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THE OFFICIAL JOURNAL OF SPAIC | SOCIEDADE PORTUGUESA DE ALERGOLOGIA E IMUNOLOGIA CLINICA

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The athlete "out of breath"

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Summary
Athletes often complain about breathing problems. This is a crucial issue due to potential implications not only on their general health, but also on their competing performance. Asthma and exercise-induced bronchoconstriction are prevalent conditions in elite athletes, which leads doctors to rely most of the times on asthma medication to treat athletes feeling "out of breath". However, there are several other conditions that may mimic asthma and cause dyspnea in athletes. Effective treatment of dyspnea requires appropriate identification and treatment of all disorders. Proper knowledge and accurate diagnosis of such entities is mandatory, since asthma medication is not effective in those conditions. Herein we review the most common differential diagnosis of dyspnea in athletes, and describe the diagnostic strategies in order to increase awareness and to improve doctor's confidence on dealing with these patients.

Introduction

Regular physical exercise and participation in sports are considered to be important components of a healthy life and are recommended for all individuals (1). However, in patients with respiratory symptoms, physical exertion is a potent stimulus that can produce episodes of airway distress. These individuals may show less tolerance to exercise due to the worsening of respiratory symptoms during exercise and this can preclude them from playing sports or attempting to keep fit. For instance, regarding asthma, evidence has shown that physical training improves cardiopulmonary fitness (2) and may even improve quality of life of both asthmatic children and their caregivers (3). Therefore, given the beneficial effects of exercise, every effort should be taken by doctors to recognize respiratory diseases and yield all actions so they are not a limitation for its practice. This issue is particularly relevant when concerning athletes. Respiratory symptoms induced by exercise have potential implications not only on athlete's general health, but also on their training capacity and competing performance.

Respiratory complaints pose several issues unique to athletes as they face special challenges managing their respiratory symptoms while practicing sport. Discrimination between physiological and pathological limitations to maximum exercise is difficult given the heavy training with extremely high level of physical fitness and maximum oxygen uptake (VO_2max) reached (4). This limits the ability of diagnosis. On the other hand, some athletes will not reveal their respiratory symptoms due to fear that the disclosure of their disorders will be detrimental. Therefore, athlete's respiratory disorders often perplex, frustrate and distress both patients and their physicians. However, as stated by International Olympic Committee, "*all care should be taken to ensure that sports do not affect the health or welfare of the participants*". So, the aim of this study is to review

several respiratory diagnostic hypotheses and how to differentiate and approach each, in order to provide the best treatment for athletes complaining of breathless. As a general purpose, we aim to demystify this subject and improve doctor's confidence on dealing with these patients.

Asthma, the most common respiratory disorder among athletes

Well known by *Aretaeus* since the year 100 AD (5), asthma induced by sport practicing is not always easy to describe and recognize. For this reason, in 2008, a Joint Task Force was established by the European Respiratory Society (ERS) and the European Academy of Allergy and Clinical Immunology (EAACI) in cooperation with GA²LEN; accordingly, exercise-induced asthma (EIA) is defined as the presence of lower airway obstruction and symptoms of cough, wheezing or dyspnea induced by exercise in patients with underlying asthma (4); the same clinical presentation occurring after exercise in subjects without asthma is called exercise-induced bronchoconstriction (EIB), and does not imply underlying chronic asthma (4). For the purposes of this document, terminology EIA or EIB will used interchangeably because, when occurring with exercise, presentation and treatment of both conditions are similar.

Substantial data show EIB occurs very commonly in athletes at all levels (6). Many studies performed in elite-level athletes have documented a prevalence of EIB varying between 30 and 70%, depending on the population studied and the methods implemented (7). In any case, asthma is definitely the most common chronic medical condition among Olympic athletes (8). Asthma is well-known to be more frequent in athletes than in general population, as well as more prevalent in elite athletes, particularly those who participate in endurance events, in swimming, and winter sports, than in recreational athletes (4,9). Several studies indicate subjects prone to EIB have increased levels of exhaled nitric oxide, leukotrienes, and airway epithelial shedding (10-12). The epithelium may play a key role in sensing the transfer of water and heat out of lower airways, but also in activation of sensory nerves (6,13). Also, atopy and type of sport are known to influence risk of asthma in athletes (9). Furthermore, in some specific sports, environmental training and competing conditions may also have a detrimental effect to airways (13).

Diagnosis is particularly relevant because of potential implications on performance both in training and competition, since airway narrowing during exercise compromises ventilatory capacity and efficiency (14). Additionally, asthma has been pointed out as a significant risk factor for unexplained death in young and healthy subjects (15), and a high proportion of asthma-related deaths have been reported in athletes associated with a sporting event (16). Besides, a subgroup of athletes who are asymptomatic present objective evidence of EIB (17), which raises the question of its potential underdiagnosis and the resulting underperformance. On the other hand, untreated or undertreated asthma results in chronic sustained inflammation associated with persistent epithelial damage, which contributes to airway remodeling and fibrotic changes, fixed obstruction and progressive lung function decline over time (18).

It has become quite clear the importance of a correct diagnosis.

But how to recognize EIA?

Clinical presentation of EIA includes wheezing, cough, shortness of breath and/or chest tightness, generally occurring within 5 to 30 minutes *after* intense exercise, sometimes, but unusually, during exercise (7,17). Gradual spontaneous improvement is common after ending exercise. Symptoms are often mild to moderate in severity and may cause impairment of athletic performance, but are often not severe enough to cause significant respiratory distress (6), which may mislead the doctor to pursue this diagnostic hypothesis.

Asthma-like symptoms in elite athletes are not necessarily associated with classic features of asthma (14). Athletes may not suffer from the obvious symptoms as regular asthmatic patients do, but rather cough (19) or some nonspecific complaints such as poor performance or "feeling out of shape", abdominal pain, headaches, muscle cramps, fatigue, and dizziness (20). So, symptoms of EIB are variable and nonspecific, and the presence or absence of specific respiratory symptoms has very poor predictive value to objectively confirm EIB (6,19,21,22).

Physical examination can reveal expiratory dyspnea, expiratory wheezing or rhonchi and other signs of bronchial obstruction such as respiratory retractions (4,23) if the athlete is observed closely after training, but it is often normal during a scheduled appointment.

Not all breathing complaints mean asthma…

Although asthma is the most frequent respiratory chronic disorder in athletes, several other clinical entities can produce similar symptoms (24,25). Overlooking these conditions might therefore lead to wrong diagnosis and unsuccessful treatments. Actually, *it is quite interesting to note that most of the elite athletes who are referred for respiratory problems do not suffer from asthma or EIB* (26). Exercise-induced dyspnea, in particular, is associated with many disease processes and is a remarkably uncommon complaint among those who suffer from EIB (24). Wheezing or stridor can also be caused by other airway abnormalities and closely mimic EIB (14). On the other hand, in the particular case of athletes, their underlying high cardiorespiratory fitness make the diagnostic process even more complex, since also a variety of rare alternative diagnosis must be considered.

Exercise-induced stridor

Vocal cord dysfunction (laryngeal obstruction or inspiratory stridor) is one of the most frequent causes of exercise-induced

dyspnea in athletes. Symptoms such as shortness of breath, increased inspiratory effort, and stridor can be caused by exercise-induced laryngeal obstruction (EILO), and in many subjects only arise during exercise (24). There is no consensus definition, but the following has been proposed: an intermittent extrathoracic airway obstruction, mainly during inspiration, leading to dyspnea of varying intensity (27). Symptoms are thought to occur due to relatively small cross-sectional area of laryngeal orifice, which may be even further reduced by negative pressure created on inspiration during heavy exercise, and the paradoxical movement (adduction instead of normal abduction) of the vocal cords during inspiration (4,24). This condition is frequently associated with psychologically stressful events such as competitions. It has also has been associated with gastroesophageal reflux (24). Elite athletes are more frequently affected than general population. Prevalence is reported between 5 and 35% of those athletes referred for routine evaluation for asthma and/or EIB (28,29). Prevalence of EILO appears to be gender related and is highest among young females (30). Differential diagnosis is important, as asthma treatment will have definitively no effect. However, it should be remarked that about half of athletes with asthma can present concomitant EIB (28).
Laryngomalacia is a less common cause of exercise-induced stridor (24). This condition is characterized by collapse of the arytenoid area with normal vocal cord motion and primarily affects female competitive athletes who abruptly develop stridor at near peak exercise (31). Females may be predisposed to collapse because larynx is shorter and narrower than in males (24).
Moreover, exertional inspiratory stridor may be caused by foreign body aspiration, poor-performance, psychogenic stridor, infectious croup, subglottic stenosis, and exercise-induced anaphylaxis, although these diagnoses are much more infrequent.

Upper respiratory tract infections

Athletes practicing regular strenuous exercise may be at increased risk of upper respiratory tract infections (URTIs) during periods of heavy exercise and for a couple of weeks following competition events (32,33). In contrast to moderate or intermittent physical activity, prolonged and intensive exertion causes changes in immunity that possibly reflects physiological stress and suppression (13). URTIs are so frequent in elite athletes that give rise to respiratory complaints over prolonged periods of time often related to competition seasons or heavy training blocks (34), and may resemble a chronic condition.

Poor physical fitness / Deconditioning

Poor physical fitness is a very frequent cause of exercise-induced breathless in children and adolescents testing because of respiratory complaints (35). It is not very common in elite athletes, but may occur during the "off season" when they become deconditioned and an increase in respiratory drive with lesser amounts of exercise may be interpreted as pathologic (14). Deconditioned subjects have a lower lactate/ventilatory threshold, accumulating lactate and increasing minute ventilation with lesser amounts of exercise; excess lactate buildup results in exercise-associated increases in ventilation and ultimately hypocapnia (24).

Physiologic limitation

Normal physiologic exercise limitation was the most common reason for exercise-induced dyspnea after cardiopulmonary exercise testing in a study by Abuhasan et al (35). It occurred in 52% of referrals for EIB; of those, two thirds had normal or above normal cardiovascular conditioning. The dyspnea is likely related to the increase in ventilation which is necessary to meet increased metabolic demands of high intensity exercise. The increase in respiratory drive and work is a normal physiologic response to exercise but may be interpreted as pathologic by subjects who find that it limits their ability to perform to their expectations (14).

Rhinitis

Elite competitive athletes have a significant increased prevalence of allergic rhinitis and present a variety of both usual and rare symptoms and signs (14). Common symptoms of rhinitis include sneezing, anterior rhinorrhea, post nasal drip / chronic cough, and nasal obstruction (36). However, in athletes, the clinical presentation of rhinitis is frequently more subtle and might present as reduced exercise performance, fatigue, poor-quality sleep, and difficulty to recover after more demanding exercise sessions (36). Especially the lower performance and the cough induced by post-nasal dripping might mislead the doctors to pursue other respiratory disease such as asthma.

Exercise-induced hyperventilation and Dysfunctional breathing

Dysfunctional breathing is defined as chronic or recurrent changes in breathing pattern that cannot be attributed to a specific medical diagnosis; symptoms include exercise-induced breathlessness, as well as a variety of other asthma-like symptoms such as dyspnea with normal lung function, chest tightness, chest pain, deep sighing, frequent yawning and hyperventilation. Exercise-induced hyperventilation is a common physiologic response to exercise but also it may be interpreted as a primary problem since it can be associated with chest tightness and shortness of breath (24,37). The decreases in end-tidal CO_2 during exercise can be associated with chest discomfort perceived as dyspnea (37). An associated personality pattern

characterized by a high degree of competitiveness among the patients with perceived dyspnea from exercise-induced hypocapnia has been pointed out (37).
A higher incidence of moderate/severe rhinitis in patients with dysfunctional breathing has been reported (38), which is not surprising considering the consequences of oral breathing. The nasal congestion could be either the cause or the result of the abnormal breathing pattern with low $PaCO_2$ levels increasing nasal resistance.

Exercise-induced arterial hypoxemia

This condition occurs especially in well-trained athletes with high VO_2max and is thought to be primarily because of diffusion limitations and ventilation-perfusion mismatch (39). Hypoxemia develops at all exercise intensities with varying patterns and is more common in aerobically trained subjects (40). It is postulated that incomplete diffusion in the healthy lung may be because of a rapid red blood cell transit time through the pulmonary capillary (4). Ventilatory requirement rises with increased ionotrophic and chronotrophic capacities of the cardiovascular system induced by physical training; however, there is a limited capability of the airways and the lungs to produce higher flow rates or higher tidal volumes with little or no change in the pressure-generating capability of inspiratory muscles (41). Exercise-induced arterial hypoxemia is defined as reduced arterial oxygenation, which may result from a fall in PaO_2 (and thus also in SaO_2), from a rightward shift of the O_2 dissociation curve without a fall in PaO_2 or from a combination of these processes (42). It may occur in up to 50% of highly trained athletes (4,43-45). By virtue of their smaller lung volumes and airway diameters, women develop more mechanical ventilatory constraints during exercise, which may result in increased vulnerability to hypoxemia during exercise (40).

Other causes

Other chronic disorders are possible to less often cause exercise-induced symptoms in athletes. Heart diseases and other respiratory disorders should be also considered. In previously healthy persons, cardiac abnormalities are a rare cause of exercise-induced dyspnea (24). On the contrary, some pulmonary abnormalities can present with exercise-induced dyspnea. Chest wall or other musculoskeletal abnormalities can impair pulmonary mechanics and *pectus excavatum* has been associated with exercise intolerance and dyspnea (35); also mild scoliosis in adolescents has been linked with abnormal ventilator response to exercise (46). Pulmonary arteriovenous malformations and interstitial lung disease are very uncommon. Obesity, which may represent a differential diagnosis to EIA in the common asthmatic patient, is rare in most athletes.

In the particular case of swimmers and scuba-divers, attention must be paid to a rare condition called swimming-induced pulmonary edema (SIPE). SIPE occurs in well-trained water athletes after a heavy water training session (4). This condition was reported early in the 70s in previously healthy swimmers who developed typical symptoms of pulmonary edema together with a restrictive pattern in pulmonary function, which remained for up to one week after the swimming incident (4,47). Pulmonary edema is the accumulation of water in the lung extravascular spaces. This reflects a breakdown of the normal homeostatic mechanisms that maintain lung fluid balance, predominantly increased hydrostatic pressures and increased capillary membrane permeability (48). During exercise, elite athletes increase their cardiac output. A combination of additional pulmonary capillary bed recruitment and capillary distension mitigate against sharp rises in pulmonary artery pressures in healthy individuals. It is postulated that in susceptible individuals, compensatory mechanisms are overwhelmed. Increased hydrostatic capillary pressures result in mechanical failure, which produces exercise-induced pulmonary hemorrhage (48).
It is important to bear in mind that *more than one condition may coexist in a given subject*. In particular, EILO can be present concomitantly in about half of the athletes with EIB (28,29).

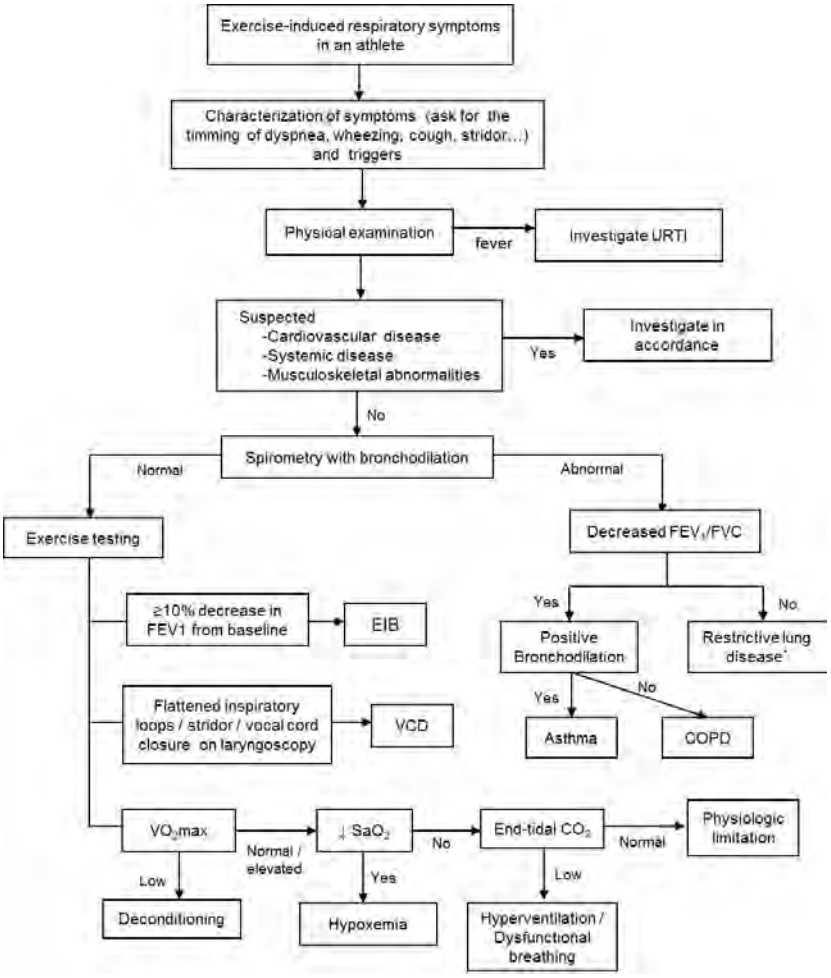
How to diagnose each respiratory disorder?

A systematic approach such as the one suggested in **figure 1** is often useful. In the specific case of competitive athletes, diagnosis of respiratory diseases poses several issues unique to this population. First, the majority of symptoms only occur when exercising at a high level of intensity and $VO2_{max}$, which are sometimes difficult to reproduce in the laboratory. To discriminate between physiological and pathological limitations to maximum exercise based on symptoms or resting exams is not easy most of the times (4). On the other hand, some athletes will not reveal their symptoms due to fear that their respiratory disorders disclosure will be detrimental (14), as others without asthma will try to secure asthma treatments in an attempt to gain a competitive advantage (7); although several studies proved that anti-asthmatic drugs do not enhance performance in healthy subjects (49), this is still a general misbelieve. Therefore, *objective evidence of respiratory diseases should always be part of these subjects' assessment.*

Asthma

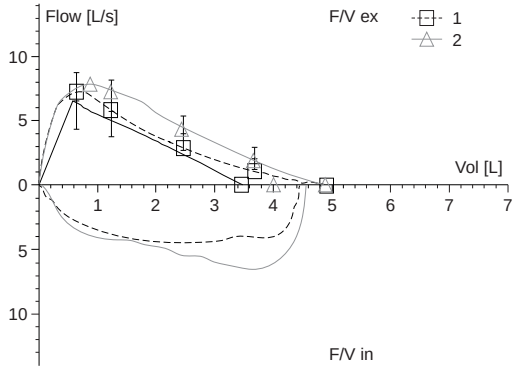
Baseline spirometry is poorly predictive of asthma in competitive athletes (14). Often they record lung function values higher than the general population and so they may appear to be within the "normal" range, although, in reality, corresponds to a pulmonary deficit on the basis of what is expected for an athlete (9,50).

***Figure 1** - Flow-chart for diagnosis of most common respiratory diseases in athletes. CO_2: carbon dioxide; COPD: chronic obstructive pulmonary disease; EIB: exercise-induced bronchoconstriction; FEV_1: forced expiratory volume in 1st second; FVC: forced vital capacity; SaO_2: oxygen saturation; URTI: upper respiratory tract infections; VO_2max: maximum oxygen uptake; VCD: vocal cord dysfunction. *consider SIPE if occurring after a heavy water training, and in the presence of hemoptysis.*



The evaluation of asthma requires therefore a combination of patient's history, clinical examination and judgment, as well as adequate tests, in a stepwise approach (4). However, as recommended by the Medical Commission of International Olympic Committee, in this special population is mandatory to obtain objective evidence to validate an asthma diagnosis by either a positive bronchodilator (**figure 2**) or bronchoprovocation test. Diagnostic tests and positivity criteria are presented in **table 1**. There are several bronchoprovocation tests that can be used and are approved for diagnosis, but the most recent guidelines recommend the use of a standardized exercise test (6). Exercise test has high specificity for asthma, but lower sensitivity. Standardization of the test is very important for the outcome: exercise load should be high, and it should also be standardized with regard to environmental temperature and humidity (6). The severity of EIB can be graded as mild, moderate, or severe if the percent fall in FEV1 from pre-exercise level is ≥ 10% to < 25%, ≥ 25% to < 50%, and ≥ 50%, respectively (6) (**figure 3**). A number of surrogates for exercise testing have been developed that may be easier to implement. These surrogates are validated for asthma diagnosis and include eucapnic voluntary hyperpnea, hyperosmolar aerosols, and dry powder mannitol (**table 1**); however, only exercise test allow for differential diagnosis using only one method. Testing directly for bronchial responsiveness using bronchial challenge with methacholine has a higher sensitivity, but a lower specificity, for asthma (23,51-

Figure 2 - *A positive bronchodilation test (reversibility of 16% and 550 mL after 400 µg inhaled salbutamol).*



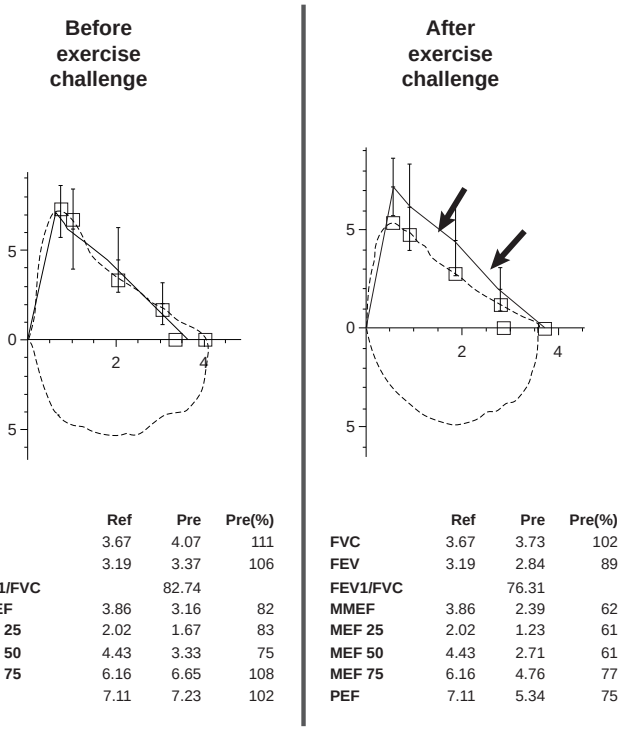
		Ref	Pre(meas)	Pre(%Ref)	Post(meas)	Post(%Ref)	Post(%Chg)
FVC	**[L]**	3.45	4.90	142.1	4.88	141.4	-0.4
FEV	**[L]**	2.92	3.45	117.9	4.00	136.7	16.0
FEV 1% FVC	**[%]**	83.21	70.37	84.0	81.95	97.8	16.5
MMEF 75/25	**[L/s]**	3.62	2.38	65.8	3.90	107.6	63.5
FEF 25	**[L/s]**	5.68	5.84	102.7	7.26	128.8	24.3
FEF 50	**[L/s]**	4.03	2.87	71.2	4.41	109.4	53.6
FEF 75	**[L/s]**	2.08	1.04	50.1	1.83	88.2	76.1
FVC IN	**[L]**	3.48	4.41	126.6	4.53	130.1	2.8
FEV6	**[L]**		4.87				
FEV 0.5	**[L]**		2.50		2.97		18.8
PEF	**[L/s]**	6.54	7.28	111.4	7.91	121.0	8.7
ATS error code			24		10		-58.3

Table 1 - *Methods for diagnosis and positivity criteria set by the International Olympic Committee to document exercise-induced bronchoconstriction in athletes.*

Method	Protocol	Positivity criteria
Bronchodilation test	FEV_1 before and 15 minutes after inhalation of a standard beta-2 agonist	Increase in FEV_1 ≥ 200mL and ≥ 12% from baseline
Bronchial provocation challenges		
Methacholine test	Provocative dose (PD_{20}) or concentration (PC_{20}) of inhaled methacholine inducing a FEV_1 decrease from baseline ≥ 20%	PC_{20} ≤ 4 mg/ml or PD_{20} ≤ 400 µg (cumulative dose), or ≤ 200 µg (noncumulative dose) in those not taking ICS PC_{20} ≤ 16 mg/ml or PD_{20} ≤ 1600 µg (cumulative dose) or ≤ 800 µg (noncumulative dose) in those taking ICS for at least 1 month
Eucapnic voluntary hyperpnoea	FEV_1 before and within 30 min of 6 min dry (or dry and cool) air inhalation at 85% of predicted maximum voluntary ventilation	≥10% decrease in FEV_1 from baseline
Hypertonic saline inhalation	FEV_1 before and after inhaling 22.5 mL of 4.5 g% NaCl	≥15% decrease in FEV_1 from baseline
Mannitol inhalation	Provocative dose of inhaled mannitol inducing a FEV_1 decrease from baseline ≥15% ($PD_{15}M$)	$PD_{15}M$ ≤ 635 mg of mannitol
Exercise challenge (field or laboratory)	FEV_1 before and within 30 min of exercise challenge achieving heart rate > 85% for at least 4 min	≥10% decrease in FEV_1 from baseline

FEV_1: Forced expiratory volume in one second; ICS: inhaled corticosteroids; NaCl: sodium chloride.

Figure 3 *- A positive bronchoprovocation challenge with exercise. A reduction of 16% in FEV1, 5 minutes after exercise challenge test is observed, representing mild (fall in FEV1 from pre-exercise level ≥ 10% and < 25%) bronchial hyperresponsiveness to exercise.*



	Ref	Pre	Pre(%)
FVC	3.67	4.07	111
FEV	3.19	3.37	106
FEV1/FVC		82.74	
MMEF	3.86	3.16	82
MEF 25	2.02	1.67	83
MEF 50	4.43	3.33	75
MEF 75	6.16	6.65	108
PEF	7.11	7.23	102

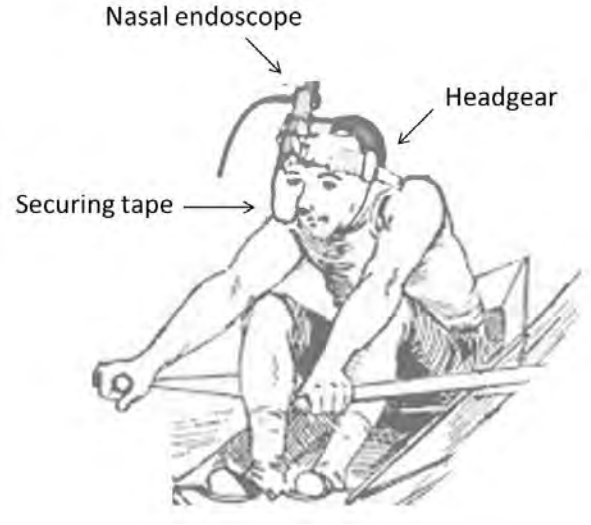
	Ref	Pre	Pre(%)
FVC	3.67	3.73	102
FEV	3.19	2.84	89
FEV1/FVC		76.31	
MMEF	3.86	2.39	62
MEF 25	2.02	1.23	61
MEF 50	4.43	2.71	61
MEF 75	6.16	4.76	77
PEF	7.11	5.34	75

53) and if negative represents a useful measurement to exclude that diagnosis (23).
Detailed information about past or current atopic disorders should be addressed and skin prick tests (SPT) or specific IgE (in case of the SPT could not be performed) should also be carried out in order to assess for atopy (14).

Exercise-induced stridor

Diagnosis may be suspected by history of inspiratory wheeze and throat tightness. Diagnosis of VCD is suggested by flow-volume loops which may reveal variable blunting of inspiratory loop (24). A ratio of less than one for maximal inspiratory flow at 50% of forced vital capacity / maximal expiratory flow at 50% of forced vital capacity, after a methacholine bronchial provocation, has been taken as suggestive of EILO (27).
Definitive diagnosis can be made by laryngoscopy during a maximum-intensity treadmill run or other exercise depending on the sport practiced (**figure 4**). That reveals audible inspiratory stridor occurring during maximum intensity and observable evidence of the paradoxical motion of the vocal cords. Typical findings from laryngoscopy are inspiratory vocal cord closure with posterior "chinking" (a small opening at the posterior aspect of the cords) or, less commonly, complete closure (24).
Laryngomalacia is differentiated from vocal cord dysfunction by fiberoptic rhinolaryngoscopy.

Figure 4 *- Laryngoscopy during a maximum-intensity rowing session.*



Upper respiratory tract infections

There are more uncertainties than evidence based facts on the nature of URTIs associated with exercise, particularly in high performance athletes (54). Physician confirmation of an infective cause, based on clinical signs and symptoms, has until recently been considered the 'gold standard', due to the costs associated with identification of the underlying causes of upper respiratory symptoms and the delay in obtaining results of investigative tests (54). However, recently, the 'gold standard' of physician verified diagnosis of URTIs has also come under scrutiny, and has been found less than ideal (55). The infectious etiology of upper respiratory symptoms has been questioned since few of them had no infectious agent identified, leading to hypothesize that symptoms might be due only to an increased inflammation state (54). A definite diagnosis of bacterial or viral infection must therefore be actively pursued with laboratory investigations.

Poor physical fitness / Deconditioning

During an exercise challenge, oxygen consumption (VO_2) peak is decreased, peak heart rate is normal/slightly decreased, the breathing reserve is > 20% of maximal voluntary ventilation, the

ventilatory equivalent for CO_2 at anaerobic threshold is normal, as well as SpO_2 (> 95%, with 4% drop during exercise) (56,57).

Physiologic limitation

Breath-by-breath analysis of gas exchange during exercise identifies exercise-induced dyspnea in very well-conditioned teenage athletes who simply reach a point of exercise limitation well beyond their anaerobic threshold and that interpret this perceived physiologic dyspnea as a pathologic condition (14,37). It is easily differentiated from poor physical fitness by maximal oxygen consumption (VO_2max).

Rhinitis

Diagnosis of allergic rhinitis in athletes is based in the concordance of a suggestive history of allergic symptoms and physical examination, supported by diagnostic tests (58,59). A thorough allergic history remains the best diagnostic tool available (59,60). In an athlete with persistent symptoms or when an allergic etiology for upper respiratory symptoms is suspected, SPT with standardized allergens or measurement of allergen-specific IgE in serum (in case SPT could not be performed) should be used (36).
Nasal challenge tests are not necessary to confirm diagnosis (36). Imaging of the nose and sino-nasal cavity is used to differentiate the source of sino-nasal symptoms, relation of sino-nasal problem with surrounding structures and the extent of the disease (59). To evaluate severity in an objective way, measurements of nasal obstruction and smell are used (58), but often unnecessary in daily clinical practice. Nasal patency can be monitored objectively using nasal peak inspiratory and expiratory flow, acoustic rhinometry that measures the volume of nasal cavity, and rhinomanometry that measures nasal airflow and pressure (59).

Exercise-induced hyperventilation and Dysfunctional breathing

A symptom-limited exercise test with a cycle ergometer has been proposed, starting with unloaded cycling and using a stepwise increase in work load of 16 W/min (56). The procedure is performed in room air according to current guidelines for exercise testing, with continuous monitoring of ECG, blood pressure and oxygen saturation. The test is continued until symptom-limitation (dyspnea), in the absence of chest pain or ECG abnormalities. Presence of an abnormal breathing pattern such as an increase in deep sigh rate in response to exercise or unsteadiness and irregularity of breathing with no evidence of bronchoconstriction on spirometry and good exercise tolerance is considered diagnostic for dysfunctional breathing (38). Abnormal breathing pattern should be assessed by the same physician in comparison with baseline breathing pattern (38).

Other proposed standardized methods for diagnosing dysfunctional breathing are Respiratory Induction Plethysmography and Manual Assessment of Respiratory Motion, a technique evaluating and quantifying breathing pattern. Both methods are able to differentiate between abdominal and thoracic breathing patterns (61).

Exercise-induced arterial hypoxemia

When EIAH is present, it usually peaks at or near maximal exercise intensity. Reduction in arterial oxygen saturation might be observed by using pulse oximetry. Noninvasive ear oximetry is commonly used in exercise studies in healthy subjects who would not be expected to desaturate < 10%. Thus the great majority of these changes lie on the relatively flat portion of the HbO_2 dissociation curve, and it is very difficult to accurately quantify changes in SaO_2, and especially in PaO_2, with this indirect measurement (42). Therefore, EIAH must be identified by direct measurements of arterial blood gases, and these measurements should be corrected to the *in vivo* arterial blood temperature (42). Arterial blood temperature is commonly measured directly or estimated from esophageal temperature. Temperature correction is very important, because temperature commonly increases ~1.5-2°C over the course of a standard progressive exercise test and even more in heavy constant-load endurance exercise (42). Failure to temperature-correct $PaCO_2$ would correspondingly overestimate ideal alveolar PO_2. Mild EIAH is defined as PaO_2 saturation of 93-95% (or 3-4% < rest), moderate EIAH as 88-93%, and severe EIAH as < 88% (42).

Other causes

Other hypothesis must follow diagnostic procedures course in accordance to what is suspected; if cardiac symptoms are present, a proper cardiac evaluation must be performed. In the presence of fatigue / weakness, consider the hypothesis of a myopathy. Physical examination may reveal other musculoskeletal abnormalities.
SIPE is easily identified by the occurrence of hemoptysis, cough and respiratory distress after heavy swimming exercises. A restrictive lung function can occur until one week afterwards (47), and infiltrates on chest radiographs may be observed.

Conclusions

It is crucial to recognize and accurately diagnose breathing complaints in athletes because it has potential implications not only on their general health, but also on their competing performance. Effective treatment of dyspnea requires appropriate identification of underlying disorder. Due to high prevalence of asthma in athletes, doctors often rely *a priori* on asthma medi-

cation to manage respiratory symptoms. Proper knowledge and accurate diagnosis of other entities is mandatory, since asthma medication is not effective in such cases. Self-reported symptoms are most of the time misleading doctors and are poor diagnostic predictors of disease. So, objective testing is frequently required and can prevent exposing patients to medications that are ineffective and have potential adverse side effects.

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Chemical research on red pigments after adverse reactions to tattoo

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tattoo; pigments; chemical analysis

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Summary
Currently, the incidence of tattooing is on the rise compared to the past, especially among adolescents, and it leads to the urgency of monitoring the security status of tattooing centers, as well as to inform people about the risks of tattoo practice. In our clinical experience, 20% of tattooed patients presented adverse reactions, like allergic contact dermatitis, psoriasis with Koebner's phenomena and granulomatous reactions, with the latter most prevalent and most often related to red pigment. Adverse reactions to tattoo pigments, especially the red one, are well known and described in literature. Great attention has to be focused on the pigments used, especially for the presence of new substances, often not well known.
For this reason, we decided to perform a study on 12 samples of red tattoo ink, obtained by patients affected by different cutaneous reactions in the site of tattoo, to analyze their chemical composition.

Introduction

The practice of tattooing is very common worldwide: more than 24% of American adults have one or more tattoos, with increasing interest and popularity also in Italy. The art of tattooing has ancient origins and was gradually linked to specific meanings like religious beliefs, tribal affiliation, loyalty to a leader, courage, therapeutic functions. Actually, the incidence of tattooing is on the rise compared to the past, especially among adolescents, and it leads to the urgency of monitoring the security status of tattooing centers, as well as to inform the people about the risks of tattoo practice (1-4).
The process of tattooing involves the repetitive piercing of the skin with ink-filled needles, with possible local or systemic complications, classified by different authors in allergic, inflammatory, infectious and neoplastic (5-7).
In our clinical experience, 20% of tattooed patients presented adverse reactions, like allergic contact dermatitis, psoriasis with Koebner's phenomena and granulomatous reactions, with the latter most prevalent and most often related to red pigment. Adverse reactions to tattoo pigments, especially the red one, are well known and described in literature (8,9).
Great attention has to be focused on the pigments used, especially for the presence of new substances, often not well known. For this reason, we decided to perform a study on 12 samples of red tattoo ink, obtained by patients affected by different cutaneous reactions in the site of tattoo, to analyze their chemical composition.

Material and Methods

The ink samples under study were labeled with nomenclature from TIR1 to TIR12 by order of arrival in laboratory, but especially because in most of them the exact chemical composition was not described.

Chemicals, Reagents and Solutions. Methanol for analysis and HPLC grade solvents were purchased from Sigma-Aldrich (Milan, Italy) and Carlo Erba (Milan, Italy). Detailed information on the analysed samples, i.e. producers, production conditions, storage method, etc., can be obtained by directly asking the correspondence author.
Chromatographic equipment. The HPTLC system (CAMAG, Muttenz, Switzerland) (10-12) consisted of Linomat 5 sample applicator using 100 mL syringes and connected to a nitrogen tank; chamber ADC 2 containing twin trough chamber 20 x 10 cm; Immersion device III; TLC Plate Heater III; TLC visualizer linked to winCATS software. Glass plates 20 x 10 cm (Merck, Darmstadt, Germany) with glass-backed layers silica gel 60 (2 µm thickness). Before use, plates were prewashed with methanol and dried for 3 min at 100 °C.
Sample preparation and application. The samples (5 µL each) were dissolved in water (1000 µL). Solutions were applied with nitrogen flow. The operating conditions were: syringe delivery speed, 10 s µL-1 (100 nL s-1); injection volume, 4 µL; band width, 8 mm; distance from bottom, 15 mm.
Development and derivatisation. The HPTLC plates were developed in the automatic and reproducibly developing chamber ADC 2, saturated with the same mobile phase, dichloromethane / methanol 9:1 (v/v), for 20 min at room temperature. The developing solvents (i.e. type of solvents and ratios) were carefully optimised before the analyses. The length of the chromatogram run was 70 mm from the point of application. The developed layers were allowed to dry on TLC Plate Heater III for 5 min at 120 °C and then derivatised with a selected solution, including anhysaldehyde (170 ml methanol, 20 ml acetic acid, 10 ml sulfuric acid, 1.00 ml anisaldehyde). Finally, the plates are warmed for 5 min at 120 °C before inspection.
Inspection. All treated plates were then inspected under a UV light at 254 or 366 nm or under reflectance and transmission white light (WRT), respectively, at a Camag TLC visualiser, before and after derivatisation.
Documentation. CAMAG DigiStore2 digital system with winCATS software 1.4.3 was used for the documentation of derivatised plates.
Stability and Validation. Sample solution of the ink were prepared and stored at room temperature for 3 days and then applied on the same HPTLC plate and the chromatogram evaluated for additional band. Similarly band stability was checked by keeping the resolved peaks and inspecting at intervals of 12, 24 and 49 h. Overlapping of bands is a typical analytical challenge for complex mixtures like multi-ingredient products. HPTLC allowed a good separation and visualisation of the constituents. Sample solutions of the extracts were found to be stable at 4 °C for at least 1 month and for at least 3 days on the HPTLC plates. Repeatability was determined by running a minimum of three analyses. RF values for main selected compounds varied ± 0.02 %. The effects of small changes in the mobile phase composition, mobile phase volume, duration of saturation were minute and reduced by the direct comparison. On the contrary, the results were critically dependent on prewashing of HPTLC plates with methanol.

Figure 1 *- HPTLC fingerprint analysis of Tattoo Ink. Mobile phase: Dichloromethane / Methanol (9:1 v/v). Visualisation: 254 nm. Before derivatisation. Tracks: 1, TIR1; 2, TIR2; 3, TIR3; 4, TIR4; 5, TIR5; 6, TIR6; 7, TIR7; 8, TIR8; 9, TIR9; 10, TIR10; 11, TIR11; 12, TIR12.*

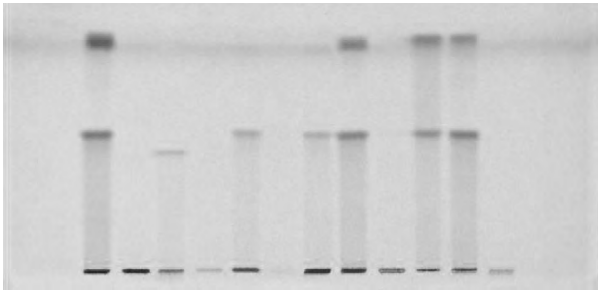


Figure 2 *- HPTLC fingerprint analysis of Tattoo Ink. Mobile phase: Dichloromethane / Methanol (9:1 v/v). Visualisation: 366 nm. Before derivatisation. Tracks: 1, TIR1; 2, TIR2; 3, TIR3; 4, TIR4; 5, TIR5; 6, TIR6; 7, TIR7; 8, TIR8; 9, TIR9; 10, TIR10; 11, TIR11; 12, TIR12.*

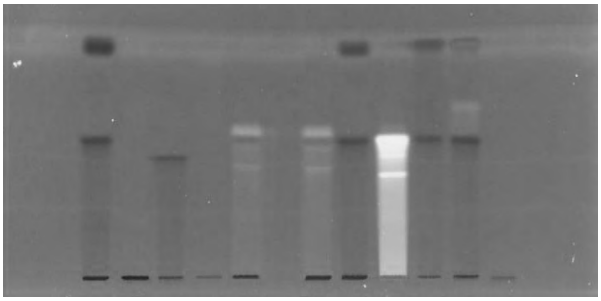


Figure 3 *- HPTLC fingerprint analysis of Tattoo Ink. Mobile phase: Dichloromethane / Methanol (9:1 v/v). Visualisation: white light. Derivatisation: anhysaldehyde. Tracks: 1, TIR1; 2, TIR2; 3, TIR3; 4, TIR4; 5, TIR5; 6, TIR6; 7, TIR7; 8, TIR8; 9, TIR9; 10, TIR10; 11, TIR11; 12, TIR12.*

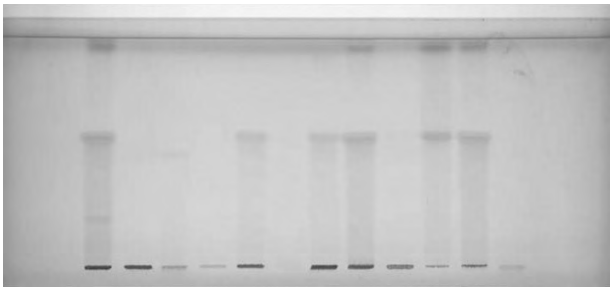
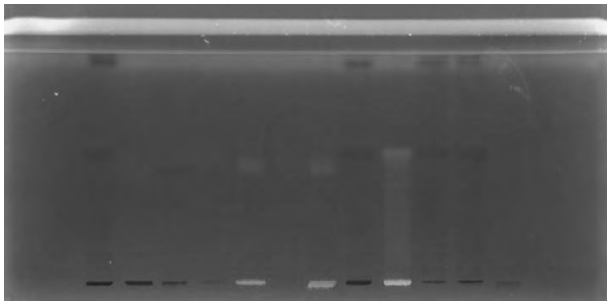


Figure 4 *- HPTLC fingerprint analysis of Tattoo Ink. Mobile phase: Dichloromethane / Methanol (9:1 v/v). Visualisation: 366 nm. Derivatisation: anhysaldehyde. Tracks: 1, TIR1; 2, TIR2; 3, TIR3; 4, TIR4; 5, TIR5; 6, TIR6; 7, TIR7; 8, TIR8; 9, TIR9; 10, TIR10; 11, TIR11; 12, TIR12.*



Results and discussion

The study identified two groups of inks: the first one consists of samples TIR1, TIR8 and TIR10, while TIR11 presents two components more; the second one includes samples TIR5, TIR7 and TIR9, very similar. However, TIR5 and TIR9 present a common spot to the other samples.

The proofs concerning solubility showed several different groups: in the first one, sample TIR2 results vey soluble in water (probably due to the presence of polar compounds). The second group includes samples TIR5, TIR6 and TIR12, which are slightly soluble / insoluble in water and in other solvents. This fact made it impossible to obtain a chromatogram.

In order to have a precise and complete profile, the HPTLC plates were read at different wavelengths (UV 254 and 366 nm, white light WRT), before and after derivatisation with anisaldehyde-sulfuric acid.

The data obtained show that only in few cases the samples have similar fingerprints. This may be due to the use of different pigments for the formulation of various red shades.

Further analyses not listed in this study as in progress investigation, reveal the presence of toxic substances in some inks tested. This fact lets us hypothesize a link between the inks used for tattooing and the different skin reactions often observed in the areas of tattoos.

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Italian Multicenter Cross-Sectional Study (AISAG) on light smoking and allergic diseases in adults

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Key words

Smoking; allergic respiratory diseases; food allergy; allergic dermatitis

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Summary

Allergic rhinitis, allergic dermatitis, and food allergy are extremely common diseases and are frequently associated to each other and to asthma. Smoking is a potential risk factor for these conditions, but so far, results from individual studies have been conflicting. On the basis of these contradictory data in the literature we have carried out a multicenter cross-sectional study to evaluate the relationship between some allergic conditions and exposure or not to active light smoking. The study was carried out between May 2013 and November 2013 in 22 different Italian hospitals. Patients with respiratory and/or food allergy, and aged 18 years and over, visited at Allergy Outpatient Clinics, were invited to participate. A total of 1586 allergic patients (21.6% smokers) with a mean age of 39.2 years (standard deviation, SD = 15.1) were included. We demonstrated that the prevalence of tobacco smoking was higher in patients with food allergy and in asthmatic patients in stage III-IV. But no other statistical differences were found at univariate analysis. The sensitization patterns of non-smokers and smokers were similar. Furthermore, tobacco smoking was associated with higher risk of food allergy and lower risk of asthma. Moreover, tobacco smoking was an independent risk factor for persistent respect to intermittent rhinitis, and for asthma GINA stage III-IV with respect to stage I-II.

Introduction

Population-based studies appear to show a relationship between smoking and bronchial hyperresponsiveness. However, the presence of asthma in adults has generally been unrelated to smoking history, possibly reflecting a false opinion about the tendency for asthmatics not to become regular smokers or to smoke less than their non-asthmatic counterparts (1). Several studies have demonstrated that active smoking increases the risk for developing asthma (2-5).

But there are also scattered studies that seem to cast doubt on the relationship between exposure to cigarette smoke and asthma / allergies. For example, it was reported that IgE levels in smokers showed a moderate inverse correlation with the degree of smoking and that the mean IgE level in ex-smokers was much lower than in current light smokers but was still higher in nonsmokers (6). It was also demonstrated that cigarette smoking is associated with high prevalence of chronic rhinitis and low prevalence of allergic rhinitis in men (7). On the basis of these contradictory data in the literature we have carried out a multicenter cross-sectional study to evaluate the relationship between some allergic conditions and exposure or not to active light smoking.

Methods

The study was carried out between May 2013 and November 2013 in 22 different Italian hospitals. Patients with respiratory and/or food allergy, and aged 18 years and over, visited at Allergy Outpatient Clinics, were invited to participate. Patients were asked about their smoking habit; non-smokers and light smokers (defined as 5-10 cigarettes per day for 5-10 years) were included in the study. The local Ethics Committee approved the study design and protocol and patients gave written informed consent.

For each subject, we collected data on age, gender, smoking habit, allergic symptoms, the pattern of respiratory sensitization, the presence of food allergy and atopic dermatitis. Asthma severity and control, and rhinitis severity were scored according to the Global Initiative for Asthma (GINA) and the Allergic Rhinitis and its Impact on Asthma (ARIA) Guidelines, respectively.

Skin prick tests (STPs) were done using a panel of standardized commercial extracts of allergens of the most common ones responsible for respiratory symptoms in Italy: pollens (*Graminaceae mix 5:* grass; *Compositae mix*; *Parietaria mix*: pellitory; *Betula pendula*: birch; hazelnut; olive, cypress), house dust mites (HDM: *Dermaptophagoides pteronyssinus* and *D. farinae*), animal danders (dog, cat), feathers mix, moulds (*Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarum, Penicillum mix*).

Serum specific IgEs were detected by currently available commercial laboratory methods (RAST and ImmunoCAP; Pharmacia AB, Uppsala, Sweden, then Phadia AB, now Thermo Fischer Scientific).

Patients were divided into two groups according to tobacco smoking: non-smokers and light smokers. Common statistical methods were used for the analysis of proportions and associations between tobacco smoking and demographic and clinical features. Furthermore, multivariate logistic regression models, adjusted for covariates, were used to investigate associations of tobacco smoking (independent variable) with allergic symptoms, food allergy, atopic dermatitis and the pattern of respiratory sensitization (dependents variable). Results are presented as odds ratios (OR) and 95% confidence intervals. The selection of variables for fitting the most parsimonious model was performed using a backward stepwise procedure, with p = 0.10 for retaining each variable in the model.

Statistical analysis

For statistical tests, *P* values lower than 0.05 were considered significant in two-tailed tests. All statistical analysis were carried out using STATA, version 12.0, software (STATA Statistics/Data Analysis 12.0 - STATA Corporation, College Station, TX, USA).

Results

A total of 1586 allergic patients (21.6% smokers) with a mean age of 39.2 years (standard deviation, SD = 15.1) were included. The majority of them were aged 35 years or less. Asthma was present in 72.2% of subjects, rhinitis in 79.4% and United Airways Disease (rhinitis plus asthma) in 47.6%. Most of asthmatic were in GINA stage I and II (89.9%), whereas among patients with rhinitis, 57.6% had intermittent symptoms. The most common respiratory allergenic sensitizations were, in decreasing order: grass (62.9%), HDM (53.3%), Betula (29.6%) and Parietaria (25.0%).

The demographic and clinical features according to smoking habits are shown in **table 1**. The proportion of tobacco smokers was significantly higher in males, subjects younger than 45 years, non-asthmatic patients, and those with persistent rhinitis. In addition, the prevalence of tobacco smoking was higher in patients with food allergy and in asthmatic patients in stage III-IV, next to statistical significance threshold. No other statistical differences were found at univariate analysis. The sensitization patterns of non-smokers and smokers were similar, as shown in **figure 1**; this was confirmed when we restricted the analysis to monosensitized patients (**figure 2**).

After adjusting for demographic and clinical features, tobacco smoking was associated with higher risk of food allergy (OR = 1.46, CI 95%: 0.97-2.19; p = 0.069) and lower risk of asthma (OR = 0.75, CI 95%: 0.57-0.99; p = 0.042). Moreover, tobacco smoking was an independent risk factor for persistent respect to intermittent rhinitis (OR = 1.51, CI 95%: 1.16-1.96; p = 0.002), and for asthma GINA stage III-IV respect to stage I-II (OR = 1.73, CI 95%: 1.09-2.74; p = 0.021). No associations were observed between tobacco smoking and other clinical characteristics in multivariate logistic regression models.

Table 1 *- Demographic and clinical characteristics according to tobacco smoking.*

Variables	Categories	Non-smokers n (%)[1]	Smokers n (%)[1]	Total n (%)[1]	P value[2]
Total		1243 (78.4)	343 (21.6)	1586	
Gender	Male	687 (55.3)	143 (41.7)	830 (52.3)	< 0.001
	Female	556 (44.7)	200 (58.3)	756 (47.7)	
Age (years)	32 ≤	428 (34.4)	128 (37.3)	556 (35.1)	0.010
	33-44	375 (30.2)	123 (35.9)	498 (31.4)	
	≥ 45	440 (35.4)	92 (26.8)	532 (33.5)	
Family history of allergy	No	877 (71.9)	241 (70.9)	1118 (71.7)	NS
	Yes	342 (28.1)	99 (29.1)	441 (28.3)	
Asthma	No	326 (26.3)	111 (32.4)	437 (27.6)	0.025
	Yes	915 (73.7)	232 (67.6)	1147 (72.4)	
GINA criteria (restricted to patients with asthma)	I-II	801 (90.7)	197 (86.8)	998 (89.9)	*0.080*
	III-IV	82 (9.3)	30 (13.2)	112 (10.1)	
Rhinitis	No	967 (77.6)	282 (22.4)	1258 (79.4)	NS
	Yes	267 (81.6)	60 (18.4)	327 (20.6)	
ARIA classification (restricted to patients with rhinitis)	Intermittent	581 (59.6)	143 (50.9)	724 (57.6)	0.009
	Persistent	394 (40.4)	138 (49.1)	532 (42.4)	
Polysensitization	No	405 (32.6)	112 (32.7)	517 (32.6)	NS
	Yes	838 (67.4)	231 (67.3)	1069 (67.4)	
United airways disease	No	646 (52.0)	183 (53.7)	829 (52.4)	NS
	Yes	596 (48.0)	158 (46.3)	754 (47.6)	
Atopic dermatitis	No	1164 (93.9)	319 (93.5)	1483 (93.8)	NS
	Yes	76 (6.1)	22 (6.5)	98 (6.2)	
Food allergy	No	1136 (91.6)	302 (88.6)	1438 (91.0)	0.082
	Yes	104 (8.4)	39 (11.4)	143 (9.0)	

[1]Column percentage,

[2]Chi square test. NS: Not statistical significant

Figure 1 *- Pattern of the sensitizations (skin test positive) between non-smokers and smokers.*

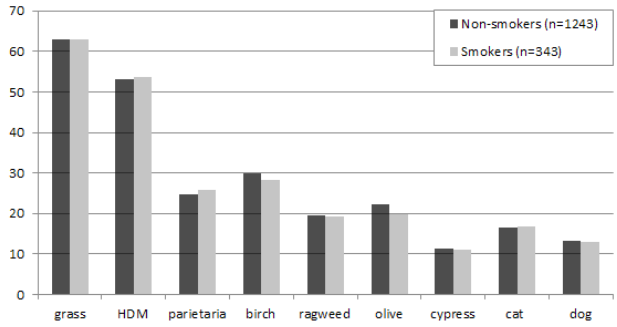
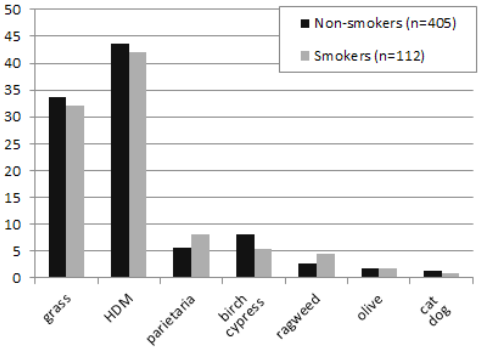


Figure 2 *- Pattern of the sensitizations (skin test positive) between monosensitized non-smokers and smokers.*



Discussion

Allergic rhinitis, allergic dermatitis, and food allergy are extremely common diseases and are frequently associated to each other and to asthma. Smoking is a potential risk factor for these conditions, but so far, results from individual studies have been conflicting. Prevalence rates for smoking in asthma are relatively close to those found in the general population and several studies have demonstrated that active smoking increases the risk for developing asthma (8-10). A 1996 longitudinal study of 5801 people born in 1958 who were part of a national British cohort has implicated smoking in the development of wheeze and asthma in young adults (8). Subjects were followed up at the ages of 7, 11, 16, 23, and 33 years. Active smoking was strongly associated with the incidence of asthma and wheezing illnesses between the ages of 18 and 33 (OR = 4.42, 95% CI 3.31-5.92) after controlling for a variety of factors, including gender, maternal age, birth order, gestational age, hay fever, eczema, father's social class, and maternal smoking. In addition, among the 880 children who developed asthma or wheezy bronchitis by age seven, relapse at age 33 after prolonged remission of childhood wheezing was more common among current smokers. A study of adolescents found that those who smoked ≥ 300 cigarettes per year had a relative risk of 3.9 for developing asthma, compared to their non-smoking peers (9).

There is also a growing body of evidence that secondhand smoke exposure is associated with the development of asthma in early life (10). Maternal smoking is the most important cause of secondhand smoke exposure, because of the greater exposure of the child to the mother than the father (11-13).

In adults, data on the effects of environmental tobacco exposure on nonmalignant lung disease are sparse. The association between passive exposure to tobacco smoke and respiratory symptoms was studied in a sample of 4197 non-smoking adults as part of the Swiss Study on Air Pollution and Lung Diseases in Adults (SAPALDIA Study) (14). Passive exposure to tobacco smoke was associated with increases in the risks of doctor-diagnosed asthma (odds ratio = 1.39), wheezing, bronchitis, and dyspnea.

Prenatal exposure to smoking may also be important, being associated with reduced pulmonary function in the infant. One study, for example, evaluated the effect of prenatal maternal cigarette smoking on the pulmonary function of 80 healthy infants shortly after birth (15). Maternal smoking was assessed by questionnaire reports and urine cotinine concentration at each prenatal visit. Pulmonary function (assessed as flow at FRC) was lower in infants whose mothers smoked compared to those whose mothers did not smoke. Another report evaluated the effect of early levels of lung function on the subsequent occurrence of a wheezing lower respiratory tract illness in the first year of life (16). Reduced pulmonary function early in life increases the risk for wheezing and subsequently for asthma later in life. It has been proposed that prenatal smoking exposes the fetus to the growth-retarding effects of tobacco and enhances airway-parenchymal dysanapsis (disproportionately small airways compared to the size of the pulmonary parenchyma). These changes may contribute to the postnatal expression of increased airway responsiveness and asthma (17).

Two other studies have examined the effects of prenatal and postnatal exposure to smoking on asthma and wheezing in children (18-19). The first study used a broad case definition to identify 620 schoolchildren aged seven to nine years in Cape Town with current asthma or wheeze in the last 12 months (18). In bivariate analyses, maternal smoking, whether defined as ever smoking (OR = 1.80), smoking during pregnancy (OR = 1.97), smoking during the first year of the child's life (OR = 1.70), or

current smoking (OR = 1.70) was significantly associated with current asthma/wheeze among the children. The number of cigarettes smoked daily by the mother and the number of household smokers were also related to current asthma/wheeze. Further strengthening these findings, the children's cotinine-creatinine ratio was significantly associated with current asthma/wheeze (OR = 1.61 for the highest quartile versus the lowest quartile). In a multivariate logistic regression model controlling for a variety of known risk factors, maternal smoking during pregnancy (OR = 1.87, 95% CI 1.25-2.81) and the number of household smokers (OR = 1.15, 1.01, 1.30) remained significantly associated with current asthma/wheeze. The second study examined the relationship between current and past exposure to maternal, paternal, and non-parental environmental tobacco smoke in the home and several measures of asthma and wheeze in a large sample of school-aged children (11,534 children) from 24 communities in the US and Canada (19). Asthma was identified based on either an active diagnosis of asthma or use of medication for asthma. Wheeze outcomes were: any wheezing, wheezing with a cold, wheezing without a cold, persistent wheeze, shortness of breath with wheeze, awakening at night by wheezing, wheezing with exercise, medication for wheeze, emergency department visit for wheeze, and hospitalization for wheeze. Children who were currently exposed had a significantly increased risk of reported wheeze with a cold (OR = 1.65), emergency department visit for wheeze (OR = 1.63), persistent wheeze (OR = 1.42), shortness of breath with wheeze (OR = 1.35), wheeze with exercise (OR = 1.24), and medication for wheeze (OR = 1.23) in past year. For most of the wheeze outcomes, there was an increasing risk associated with increasing number of smokers in the home and number of cigarettes smoked in the home per day. Active asthma was significantly associated with expo-sure to environmental tobacco smoke in pregnancy only (OR = 2.70, 95% CI 1.13-6.45), and no significant association was found for currently exposed children.

Cigarette smoking and asthma interact to induce important adverse effects on clinical, prognostic and therapeutic outcomes (20-25). Active smokers, particularly females, are at risk of developing asthma. Smokers with asthma experience worse asthma control than nonsmokers with asthma. Mechanisms for the adverse effects of smoking in asthma include altered airway inflammation and corticosteroid insensitivity. Finally, in a recent systematic review and meta-analysis, it were observed very modest associations between smoking and some allergic diseases among adults (26). Among children and adolescents, both active and passive exposure to second hand smoke were associated with a modest increased risk for allergic diseases, and passive smoking was associated with an increased risk for food allergy. In our study we demonstrated that the prevalence of tobacco smoking was higher in patients with food allergy and in asthmatic patients in stage III-IV. But no other statistical differences were found at univariate analysis. The sensitization patterns of non-smokers and smokers were similar. Furthermore, tobacco smoking was associated with higher risk of food allergy and lower risk of asthma. Moreover, tobacco smoking was an independent risk factor for persistent respect to intermittent rhinitis, and for asthma GINA stage III-IV respect to stage I-II. Additional studies with detailed measurement of exposure and better case definition are needed to further explore the role of smoking in allergic diseases. In conclusion, quitting smoking can improve symptoms and lung function, but the low rates of smoking cessation highlights the need for improved strategies for managing these patients. Clinical trials assessing new therapies for asthma need to enroll smokers to identify treatments that are effective in the asthma smoking phenotype.

Limits of the study

We are aware that our research may have an important limitation. The association between tobacco smoking and asthma could be affected by reverse causality bias: subjects with asthma are less inclined to start smoking than not asthmatic subjects, and probably smokers tend to quit smoking at the onset of asthmatic symptoms. A prospective cohort study of subjects without asthma and/or allergy should be appropriate to disentangle this topic, though it requires large sample and long times of observation. Finally, we have not considered in the study the problem of "secondhand" smoke during pregnancy or in early childhood and the and its potential consequences in adulthood.

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Secondary hypogammaglobulinemia in Waldmann's disease treated with subcutaneous immunoglobulins

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Key words

primary intestinal lymphangectasia; Waldmann's disease; secondary immunodeficiencies; subcutaneous immunoglobulins; protein-losing enteropathy

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Summary

Primary intestinal lymphangiectasia (PIL) is rare disorder characterized by congenital malformation or obstruction of intestinal lymphatic drainage; it is responsible for protein losing enteropathy leading to lymphopenia, hypoalbuminemia and hypogammaglobulinemia. A low-fat diet associated with medium-chain triglyceride supplementation is the cornerstone of PIL management. The administration of intravenous immunoglobulins does not always lead to satisfactory plasma levels and therefore the replacement therapy with immunoglobulins is controversial. We describe here the case of a patient with PIL and severe hypogammaglobulinemia treated with immunoglobulins. The striking aspect of this case is the clinical and serological benefit obtained with the subcutaneous compared to the intravenous immunoglobulins administration.

Introduction

Primary intestinal lymphangectasia (PIL), also known as Waldmann's disease, is a rare disorder characterized by dilated intestinal lymph vessels resulting in lymph leakage into small bowel lumen that leads to protein-losing enteropathy and ultimately to lymphopenia, hypoalbuminemia and hypogammaglobulinemia (1). The last one is not only due to protein loss, but also to B cells defect characterised by a decreased number of B lymphocytes, a defective production of immunoglobulins and a poor antibody response (2,3). Therefore, in patients with PIL, the risk of pyogenic bacterial infections and the occurrence of malignancy, especially lymphomas, is increased. Even if low fat diet associated with supplementary medium chain tryglicerides (MCT) remains the cornerstone of PIL medical management (4), other medications have recently been taken into consideration (i.e. antiplasmin and octreotide) whose efficacy is variable and insufficiently evaluated (1). Among the therapeutic options, the replacement therapy with immunoglobulins is controversial but needs to be considered in patients with severe hypogammaglobulinemia and recurrent infections. Indeed, in these patients the administration of Intravenous Immunoglobulins (IVIg) does not always guarantee the achievement of satisfactory plasma levels because of both pharmacodynamics of IVIg and loss of the immunoglobulins from the gastrointestinal tract.

We describe here the case of a young man with PIL, who developed secondary immunological abnormalities, treated with the specific diet and Subcutaneous Immunoglobulins (SCIg) obtaining clinical and immunological improvement. According to our knowledge this is the first report of a case of PIL treated with SCIg.

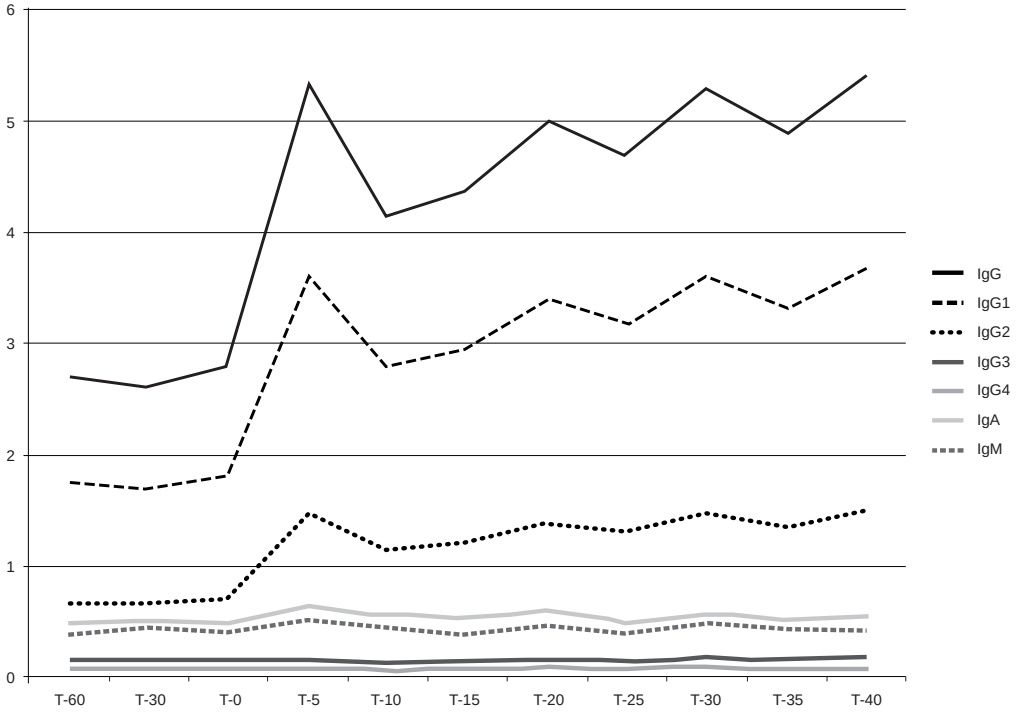
Case report

MA, a 26 years old Caucasian man, was diagnosed to have PIL with secondary immunological defect and congenital peripheral oedema at the age of 3. At birth he presented with right arm and leg pitting oedema and, at 7 months of age, he underwent a derivative lymphatic microsurgery in the upper arm. The second surgery was not performed because of immunological defects: decreased number of T and B cells and severe hypogammaglobulinemia. At the age of 3 years, the patient presented clinical signs of malabsorption such as abdominal pain, nausea, vomiting, loss and inability to gain weight, growth retardation and fatigue. Therefore, the patient underwent gastroduodenoscopy and duodenum biopsies which revealed diffuse dilated mucosal and submucosal lymph vessels, confirming the diagnosis of PIL. The patient started a specific diet with medium chain tryglicerides obtaining clinical improvement. A new laboratory evaluation was also performed and confirmed the previous findings of immunological abnormalities such as hypogammaglobulinemia, with an increase number of NK cells and decreased number of B and T cells. Moreover, a nearly absent response to "in vitro" stimulation and to vaccines was observed.

The patient experienced good health till the age of 23 years, when suddenly he started to complain of recurrent upper and lower airways infections. The patient was treated with several antibiotics without clinical benefit. He performed a chest and paranasal sinuses CT scan that showed several bronchiectasis and nasal turbinates' mucosal hyperplasia. A new evaluation of the immunological defects confirmed severe hypogammaglobulinemia (IgG 2.28 g/L, IgG1 1.82 g/L, IgG2 0.70 g/L, IgG3 0.17 g/L, IgG4 0.11 g/L, IgA < 0.06 g/L, IgM < 0.05 g/L) and a lymphocytopenia with B and T cell depletion (WBC 4160/mmc, lymphocytes 420/mmc). Therefore, we decided to start the administration of IVIg as replacement therapy (0,4 g/kg per month). Even if this treatment obtained a slight improvement in the recurrence of infections, the serum IgG levels remained unsatisfactory (**figure 1**). In order to gain higher titres of IgG and a better quality of life we switched to SCIg (10 g every 10 days) with very good results on the persistence of acceptable levels of IgG (**figure 1**).

The striking aspect of this case is the clinical and serological benefit on immunodeficiency obtained with the SCIg compared to the IVIg administration (**figure 1**).

***Figure 1* - Plasma levels of Immunoglobulins before and after the administration of SCIg**. *The beginning of SCIg administration is indicated as T0. Before T0 the plasma levels of Ig was evaluated monthly. From T0 the dosage of plasma Immunoglobulins was performed every 5 days (T5; T10; T15…)*



Discussion

Intravenous Immunoglobulins are a therapeutic compound obtained from the serum IgG fraction pooled by several thousands of healthy donors. It has been used for years as a replacement therapy in a wide range of primary and secondary immunodeficiencies, and represents the first therapeutic option for antibody deficiencies (5). Immunoglobulins can be administered via SC or IV route; SCIg preparations were introduced in the 1980s in US and Europe. However, slow infusion technique and low concentration of available preparations at that time, made SCIg less attractive than IVIg to patients and healthcare professionals. Therefore, IVIg, which allowed infusions of higher monthly doses, became the best route of administration. Pure and highly concentrated SCIg preparations with relatively low viscosity that allow a relatively rapid administration have recently been developed. Whereas IVIg is infused every 3-4 weeks, SCIg is administered once a week or biweekly, with the total IVIg monthly dose divided in smaller doses. The SC administration is characterised by a progressive release of IgG into the circulation obtaining a more stable serum IgG levels. Moreover, a multicentre European study showed that SCIg increases serum IgG levels of 17.7% compared to IVIg using equivalent doses of Ig, in patients who switched from the IV to the SC route (6). Subcutaneous Immunoglobulins are easy to use and to self-administer, providing patients with flexibility and improved quality of life. Furthermore, patients require less assistance from healthcare professionals, reducing the cost associated with Ig replacement therapy, and take a greater control over their treatment.

For all these reasons and since the patient had not lymphangectasia on the abdominal wall, we chose to shift form IVIg to SCIg replacement therapy.

Among all the SCIg preparations available, we used Hizentra®, a 20% (200 g/L) ready-to-use liquid preparation of polyvalent human IgG for subcutaneous administration. Currently, it is the only 20% SCIg therapy approved by the US Food and Drug Administration and European Medicines Agency for treatment of primary and secondary immunodeficiencies.

The choice of Hizentra® lead us to perform the administration of SCIg once every 10 days, for a total amount of 30 g of Ig per month (the same monthly dosage previously used by the patient via IV route). As shown in **figure 1**, using the new therapeutic regimen we have observed a progressive increase in plasma levels of IgG, which slowly edged up and stabilized around 6 g/L (IgG1 3.67 g/L, IgG2 1.51 g/L, IgG3 0.19 g/L, IgG4 0.11 g/L), result never observed before using the IVIg route.

We now want to evaluate whether SCIg administration leads not only to a more stable levels of circulating Ig, but also to modulation of immune response as shown for IVIg (7-9).

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Erythema multiforme caused by sildenafil in an HIV(+) subject

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Key words
Sildenafil, HIV, drug-allergy, erythema multiforme, patch-test

Summary
Erythema multiforme is mainly caused by drug allergy and infections. This is the case of a HIV-positive, 49-year-old male, recently cured for syphilis, that presented erythema multiforme minor, five days after taking sildenafil. He had a fast recovery, only with the use of antihistamines. Cell-mediated allergy to sildenafil was confirmed six months later, with the use of patch-tests.

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Introduction

Erythema multiforme (EM) is an acute, self-limited skin eruption, mainly caused by infections and drug allergy (1). It is considered a type IV hypersensitivity reaction and its manifestations are within a wide spectrum of severity, summarized in a 'minor' vs 'major' classification. Viral (most commonly HSV), bacterial (Mycoplasma pneumoniae) and fungal infections (histoplasmosis) are precipitating factors of EM (1).
EM is rarely related to HIV and is not a common manifestation of drug allergy to antiretrovirals (2). Setting the diagnosis of EM in an HIV(+) patient can be challenging and anamnesis is its cornerstone.
The following is the case of an HIV-positive patient that presented EM minor, five days after the use of sildenafil.

Case report

A 49-year-old male was referred to my Allergy Clinic due to pruritus and mild erythema, limited to palms and feet, occurring for about twelve hours. No other symptoms were reported and no abnormal signs were noticed upon physical examination. He had been diagnosed with HIV four years before, with his first CD4 cell count being very low (52/mm^3). Soon after the diagnosis HAART treatment was prescribed, including tenofovir plus emtricitabine (co-formulated), atazanavir and ritonavir. The patient was still under the same antiretroviral treatment and his latest CD4$^+$ count was 620/mm^3, with an undetectable HIV viral load.
He reported to have been treated with ceftriaxone two months earlier, for syphilis. Such therapy had been completed more

than 45 days before, so the correlation of the antibiotic with the appearance of dermatitis was rather improbable. Two days before the appearance of the dermatitis he had used a new body lotion, so contact dermatitis was suspected, although the problem was limited to the extremities. Cetirizine (10mg/once daily) was prescribed and he was given instructions not to use the lotion and to be re-examined if dermatitis insisted.

The next morning he woke up due to intense pruritus and he soon noticed an extensive maculopapular rash, covering the extremities, abdomen, thorax and gluteal region, less extended to his back and neck. He was soon re-examined; besides the diffuse morbilliform eruption, a single target-lesion was detected in his right palm. No facial involvement was noted but there were oral mucosal lesions, causing a tingling sensation. No epidermal detachment or blisters were detected. The diagnosis of erythema multiforme was set and blood tests (complete blood count, a comprehensive metabolic panel, ESR and HSV 1 & 2 serology) were ordered.

When anamnesis was asked again he reported having used a tablet of sildenafil 100 mg, as an aphrodisiac, five days before the initial rush. He recalled having used sildenafil just once again, one year ago. Since herpes is often the cause of EM he was asked for herpes labialis, but he reported that he had no signs of it, at least during the previous six months.

He was released with cetirizine 10mg/twice daily for 7 days and a cream containing polidocanol and urea. He was advised to communicate promptly in the case that any vesicles would appear. His blood tests resulted to be normal.

He was re-examined 3 days later and skin's improvement was obvious, while pruritus was mentioned to be much milder. The total duration of the lesions was less than two weeks.

A patch test was performed, six months after the initial diagnosis, using sildenafil at 10% in petrolatum. It resulted positive confirming a cell-mediated drug allergy.

Discussion

The initial appearance of symmetrical maculopapular and "target" lesions on the extremities is typical in EM, usually spreading from acral to proximal areas and finally to the trunk. The pruritic -limited to the extremities- erythema, observed as first symptom in our case, was atypical for EM, while it is common in irritant or allergic contact dermatitis.

HIV-infected patients have a higher risk of developing cutaneous reactions, presumably as a result of immune dysregulation and altered drug metabolism (2). Antiretroviral drugs, as well as other medications, are often the etiology of EM in HIV-positive patients (2, 3). Antiretroviral therapy may cause various allergic skin reactions -from mild maculopapular lesions to toxic epidermal necrolysis (TEN)- but these reactions usually develop within the first 10 weeks of the initiation of therapy (2). The regular daily uptake of antiretroviral medications makes antiretrovirals an unlikely cause of drug allergy. On the contrary discontinuation of these drugs has been reported to cause EM as a result of viral replication (3).

Treponema pallidum has also been reported to cause EM, while secondary syphilis may also present with signs resembling EM, in HIV-positive patients (4-6). In such cases distinguishing EM due to *Treponema pallidum* from secondary syphilis, offers an intriguing differential diagnosis to the clinician. Nevertheless, all cases of HIV-positive patients with EM or EM-like symptoms associated with syphilis, resolve only after the use of antibiotics (4-6). In our case repeating antibiotics might have been an alternative in the case that lesions would not resolve fast, just by the use of antihistamines. It appears that treatment for syphilis had been successful for our patient and that syphilis was not related with the occurrence of EM, two months later.

Although allergic reactions to sildenafil are rare, a single case of TEN after a high intake of it has been published (7). Ours is the first case of EM associated with sildenafil. The time lapse of EM's onset, the lack of other newly-introduced drugs and the absence of an infection during the last weeks, drove to the diagnosis. Antihistamines controlled the pruritus and have probably helped to accelerate the anyhow self-limiting course of EM (1).

Serology for HSV types 1 and 2 can help to set the diagnosis in many EM cases, specially when a relavant recent anamnesis is reported, however they are not considered necessary (1, 8). Recurrent attacks of EM are expected in HSV-positive cases, requiring prevention with acyclovir (8). Skin biopsy is indicated only in persisting cases and can set the diagnosis in EM and EM-like eruption associated with *Treponema pallidum* (4, 5). When bacteria are the infectious agents, the use of antibiotics is necessary, offering a prompt resolution of the skin lesions.

In non-IgE mediated drug allergies, no well-standardized allergy tests exist for each drug. In order to confirm the diagnosis general instructions on patch testing for non-immediate drug eruptions were followed, and were proved to be useful (9). Theoretically immunodeficiency might affect the outcome of patch tests, however they are successfully used in HIV(+) patients (10). Other tests for cell-mediated allergy, like the lymphocyte transformation test, may be more reliable.

Stopping drugs, if feasible, is a first step to the diagnosis. However, stopping or switching antiretrovirals in the case of EM can be tried only during the first months of their introduction. The same practice stands also for other medications of regular-daily intake, while antibiotics or other drugs used occasionally, are more likely to cause drug allergy.

Concluding, clinical examination and anamnesis are the cornerstone of drug allergy diagnosis, specially when there are limited laboratory exams to confirm it.

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Can the presence of cat/dog at home be considered the only criterion of exposure to cat/dog allergens? A likely underestimated bias in clinical practice and in large epidemiological studies

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Key words

Allergen exposure; allergic rhinitis; allergic sensitization; bronchial asthma; cat, dog, cat/dog allergy, hypersensitivity; pet

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Summary

An important aspect of allergic sensitization to furry animals is the association of dog and cat exposure in early childhood with the incidence of respective allergies later in life. This topic is very controversial, because some authors have found a "facilitating" effect, while others have noticed a "protective" or even no significant effect in individuals living in urban areas. It is likely that some biases could be responsible of these contradictory findings. Cat/dog ownership or their presence in indoor environments are considered usually the main criteria to assess the exposure to these pets in studies' questionnaires. Even in clinical practice "are there animals at home?" is the common query usually done when collecting anamnestic data.

In our opinion, these commonly used questions should not be considered the main index of exposure to pet allergens, because they can lead to erroneous interpretation of the clinical significance of positive skin prick tests for pet allergens as well as of the real risk of exposure to allergens of dog/cat in epidemiological studies. Consequently, we suggest a new, more realistic, classification of modalities of exposure to pet allergens in "real life" based on five possible conditions.

Although domestic animals have been kept in our homes for thousands of years, the last one hundred years have seen two major developments: firstly, the combination of clean water, shoes, separation from animals, and helminth eradication that we refer to as hygiene, and secondly, the lifestyle changes associated with overheated, airtight homes and indoor sedentary entertainment. The result has been a dramatic increase in immediate hypersensitivity to indoor allergens including those of common pets (1).

Exposure to animal allergens constitutes a relevant risk factor for the development of allergic sensitization and respiratory allergic diseases, such as asthma and rhino-conjunctivitis in susceptible individuals (2,3). Cats and dogs are the most common pets living indoor environments and the frequency of their ownership is highly variable in Europe ranging from 7.2 to 35% for the cats and from 5.4 to 35% for the dogs (4). The prevalence of allergic sensitization to cats/dogs varies in different countries according to cultural differences, environmental factors and rate of pet ownership.

An important aspect of allergic sensitization to furry animals is the association of dog and cat exposure in early childhood with the incidence of respective allergies later in life. This topic is very controversial because some authors have found a "facilitating" while others have noticed a "protective" or even no significant effect in individuals living in urban areas (5-16). On the contrary, it is widely recognized that early exposure to animals,

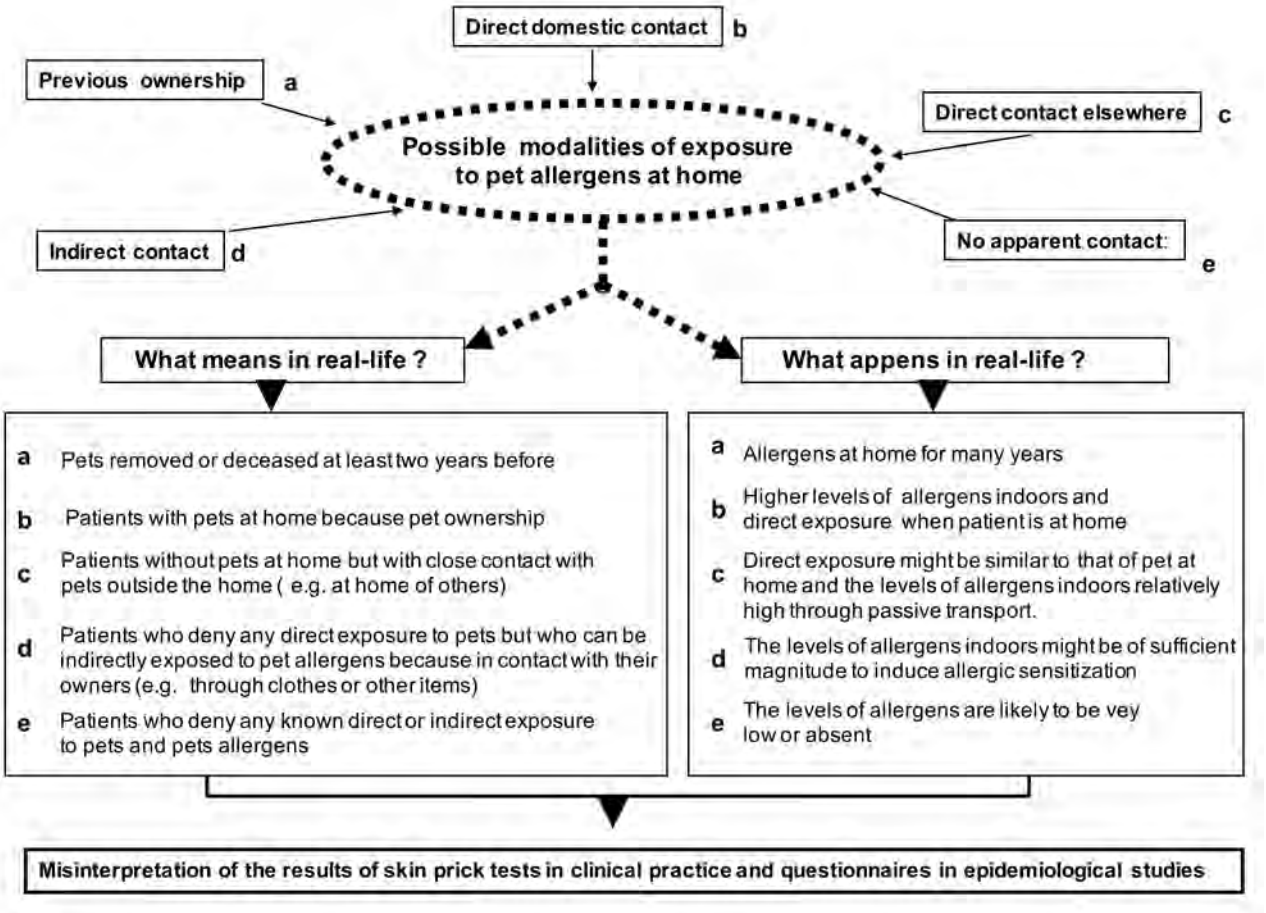
especially cattle, in the farms may induce a "protective" effect on development of respiratory allergy later in life (17,18). It is likely that some biases could be responsible of these contradictory findings. Recent studies suggested that the seemingly protective effect of pet exposure might be a result of a "healthy pet keeping effect" or a "selective avoidance" because parents with asthmatic diseases tend to keep their child from being exposed to cats/dogs to avoid a possible allergic sensitization (19,20).

In our opinion, a serious and underestimated limitation to the conclusions of these particular studies should be acknowledged. Cat/dog ownership or their presence in indoor environments is usually considered the main criteria to assess the exposure to these pets in study questionnaires. Even in clinical practice, "are there animals at home?" is the common query usually done when collecting anamnestic data.

These commonly used questions should not be considered the main index of exposure to pet allergens and, consequently, the main risk factor for allergic sensitization either in clinical practice or in large epidemiological studies.

Dynamic distribution of the main pet allergens indoors is complex, depending on production, aero-dispersion, sedimentation and passive transport through clothes and other items. These variables determine a diffuse presence of pet allergens (indirect exposure) also in indoor environments without pets and in those where pets are no longer present for a long time (e.g. voluntary removal or re-location, natural death etc.) (21). The higher is the frequency of pet ownership in a given community the higher will be the degree of pet allergen contamination of pet-free private homes (22). In this context, also in some public places (schools, day care centres, means of transport etc.), passive transfer constitutes the exclusive modality of common pet or other animal allergens contamination (23-25). Finally, several studies have shown that amounts of pet allergens passively transferred in pet-free environments are of sufficient magnitude to induce

Figure 1 *- Suggested modalities of exposure to pet allergens and possible consequences in "real-life".*



allergic sensitization in susceptible, atopic individuals, and to trigger respiratory symptoms in already and highly pet-sensitized subjects (26,27). In developed countries, the consequence of pet allergen ubiquity is a persistent stimulation of airways similar to that induced by dust mite, that would increase the risk of allergic sensitization either directly or by a cross-reaction mechanism involving albumins and lipocalins (28-30).

On the base of this background, we suggest a new, more realistic classification of possible modalities of exposure to pet allergens (**figure 1**).

Figure 1 clearly shows that only the condition "b" is reported usually in collecting anamnestic data during clinical consultations, and in the questionnaires utilized for large epidemiological studies on the relationship between early exposure to pet allergens and subsequent enhancing or protective effect on allergic sensitization to these allergens. In the conditions "a, c and d", the presence of pets at home is considered "formally negative" in patients' responses and in the questionnaires but the level of exposure (direct / indirect) to pet allergens could be outstanding. Only the condition "e" should be considered at the lower risk of pet allergen exposure after having certainly excluded any direct as well as "indirect contact". It is evident that these biases can lead to erroneous interpretation of the clinical significance of positive skin prick tests for pet allergens as well as of the real risk of exposure to allergens of dog / cat in epidemiological studies.

We have used this classification of exposure either for common / less common pets, pests or for a bigger animal such as horse, in this last case with some modifications. In these studies we have shown the role of these different modalities of exposure on the prevalence of allergic sensitization to several furry animals (31-38).

We think that our classification could be of particular importance to evaluate the modality of pet exposure at home in all countries characterized by a high frequency of pet ownership. It is likely that, in these countries, the "average amount" of pet allergens indoors could be high (or very high in some particular conditions) also in the absence of a pet at home. In conclusion, the magnitude of exposure to pet allergens at home is not related exclusively to pet ownership / presence of a pet indoors but it can be also relevant without a pet living with the inhabitants. These considerations should be taken into account during the planning of epidemiological studies on the relationship between exposure to pet and development of allergic sensitization to pet allergens. In clinical practice, a real assessment of the risk and clinical significance of allergic sensitization to pet allergens is crucial for the management of patients (pet-avoidance measures, allergen immunotherapy, pharmacological treatment of respiratory symptoms etc.).

We believe that the topic of animal allergy is very important not only for clinical but also for emotional implications in all pet-owner patients and especially in children. The love for animals in general and for pets in particular is increasing worldwide, so we wish to underline the necessity for an adequate assessment of risk factors for allergic sensitization and possible prevention strategies by using a more realistic evaluation of possible modalities of exposure.

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