Airway inflammation in the elite athlete and type of sport

Running title: Neutrophilia in water training athletes.

Key words: Airway inflammation, Sputum induction. Neutrophilia. Eosinophilia.

Swimming.

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Abstract:

Background: The prevalence of asthma and bronchial hyperresponsiveness is greater in elite athletes than in the general population and its association with mild airway inflammation has recently been reported.

Objective: To study the relation between the type of sport practiced at the highest levels of competition (on land or in water) and sputum induction cell counts in a group of healthy and asthmatic individuals.

Material and methods: Fifty athletes were enrolled. We analyzed medical history, results of methacholine challenge tests and sputum induced by hypertonic saline.

Results: Full results were available for 43 athletes, who were classified by asthma diagnosis and type of sport (land or water sports). Nineteen were healthy (10 land and 9 water athletes) and 24 were asthmatic (13 land and 11 water athletes). Although the eosinophil counts of healthy and asthmatic individuals were significantly different (mean difference 3.1%, confidence interval [CI] 0.4 to 6.2, \( P=0.008 \)), ANOVA showed no effect on eosinophil count for either diagnosis of asthma or type of sport. However, an effect was observed for neutrophil counts (analysis of variance: \( F=2.87, P=0.04 \)). We also detected a significant correlation between neutrophil counts and both duration of training and bronchial hyperresponsiveness among athletes exposed to water (Spearman’s rank correlations, 0.36 and 0.47, \( P=0.04 \) and 0.04, respectively).

Conclusions: Elite athletes who practice water sports have mild neutrophilic inflammation, whether or not asthma is present, related to the degree of bronchial hyperreactivity and the duration of training in pool water.
INTRODUCTION

The prevalence of asthma and bronchial hyperresponsiveness is greater among elite athletes than in the general population [1]. The prevalence is even higher among certain groups of athletes (long-distance runners, cyclists, skiers and swimmers) than among athletes in general [2].

The underlying inflammation in such athletes is quite similar to the pattern seen in asthmatics. This eosinophilic inflammation in some athletes have been related to the presence of asthma, atopy, type of sport and season of and duration of training [3]. The reason for inflammation is unknown, although a possible role for increased ventilation (of up to 200 L/min) has been suggested, as such an increase would extend the athlete’s exposure to cold air, airborne allergens or environmental irritants, such as the chlorine derivatives at the surface of pool water where swimmers train [1]. Inflammation can be reversed, at least partially, when the athlete’s period of active training comes to an end [4], suggesting that it is at least partly caused by participating in the sport.

Helenius et al [1] observed that increased ventilation in a group of cyclists was related to the appearance of bronchospasm, and we have demonstrated that the prevalence of asthma and bronchial hyperreactivity is higher among swimmers [5,6]. However, the relation between type of sport, amount of exposure to environmental allergens or irritants and the type of bronchial inflammation has been insufficiently studied.

Our aim in this study was to identify the effect of type of sport and duration of training in hours (as an indicator of level of exposure) on the features of airway inflammation in a group of healthy and asthmatic elite competition athletes.
MATERIAL AND METHODS

Patients

Fifty elite athletes were studied. Twenty-three were healthy and 27 were asthmatic. The asthmatic athletes were monitored by means of a specific questionnaire [1] and were stable during the study period. All were nonsmokers, were free of any other known diseases, and trained at the Centre d’Alt Rendiment (CAR – High Performance Center) in Sant Cugat, near Barcelona, Spain. The athlete agreed to enroll after a personal interview, at which his or her coach was also present. Written informed consent was obtained from all the subjects and the study was approved by the Hospital Ethics Committee.

Methods

Part of the study was performed at the physiology laboratory of the CAR, where the subjects were recruited. There we obtained a medical and personal history (symptoms, history of asthma and allergies, treatment, sport practiced and number of hours of training per week) and performed a physical examination and a methacholine bronchial challenge test. All participants also went to the laboratory at Hospital de la Santa Creu i de Sant Pau, where they were interviewed about control over their asthma, a skin prick test was performed and sputum was induced using the procedure described below. The explorations were performed in two different days separated by a maximum of two weeks but clinical stability was ensured.

The patients were classified by diagnosis, as healthy or asthmatic, and by type of sport as being exposed to water (22) or not exposed (land sports, 28). A diagnosis of asthma was given if present or past symptoms of asthma were reported and the FEV₁ fell 20% from the baseline when the methacholine concentration was less than 8 mg/mL [8]. Land athletes
were engaged in sports outside water (track and field, cycling, judo, Taekwondo, football) whereas water athletes practiced sports that unfolded entirely or partially in pools (swimming, underwater hockey and water polo). All of them considered as summer sports.

**Prick test:** A standard skin prick test of each athlete applied the respiratory allergens that are most common in Spain (Bial-Aristegui, Bilbao, Spain) and the number of aeroallergen-positive findings per subject was recorded. A test was considered positive when the wheal was equal to or greater than 3 mm in diameter in comparison with the negative control.

**Methacholine challenge test:** Spirometry was performed with a Datospir 500 (Sibelmed, S.A., Barcelona, Spain) following the recommendations of the Spanish Society of Respiratory Diseases and Thoracic Surgery [9]. The methacholine provocations were performed by adapting the continuous method described by the European Respiratory Society [10]. A Hudson-type nebulizer was used to spray recently prepared solutions of methacholine in isotonic saline solution at concentrations ranging from 0.01 to 32 mg/mL. The mass median aerodynamic diameter of the nebulizer chamber was between 1.5 and 3 µm and the air flow rate was 7 L/min. The chamber was filled with 2 mL of the appropriate solution with an oxygen flow of 7 L/min and the device was connected to a unidirectional valve inserted into the mouth of the subject, who inhaled the circulating aerosol at a normal breathing rate for 2 minutes with nostrils occluded by a nose clip. Lung function measurements were recorded at 30 seconds and were confirmed at 90 seconds after each inhalation. The process was repeated with increasing concentrations until we observed a fall in FEV\textsubscript{1} of 20% from the baseline value (PC\textsubscript{20}) or until the maximum concentration was reached. The PC\textsubscript{20} was computed from the methacholine dose-response curve in
relation to the methacholine concentration by linear interpolation on a log scale. If a patient’s FEV₁ never fell 20% from baseline, the PC₂₀ that was assigned arbitrarily was one dose higher than the maximum methacholine concentration administered (32 mg/mL).

Sputum sample induction and processing: Sputum was induced by hypertonic saline solution to obtain cell counts according to the standard procedures of our laboratory [11]. Briefly, we administered a short-acting inhaled beta 2-adrenergic agonist and 10 minutes after bronchodilation, the subjects inhaled hypertonic saline solutions at concentrations of 3% and 4% for 7 minutes each. The subject’s status was monitored spirometrically at the beginning and end of each inhalation period. Within 2 hours mucous in saliva was sampled and processed with dithiothreitol (Sputalysin; Calbiochem Corp., San Diego, CA, USA) and phosphate buffered saline. The cell suspension was filtered and stained with Trypan Blue for cytometric evaluation of the number of cells per gram of sputum, cell viability and the number of squamous cells contaminating from the upper airways. The suspension was centrifuged and the sediment was used to obtain differential cell counts (macrophages, eosinophils, neutrophils, basophils, lymphocytes and bronchial epithelial cells) using a May-Brünwald-Giemsa stain. Cell count reference values were those previously described by Belda et al [12].

Statistical analysis: The SPSS statistical package, version 10.0 (1999), was used to analyze the data. Descriptive statistics were expressed as means with standard deviations between parentheses for each variable. An analysis of variance (ANOVA) was carried out to detect the effects of type of sport (land or water) and of diagnosis (healthy or asthmatic). Spearman’s rank linear correlation coefficient (r) was used to determine relations between variables and comparisons were made using Man-Whitney U tests.
RESULTS

Induced sputum samples viable for processing were obtained from 43 of the initial 50 subjects. No significant differences were found between the studied subjects and the 4 healthy and 3 asthmatic athletes who withdrew. The induction procedure was well tolerated by all participants. The final population sample was composed of 19 healthy controls and 24 asthmatic patients. Among the healthy controls, 10 engaged in land sports and 9 in water sports. Among the asthmatics 13 practiced land sports and 11 participated in water sports. Atopy was present, with sensitivity to a mean of 2 agents, in 63% of the study sample (27/43) but only 3 healthy controls and 5 asthmatics had allergic rhinitis symptoms. All the asthmatics needed rescue doses of short-acting beta 2-adrenergic agonists, 50% of them (12/24) were receiving regular doses of inhaled corticosteroids, and 37.5% used long-lasting beta 2-adrenergic agonists (9/24). All subjects, including the asthmatics, had normal lung function and 11 asthmatics were classified as having mild intermittent asthma, 6 as having mild persistent asthma and 7 as having moderate persistent asthma. **Table 1** shows the characteristics of subjects in both groups.
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Healthy athletes</th>
<th>Asthmatic athletes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sport</td>
<td>Land</td>
<td>Water</td>
</tr>
<tr>
<td>N: 43</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>7/3</td>
<td>9/0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>23 (5)</td>
<td>27 (10)</td>
</tr>
<tr>
<td>Methacholine (PC_{20}, mg/mL)</td>
<td>&gt; 32</td>
<td>&gt; 32</td>
</tr>
<tr>
<td>Training (h/week)</td>
<td>21 (3)</td>
<td>10 (8)</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>112 (20)</td>
<td>115 (2)</td>
</tr>
<tr>
<td>FEV1 (%)</td>
<td>108 (19)</td>
<td>113 (11)</td>
</tr>
<tr>
<td>Prick +/-, n +</td>
<td>6/4, 2(2)</td>
<td>6/3, 2(2)</td>
</tr>
<tr>
<td>ACQ</td>
<td>0.4 (0.5)</td>
<td>0.3 (0.5)</td>
</tr>
<tr>
<td>Budesonide (N), (µg/day)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salmeterol (N), (µg/day)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 1.**
Anthropometric and clinical characteristics of both healthy and asthmatic patients. Data are mean (SD), included log transformed methacholine. Prick +/-, n +: number of subjects with a positive/negative prick test, number of positive pricks per subject. ACQ: asthma control questionnaire [7]. N, µg/day: number of subjects taking salmeterol and daily doses in micrograms.
The differential cell counts from sputum samples were a mean 6.9 (8.8) million total cells/g for healthy subjects and 8.2 (8.1) million/g for asthmatics ($P=0.14$). The percentage of eosinophils was 0.2% (0.3%) for healthy controls and 3.5% (7.8%) for asthmatics (Mann-Whitney U, mean difference 3.1%; 95% confidence interval [CI] 0.4-6.2, $P=0.008$). The percentage of neutrophils was 40.3% (20.1%) in healthy controls and 51.1% (20.2%) in asthmatics ($P=0.14$). The findings for each type of cell are shown in Table 2 by diagnosis and by type of sport.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Healthy athletes</th>
<th>Asthmatic athletes</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCC (mill/g)</td>
<td>Land</td>
<td>Water</td>
</tr>
<tr>
<td></td>
<td>4.5 (4.8)</td>
<td>9.5 (11.5)</td>
</tr>
<tr>
<td></td>
<td>8.3 (9.0)</td>
<td>7.8 (7.1)</td>
</tr>
<tr>
<td>BEC (%)</td>
<td>3.5 (6.0)</td>
<td>1.3 (1.7)</td>
</tr>
<tr>
<td></td>
<td>3.6 (9.2)</td>
<td>0.3 (0.7)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.1 (0.3)</td>
<td>0.2 (0.3)</td>
</tr>
<tr>
<td></td>
<td>3.9 (10.0)</td>
<td>2.7 (3.5)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>30.5 (15.7)</td>
<td>51.1 (19.6)</td>
</tr>
<tr>
<td></td>
<td>47.3 (19.6)</td>
<td>53.3 (21.9)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>1.5 (1.0)</td>
<td>2.5 (1.0)</td>
</tr>
<tr>
<td></td>
<td>3.1 (2.2)</td>
<td>2.9 (2.3)</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>49.8 (18.8)</td>
<td>44.8 (18.8)</td>
</tr>
<tr>
<td></td>
<td>42.1 (15.1)</td>
<td>40.7 (18.6)</td>
</tr>
</tbody>
</table>

Table 2. Differential cell counts in induced sputum for the healthy and asthmatic athletes studied. TCC (mill/g): total cell count (millions of cells per gram of sputum sampled). BEC: bronchial epithelial cells.

**Effects of exposure to water and of diagnosis on sputum cell counts**

No significant effect of diagnosis or exposure to pool water on eosinophil counts was observed in the ANOVA; nor was there a significant interaction between those two factors and eosinophilia. There was, however, an overall effect of diagnosis (healthy/asthmatic) and environmental exposure (water/land) on neutrophil percentages ($F:2.87; P=0.04$) with a significant individual effect of environment. However, there was no interaction between the two variables (Fig. 1).
Relation between duration of training and bronchial hyperresponsiveness or inflammation

Duration of training in hours, adjusted by diagnosis, was significantly related to bronchial hyperresponsiveness (methacholine PC<sub>20</sub>) among athletes exposed to pool water (r = −0.67, P = 0.01), but no such correlation was observed for athletes engaging in land sports (r = −0.07, P = 0.30) (Fig. 2). Duration of training also correlated with neutrophil counts in water athletes (r = 0.36, P = 0.04) but not in land athletes (r = −0.16, P = 0.45) (Fig. 3). The correlation between neutrophil counts and magnitude of bronchial hyperresponsiveness of water athletes as reflected by methacholine PC<sub>20</sub> was r = 0.47 (P = 0.04) for water athletes and r = −0.35 (P = 0.15) in land athletes but it was not observed with eosinophil counts.
Figure 2.
Relation between the number of hours of training and neutrophil concentrations (percentages) in sputum from athletes engaged in land sports (white) or water sports (black). The lines show the estimated linear regressions.

Figure 3
Relation between the methacholine bronchial challenge test findings (PC_{20}) for athletes participating in land sports (white) and water sports (black). The lines show the approximate linear regressions.
DISCUSSION

Our study demonstrated a neutrophilic inflammatory effect related to greater exposure to pool water measured by duration of training in hours that was observable in elite athletes engaged in water sports. The observed inflammatory effect, which was probably caused by the pool environment, was greater than the theoretical intrinsic effect (eosinophilic or neutrophilic) produced by the asthma itself.

The inflammatory effect of environment was not reflected on eosinophil counts when adjusted by the fact that asthmatics had more eosinophils than controls. Two explanations may account for that observation, which was not corrected after adjusting by atopy or inhaled steroids use. One would be that our sample size was insufficient to detect significant differences for eosinophil counts, a variable which varied widely among asthmatic individuals. This could explain why Mann-Whitney analysis showed significant differences between diagnosis, but ANOVA, when considering the effect of environment, did not show effect of diagnosis. A second explanation would lie in the fact that the asthma of all the patients under study was under control and 50% of them were taking regular inhaled corticosteroids, two situations that are associated with low eosinophilia.

Of great importance for verifying the effect of environment on neutrophilia was the significant relation between degree of exposure to pool water measured in number of hours per week of training and neutrophil counts in sputum. The effect was present in spite of the relative “few hours” of pool training undergone by healthy water sport athletes when compared with asthmatic water sport athletes.

The ultimate reason for neutrophil recruitment in the airways of subjects long time exposed to water pool environment is unknown. It has been largely studied that chlorine derivatives are one of the main components of this environment when swimming in indoor water pools.
In addition, we showed that swimmers are exposed to huge amounts of chlorine derivatives during regular training, which is related to the degree of airway responsiveness [15]. Unfortunately, we did not measure chlorine derivatives in this study, but we think that the cause of airway neutrophil recruitment associated to airway hyperresponsiveness is the chlorine derivatives. Supporting this speculation, it has been published that the appearance of respiratory symptoms related to exposure to chlorine derivatives in the workplace or from pool water is a well-known phenomenon [16,17]. In this way, recent studies on the association of inflammation and lung injury in children following the swimming pool attendance show more drastically information and are consistent with the hypothesis implicating pool chlorine in the rise of childhood asthma in industrialized countries [18-20]. The effect is at its strongest among elite athletes, particularly during training in periods prior to competition, when they spend 8 hours per day in a microenvironment of chlorine gases equivalent to the maximum dose allowed under Treshold Limited Value (TLV) [21]. Similarly, in the study by Helenius et al [22] demonstrated that the adjusted odds ratios for asthma and bronchial hyperresponsiveness were much higher among swimmers and long-distance runners than among athletes in sports demanding speed or power.

Increased counts of both eosinophils and neutrophils in the airways of elite athletes have been demonstrated for various sports and situations: swimming [23], cross-country skiing [24] and marathons [25]. Our data are consistent with such findings and suggest a stronger role for the effect of pool water (exposure to chlorine) than for the fact of engaging in a sport of equal or greater intensity but on land. The ANOVA of individual effects for each factor also shows that the direct irritative effect of chlorine from pools is greater than that of being asthmatic or not.
The pathogenic mechanism by which neutrophils from blood would be attracted to the airways is not well understood. Kivity et al [26] demonstrated a clear relation between the concentration of cysteinyl leukotrienes (C4, D4 and E4) in supernatants from sputum preparations and the eosinophil and neutrophil counts in induced sputum from asthmatics with exercise-induced bronchospasm, whereas such a relation was not seen for other types of asthma, suggesting that cell attraction is attributable to these mediators. However, Bonsignore et al [25,27] and Kanazawa et al [28,29] suggested that nitric oxide be considered responsible for this type of inflammation, while other authors have shown that the magnitude of exercise-induced bronchoconstriction in a group of asthmatics was not related to either intensity of inflammation or bronchial hyperresponsiveness [30]. The explanation for such discrepancies may lie in differences among the subjects studied, the different sports involved, the environments in which they take place or the clinical and medical situation of the subjects. [31].

In our study we saw more marked eosinophilia and neutrophilia in asthmatic athletes, but statistical analysis suggested a greater role on neutrophilia for the training environment than for the underlying disease. We conclude that ongoing, steady exposure to water pool environment (probably due to chlorine gases), reflected in our study by hours of training, may be the source of predominantly neutrophilic inflammation. Further studies designed to measure concentrations of environmental chlorine as well as of mediators that are theoretically implicated in this type of inflammation are needed to elucidate pathogenic processes. At the same time, these theoretical mechanisms suggest that exploring therapeutic approaches involving antileukotrienes would be justified.
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REFERENCES


What is already known on this topic
The prevalence bronchial hyperresponsiveness is greater among elite athletes than in the general population and the underlying inflammation in such athletes is quite similar to the pattern seen in asthmatics, involving a predominance of eosinophils and has been related to the presence of atopy, type of sport, the season and the duration of training.

What this study adds
This study draws attention on the presence of inflammation modulated by neutrophil, not eosinophil, and its relation to the degree of bronchial hyperreactivity and the duration of training in the swimming pool in the athlete who practices aquatic sports whether or not asthma is present, perhaps due to exposure to chlorine derivatives.
Airway inflammation in the elite athlete and type of sport

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