens while young adults are more sensitized to indoor allergens.

4.1 Future perspectives

As this is an ongoing study we will continue to recruit and analyse volunteers in order to achieve a bigger sample to allow us to extrapolate our data to the general population. Furthermore, volunteers with positive qusetionnaires for AR will be frther studied in terms of symptoms features, as recommended by the ARIA guidelines and using other complementary diagnostic means to confirmate the diagnosis.

Chapter 5

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Annexes

I- Informed consent

ESTUDO DA PREVALÊNCIA DE ATOPIA NUMA POPULAÇÃO DE IDOSOS E ADULTOS JOVENS.

FOLHA DE INFORMAÇÃO DOS VOLUNTÁRIOS

(conforme "Declaração de Helsínquia", da Associação Médica Mundial, 1964)

A alergia é uma reacção exagerada do sistema imunitário ao contacto com proteínas comuns do meio ambiente que, na maior parte das pessoas, não provocam reacção. Pode manifestar-se, entre outras doenças, por asma, rinite, conjuntivite ou dermatite atópica.

Como é importante saber a percentagem de pessoas na região da Beira Interior que têm alergias, levamos a cabo o presente estudo, desenvolvido pela Universidade da Beira Interior, para o qual agradecemos a sua participação.

Para o estudo necessitamos da sua colaboração, através do preenchimento de um questionário, da realização de testes cutâneos de alergia e ainda da colheita de uma pequena quantidade de sangue (20 ml).

Os testes cutâneos de alergia são uma técnica muito segura, frequentemente usada. Consistem na colocação de uma pequena gota de proteínas do ambiente no antebraço. Uma lanceta com uma ponta de 1mm é então usada para introduzir a gota na pele. Caso haja alergia formar-se-á uma pequena pápula associada a alguma comichão, que desaparecem passado pouco tempo.

A colheita de sangue é uma técnica de rotina, sem riscos, que acarreta um desconforto mínimo.

Os testes e a colheita de sangue serão efectuados por médicos com vasta experiência.

Este estudo poderá ajudar esclarecer melhor a frequência e tipo de doenças alérgicas na região da Beira Interior.

Caso assim o deseje, poderá recusar participar neste estudo a qualquer altura, sem que isso prejudique os seus direitos em termos de assistência hospitalar.

Os resultados deste estudo poderão ser consultados pelos responsáveis científicos do projecto de investigação e ser publicados em revistas científicas. No entanto, os dados de carácter pessoal serão mantidos confidenciais.

ESTUDO DA PREVALÊNCIA DE ATOPIA NUMA POPULAÇÃO DE IDOSOS E ADULTOS JOVENS.

Eu, abaixo assinado (nome completo do voluntário)

_____

_, compreendi a explicação que me foi fornecida acerca do meu caso clínico e do método ou tratamento que se tenciona instituir, tendo-me sido dada a oportunidade de discutir e fazer as perguntas que julguei necessárias.
Por isso, consinto que me seja aplicado os métodos propostos para o estudo actual.

Data: ____/___/_____
Assinatura: _____

Testemunha (caso haja)
Data: ____/___/_____
Assinatura: _____

Eu, abaixo assinado, _____, investigador responsável, certifico que foram postas à disposição, informações respeitantes ao estudo supracitado, "de modo simples, inteligível e leal", conforme o disposto no Decreto-Lei nº 97/94, de 09 de Abril.

Data: ____/___/_____
Assinatura: _____

II- Questionnaire

DADOS PESSOAIS
CÓDIGO: _____
NOME:
_____
MORADA: _____
TELEFONE: _____ DATA NASCIMENTO: _____

QUESTIONÁRIO: Sim ❑ Não ❑

TESTES CUTÂNEOS: Sim ❑ Não ❑ Inconclusivos ❑

COLHEITA DE SANGUE: Sim ❑ Não ❑
ARMAZENADOS:
CÉLULAS: Sim ❑ Não ❑ _____
SORO: Sim ❑ Não ❑ _____

DADOS PESSOAIS

CÓDIGO: _____

Sexo: Feminino ❑ Masculino ❑

Local de residência: _____

Locais onde viveu anteriormente:

Infância:	Campo❑	Cidade❑
Idade Adulta:	Campo ❑	Cidade ❑

Habilitações Académicas (anos de estudo):

Não estudou	❑
- de 4 anos	❑
De 4 a 9 anos	❑
De 9 a 12 anos	❑
+ de 12 anos	❑

1. QUESTIONÁRIO SOBRE ASMA

(Todas estas perguntas se referem a situações em que <u>não está</u> constipado/a ou com gripe)

1.1 Alguma vez teve pieira ou 'gatinhos no peito'?

Sim ❑ Não ❑

(Se respondeu "Não", por favor passe à pergunta 2.)

1.2 <u>Nos últimos 12 meses</u>, teve "gatinhos" ou "pieira" no peito?

Sim ❑ Não ❑

1.3 <u>Nos últimos 12 meses</u> tomou medicamentos para tratar a asma?

Sim ❑ Não ❑

1.4 Que factores agravam os seus "gatinhos" ou pieira?

Alterações de temperatura (frio/quente)	❑	Pólenes	❑
Pó da casa	❑	Comidas/Bebidas	❑
Fumo do Tabaco	❑	Outros Fumos	❑
Emoções	❑	Roupa de Lã	❑

Medicamentos	❑	Cheiros intensos (perfumes, detergentes, lixívia)	❑
Constipações/Gripes	❑	Animais de estimação	❑
Trabalho	❑	Exercício Físico	❑
Outros	❑	Quais? _____	

1.5 Os seus sintomas de pieira ou falta de ar surgem:

Durante todo o ano ❑

Só em parte do ano:

Inverno (altura do Natal e do frio) ❑

Primavera (rebentar da flor) ❑

Verão (tempo quente) ❑

Outono (cair da folha) ❑

1.6 Alguma vez teve "pieira", tosse seca ou falta de ar durante ou depois de fazer exercício?

Sim ❑ Não ❑

1.7 Nos últimos 12 meses teve "pieira", tosse seca ou falta de ar durante ou depois de fazer exercício?

Sim ❑ Não ❑

2. QUESTIONÁRIO SOBRE RINITE E CONJUNTIVITE

(Todas estas perguntas se referem a situações em que não está constipado/a ou com gripe)

2.1 Alguma vez teve espirros, o nariz "a correr" ou o nariz tapado sem estar constipado ou com gripe?

Sim ❑ Não ❑

(Se respondeu "Não" por favor passe à pergunta 3.)

2.2 Nos últimos 12 meses teve espirros, o nariz "a correr" ou o nariz tapado sem estar constipado ou com gripe?

Sim ❑ Não ❑

2.3 Nos últimos 12 meses estes problemas de nariz eram acompanhados de comichão nos olhos?

Sim ❑ Não ❑

2.4 Que factores agravam estes problemas do nariz?

Alterações de temperatura (frio/quente)	❑	Pólenes	❑
Pó da casa	❑	Comidas/Bebidas	❑
Fumo do Tabaco	❑	Outros Fumos	❑
Roupa de Lã	❑	Medicamentos	❑

Cheiros intensos (perfumes, detergentes,lixívia) ❑
Animais de estimação❑
Trabalho ❑
Outros ❑ Quais? _____

2.5 Os seus sintomas do nariz surgem:

Durante todo o ano ❑
Só em parte do ano:
Inverno (altura do Natal e do frio) ❑
Primavera (rebentar da flor) ❑
Verão (tempo quente) ❑
Outono (cair da folha) ❑

3. OUTRAS ALERGIAS

3.1 Alguma vez teve alergias a algum alimento?

Sim ❑ Não ❑

3.2 Alguma vez teve alergias a algum medicamento?

Sim ❑ Não ❑

3.3 Alguma vez teve alguma reacção exagerada à picada de uma abelha ou vespa?

Sim ❑ Não ❑

4. QUESTIONÁRIO SOBRE PROFISSÃO E PASSATEMPOS

4.1 Qual é a sua profissão? _____

4.2 Neste momento encontra-se:

No activo ❑ Reformado ❑ Desempregado ❑

4.3 Alguma vez trabalhou em:

Indústria têxtil ❑ Minas ❑ Agricultura ❑

4.4 Que passatempos tem?

Outdoor 1 (caça, pesca, caminhadas, desp. ar livre) ❑

Outdoor 2 (jardinagem) ❑

Indoor (trab. com lãs, arraiolos) ❑

5. QUESTIONÁRIO SOBRE RESIDÊNCIA

5.1 Como é a sua residência?

Urbana ❑

Rural (aldeia/vila) ❑

Rural (quinta) ❑

5.2 A sua casa é alcatifada?

Sim ❑ Não ❑

5.3 A sua casa tem fungos/bolores nas paredes/tecto?

Sim ❑ Não ❑

5.4 Tem animais?

Não ❑
Sim, no quintal ❑
Sim, em casa ❑

5.5 Que animais tem?

Cão ❑
Gato ❑
Pássaros ❑

6. QUESTIONÁRIO SOBRE HÁBITOS TABÁGICOS

6.1 É fumador?

Sim ❑ Quantos cigarros fuma por semana? _____
Não ❑
Ex-fumador ❑ Há quanto tempo deixou de fumar? _____

6.2 Alguém fuma regularmente dentro de sua casa?

Alguém fuma regularmente no seu local de trabalho?

Não ❑
Em casa ❑
No trabalho ❑
Ambos ❑

7. QUESTIONÁRIO SOBRE MEDICAÇÃO

7.1 Actualmente toma medicamentos?

Sim ❑ Não ❑

7.2 Que medicamentos toma?

Medicamentos para asma ❑
Medicamentos para rinite ❑
Beta-bloqueantes ❑
iECA (Inibidores enzima conversão angiotensina) ❑
Antidepressivos ❑
Imunomoduladores (corticosteróides) ❑
Anti-inflamatórios ❑

8. QUESTIONÁRIO SOBRE ANTECEDENTES FAMILIARES

Assinale na tabela com um X as alergias que conheça na sua família:

Familiar	Asma/bronquite asmática	Rinite alérgica
Pai		
Mãe		
Irmãos		
Avós Paternos		
Avós Maternos		

Chegou ao fim do nosso questionário.
Obrigado pela sua colaboração.

III- SPT information

TESTES CUTÂNEOS

CÓDIGO: _____
DATA: _____

Tomou antihistamínicos ou antidepressivos tricíclicos há menos de 7 dias?
Sim ❑ Não ❑
Tem aplicado corticosteróides tópicos na pele?
Sim ❑ Não ❑

Controlo Negativo (Diluente)	
Controlo Positivo (Histamina)	
Dermatophagoides pteronyssinus	
Olea europea	
Mix Pólenes IV (Gramineas) Grass	
Mix Pólenes II (Ervas) Weed	
Parietaria judaica	

Voluntário sensibilizado a aerolergénios?
Sim ❑ Não ❑ Testes Inconclusivos ❑