Original Article

Seasons can influence the results of the methacholine challenge test

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Abstract:

OBJECTIVE: This study tried to evaluate whether a methacholine test may be influenced by the seasons.

METHODS: We considered 4826 consecutive subjects with normal spirometry (50.53% males; age: 35.1±16.2; forced expiratory volume in one second: 99.5±13.0%) who underwent a methacholine test for suspected asthma symptoms between 2000 and 2010. They were subdivided into four groups, like the seasons, according to the test dates.

RESULTS: A total of 1981 (41%) resulted normal (no PD_{20} was obtained with 2400 μg of methacholine); the others showed a mean $LogPD_{20}$ of 2.52±0.5 μg. The number of subjects with bronchial hyper-responsiveness (BHR) found in autumn (789, 62.3%) was higher than in summer (583, 56.7%; *P*=0.03). A higher number of females and overweight/obese subjects showed a BHR in autumn compared with the other seasons. The spring mean $LogPD_{20}$ value (2.48±0.48 μg) was lower if compared with the one measured in summer (2.59±0.49 μg; *P*=0.05). $LogPD_{20}$ value was lower in females and non-smokers in spring compared with summer (*P*<0.05). Overweight/obese non-smokers showed a lower $LogPD_{20}$ in spring and autumn compared with that in summer (*P*<0.05). Autumn was a risk factor (OR: 1.378; *P*=0.001) for BHR (using a PD_{20}<2 400 μg as BHR limit), while spring (OR: 1.330; *P*=0.021) and autumn (OR: 1.331; *P*=0.020) were risk factors for a more severe BHR (using a PD_{20}<400 μg as BHR limit).

CONCLUSION: There was a higher probability of finding BHR in outpatients with suspected asthma in autumn and spring compared with summer. Spring is the season where BHR may be more severe. Females and overweight/obese subjects were those mainly involved in this seasonal variability of BHR.

Key words:

Airway, asthma, bronchial hyper-responsiveness, methacholine challenge test, season

Bronchial asthma is a chronic inflammatory disease characterized by a variability of bronchial obstruction. In order to confirm asthma diagnosis, it is sometimes necessary to perform a methacholine challenge test in subjects with suspected asthma symptoms but with a normal baseline spirometry and without a significant response after administering bronchodilators.[1]

There is some evidence that asthma—and especially bronchial hyper-responsiveness (BHR)—may be influenced by the seasons. In fact, patients with allergic rhinitis or asthma have allergen-related seasonal changes in BHR.[2,3] During pollen exposure, a decrease in provocative concentration of methacholine causing a 20% fall in lung function (PC_{20}) was found compared with what had been detected during the previous season in hyper-responsive patients with rhinitis and/or asthma.[3-6] Mite-allergic asthmatic patients showed an increased non-specific BHR in autumn, a possible consequence of a higher indoor mite concentration.[3,7,8] Especially in cold seasons, respiratory infections might also increase BHR in asthmatics.[9-11] Furthermore, climatic variations of air temperature and above all of humidity might influence the BHR and exercise-induced bronchospasm.[12,13]

Therefore, seasons seem to influence the results in the methacholine challenge test. Fruchter and Yigla[14] had already shown that the percentage of positive methacholine challenge tests was higher in winter and spring in a selected cohort of young adult patients. However, this seasonal variability may be different in the various latitudes (different vegetation and climates). We already know that age, sex, and smoking habits can influence hyper-responsiveness,[15-18] but we do not know if a methacholine test will give different results for males and females, young and elderly subjects, smokers and non-smokers, if performed in the different seasons.

The aim of this study was to evaluate if there was a seasonal variability in the prevalence of BHR and its severity measured with the methacholine test in a large number of subjects with suspected asthma living in Tuscany Italy, an area with a temperate climate.

Methods

Subjects

For the purpose of this study, we extracted from the spirometer data files of our pneumology departments of Grosseto and Arezzo (Tuscany)

5023 consecutive methacholine challenge tests performed between 2000 and 2010 and retrospectively analyzed. All these tests had been carried out to confirm or exclude an asthma diagnosis. In fact, all patients had suspected asthma symptoms (unexplained episodes of cough and/or wheezing and/or dyspnea) with a normal spirometry and therefore they were subjected to a methacholine challenge test. Four thousand eight hundred twenty-six subjects (2439 M; mean age 35.1±16.2; mean±SD of forced expiratory volume in one second [FEV_1]: 101.1±12.9%; mean±SD of forced vital capacity [FVC]: 99.5±13%; mean±SD of FEV_1/FVC: 86±7.1) were suitable for the study because they completed the test. One hundred ninety-seven subjects were excluded as they had not finished the test: Some were intolerant to testing, others interrupted the challenge because they had shown a fall in FEV_1>10% with the buffer solution. In those few who had repeated the methacholine test several times, only the first challenge was taken into consideration.

For every test, FEV_1, FEV_1/FVC, FVC, cumulative provocative dose of methacholine causing a 20% fall in FEV_1 ($PD_{20}FEV_1$), smoking habits, body mass index (BMI), and the date of when the test had been carried out were taken into account.

The BMI value of 25 was used as a cut-off to subdivide normal weight or underweight (BMI<25) subjects from those overweight or obese (BMI>25). International cut-off points for BMI, to assess overweight and obese children, were used to subdivide those aged <18 years into underweight-normal or overweight-obese subjects.[19]

All methacholine tests had been performed randomly during any of the four seasons in the period of time taken into consideration for our study. The test was carried out after a waiting list period of 2 to 3 months. Sometimes, subjects underwent the test even some years after the onset of symptoms. The methacholine test was postponed for at least 4 weeks when subjects had shown an exacerbation of symptoms or an airway infection. B_2-agonist bronchodilators and inhaled or systemic corticosteroids were not taken 24 hours and 3 weeks before the methacholine test, respectively. Antihistamines were suspended at least 10 days before the test. The use of the data recorded in each spirometer which were necessary for this study and its protocol were approved by the local ethical committees.

Season classification

The methacholine challenge tests were divided into the four seasons according to the date in which they were carried out For each patient the exact date when the Methacholine challenge test (MCT) was performed was available. This date was considered to categorize all subjects into four groups according to the season when the Methacholine challenge test (MCT) was performed. The classification of the seasons was as follows: January 1 to March 31 was considered as winter; April 1 to June 30, spring; July 1 to September 30, summer; October 1 to December 31, autumn. This classification of the seasons was fundamentally based on the different temperatures that occur normally during the year in the Mediterranean area where Italy is located. In our area (Grosseto and Arezzo), the mean temperature measured in winter, spring, summer, and autumn between the year 2000 and 2010 were 7.7°C, 17.8°C, 22°C, and 11.7°C, respectively (data from the Italian Air Force Meteorological Service).

Methacholine challenge test

The challenge test was performed by using a dosimeter method.[20] The same kind of instrument and method were used in Grosseto and Arezzo between the year 2000 and 2010. Methacholine sulfate was provided by Lofarma (Milan, Italy) and administered in aerosol form by a MEFAR MB3 dosimeter (output: 9 µl/puff; MEFAR Elettromedicali Brescia, Italy) using a model MB2 ampoule. Buffer and methacholine were diluted with distilled water and then two different progressive methacholine solutions were prepared for the test: An ampoule with a methacholine concentration of 4 mg/ml (40-µg inhalation dose) and another with a concentration of 40 mg/ml (400 µg inhalation dose). The buffer solution was administered first followed by 40 µg of methacholine, increasing the doses step by step until $PD_{20}FEV_1$ was obtained or until the maximum dose of muscarinic agonist was reached. FEV_1 was measured after administering 40, 80, 120, 240, 400, 800, 1 600, 2 400, or 3200 µg of cumulative methacholine doses. The doses were taken from the first ampoule up to 400 µg and from the second up to 2400 or 3200 µg. At the end of exhalation during tidal breathing, the patients inhaled methacholine slowly and deeply from the nebulizer in a time of 5 seconds and then held their breaths for another 5 seconds. The test was interrupted if a fall in FEV_1>10% was obtained with the buffer solution. The time interval between the two steps was 2 minutes, calculated from the start of one step to the next. FEV_1 was measured at 30 and 90 seconds after the nebulization. An acceptable-quality FEV_1 at each time point was obtained. No more than two maneuvers after each dose was performed and the highest FEV_1 value was taken into consideration. During the first years (until the end of 2008), the maximum cumulative dose of methacholine used in the challenge was 3200 µg, whereas in the last two years (2009-2010), it was reduced to 2400 µg. As not all subjects had used the same methacholine dose cut-off and according to guidelines,[1] a high dose of methacholine is not necessary to diagnose BHR (for the high probability of a false positivity), we arbitrarily selected a cumulative 2400 µg dose as the cut-off value for all to identify hyper-responsive patients. Therefore, BHR was defined by a 20% fall in FEV_1 following a challenge with a cumulative methacholine dose $\leq$2400 µg.

Seeking to follow the indications of guidelines[1] and adapting these to our data, we also took into consideration subjects with $PD_{20} \leq 100$, $100 < PD_{20} \leq 400$, and $PD_{20} > 400$ affected by moderate-to-severe, mild, and borderline BHR to evaluate the effect of the season on different levels of BHR.

FEV1 was measured before and during the challenge using a spirometer HP 47120E Pulmonary System Desk (Hewlett Packard, Waltham, Massachusetts, USA). PD_{20} FEV_1 was calculated by linear interpolation of the dose response curves. The FEV_1 measured before inhaling the buffer solution was considered as the baseline value, whereas the FEV_1 measured after the buffer solution was used as the referral to calculate FEV_1 decrease and therefore obtain the PD_{20} value. FEV_1 and FVC were expressed as percentages of the predicted value, whereas FEV_1/FVC was reported only as a ratio (reference equation: CECA, 1971).

Statistical analysis

Categorical variables are expressed as percentages. All continuous variables are expressed as mean values,

accompanied by their standard deviations. PD_{20} was logarithmically transformed and therefore expressed as mean and standard deviation. The prevalence of BHR and PD_{20} values, found in the different seasons and in every month, were compared in each sub-group considered. The seasonal and monthly comparisons among the various groups were made by the ANOVA and Chi-square where appropriate. Post-hoc prevalence comparisons were made by the χ^2 test with Bonferroni correction; the Bonferroni test was also used as a post-hoc test for non-parametric data. Baseline FEV_1%, FVC%, and FEV_1/FVC measured in the various seasons were compared by ANOVA test with Bonferroni test as post-hoc analysis.

The logistic binary regression model was used to evaluate whether age, sex (females in comparison with males), smoking habits, BMI (overweight/obese compared with underweight/normal weight subjects), FEV_1, and the seasons could be independent BHR risk factors, using PD_{20} < or >2400 μg as a limit to identify hyper-responsive or normal subjects. Another model was also applied to evaluate whether the above-mentioned covariates were also a risk for a higher degree of severity in hyper-responsiveness (using 400 μg as a limit of low or high level of hyper-responsiveness). All above-mentioned covariates were included in the models. The risk factor for BHR in winter, spring, and autumn were calculated by comparing these seasons with the summer season which was used as a reference in the logistic regression model. Logistic regression models were used only for those subjects whose smoking history was known (4 169 subjects). *P* values <0.05 were considered as statistically significant. The statistical packages SPSS (16.0) and MedCalc (9.0.1) were used for the analysis.

Results

Among the 4826 subjects (50.53% males), 1 981 (41%) had a normal BHR as their PD_{20} was not obtained after inhaling the maximum dose of methacholine provided by the challenge. All the other BHR subjects showed a $LogPD_{20}$ of 2.52±0.5 μg. Current smokers were 831 (20%), but the smoking histories of 672 subjects were unknown [Table 1]. Methacholine challenges were performed less frequently during summer (1.029 - 21.3%; *P*<0.05) than during the other seasons: 1256 (26%) in winter, 1275 (26.4%) in spring, and 1266 (26.2%) in autumn [Table 1]. Among the seasons, no differences were found in the values of FEV_1 (% of predicted) and FVC (% of predicted); only the FEV_1/FVC ratio measured in summer was higher in comparison with autumn (*P*=0.022; [Table 1]). One hundred four subjects showed a PD_{20} between 2400 and 3 200 μg, but having chosen a 2400 μg cumulative methacholine dose as the normal cut-off value, they were considered as subjects with normal reactivity.

Subjects with BHR were more frequently found in autumn (789 subjects - 62.3%) than in summer (583 subjects - 56.7%; *P*=0.03; [Table 2]). There was no difference in the proportion of hyper-responsive subjects among males in the different seasons. However, a higher proportion of hyper-responsive females was found in autumn rather than in the other seasons (*P*=0.003). We also found that overweight or obese hyper-responsive subjects were significantly higher in number in autumn than in the other seasons (*P*=0.017). Non-smoking hyper-responsive subjects were less in summer than in autumn (*P*=0.001).

When subjects with severe-to-moderate, mild, and borderline BHR were taken into consideration, no differences were found in the prevalence of BHR among the four seasons [Figure 1]. Also, when these groups were subdivided into smokers and non-smokers, we did not find any differences in the prevalence of BHR among the four seasons.

The mean $LogPD_{20}$ value, which we found in spring, was lower in comparison with the one measured in summer (*P*=0.05) when subjects were considered as a whole [Table 3]. In particular, $LogPD_{20}$ was lower in females (*P*=0.042) and in non-smokers (*P*=0.030) in spring compared with that in summer. In child-adolescent subjects (age <20 years old), the $LogPD_{20}$ value measured in autumn was lower in comparison with the one observed in winter (*P*=0.048).

When a sub-analysis was performed considering smoking as a confounding factor, we found a lower $LogPD_{20}$ value both in smoking and in non-smoking females in spring compared with summer (*P*<0.05). In non-smoking subjects aged between 20 and 35 years, the value of $LogPD_{20}$ was also lower in spring

Table 1: Characteristics of 4,826 subjects who performed a methacholine challenge test at baseline

	January to March (Winter) (%)	April to June (Spring) (%)	July to September (Summer) (%)	October to December (Autumn) (%)	Total (%)	*P*
No. of subjects (% of total)	1256 (26.0)*	1275 (26.4)°	1029 (21.3)*°#	1266 (26.2)#	4826 (100)	0.001
Age	36.1 ± 16.6	34.5 ± 16.1	35.0 ± 15.8	34.9 ± 16.1	35.1 ± 16.2	0.084
BMI	24.8 ± 4.6	24.7 ± 5.1	24.5 ± 4.5	24.7 ± 4.7	24.7 ± 4.7	0.598
Males	614 (48.9)*	692 (54.3)*	505 (49.1)	628 (49.6)	2439 (50.5)	0.020
Females	642 (51.1)*	583 (47.7)*	524 (50.9)	638 (50.4)	2,387 (49.5)	0.020
[a]Current smokers	212 (19.3)	215 (19.3)	170 (19.7)	234 (21.7)	831 (20)	0.431
[a]ex-smokers	81 (7.4)	69 (6.2)	76 (8.8)	69 (6.4)	295 (7.1)	0.707
Overweight/obese subjects	551 (43.9)	540 (42.4)	412 (40)	551 (43.5)	2054 (42.6)	0.255
FVC % of predicted	98.8 ± 13.3	99.4 ± 13.4	99.7 ± 12.8	100 ± 12.6	99.5 ± 13.0	0.090
FEV_1% of predicted	100.6 ± 13.1	101.3 ± 13.1	101.6 ± 12.8	101 ± 12.6	101.1 ± 12.9	0.333
FEV_1/FVC	86 ± 7.0	86.3 ± 7.1	86.3 ± 7.1*	85.5 ± 7.1*	86 ± 7.1	0.022

BMI = body mass index; FEV_1 = forced expiratory volume in one second; FVC = forced vital capacity; FVC = forced vital capacity. Data were expressed as proportion and mean±standard deviation; % of groups represents the percentage of subjects belonging to the season in which the methacholine tests were performed. Mean comparisons were made by using ANOVA test and a post-hoc analysis with Bonferroni test. Proportion comparisons were made by using χ^2 test and a post-hoc analysis by χ^2 test with Bonferroni correction; *°#statistically significant differences between groups when they were compared. [a]evaluated on 4 154 patients

Table 2: Prevalence of bronchial hyper-responsiveness obtained in the different seasons in the various sub-groups

	January to March (Winter) (%)	April to June (Spring) (%)	July to September (Summer) (%)	October to December (Autumn) (%)	*P*
All BHR subjects (PD_{20}<2400 μg)	727/1256 (57.9)	746/1275 (58.5)	583/1029 (56.7)*	789/1266 (62.3)*	0.03
Males	353/614 (57.5)	399/692 (57.7)	273/505 (54.1)	360/628 (57.3)	0.58
Females	374/642 (58.3)*	347/583 (59.5)°	310/524 (59.2)#	429/638 (67.2)*°#	0.003
Age <20 years old	172/253 (68.0)	190/281 (67.6)	127/209 (60.8)	170/258 (65.9)	0.347
Age between 20 and 35 years old	225/383 (58.7)	266/431 (61.7)	198/350 (56.6)	272/433 (62.8)	0.271
Age between 36 and 51 years old	202/361 (56.0)	170/332 (51.2)	160/276 (58.0)	209/342 (61.1)	0.071
Age >/=52 years old	128/259 (49.4)	120/231 (51.9)	98/194 (50.5)	138/233 (59.2)	0.137
Normal weight or underweight	416/705 (59.0)	442/735 (60.1)	350/617 (56.7)	435/715 (60.8)	0.452
Overweight or obese	311/551 (56.4)*	304/540 (56.3)°	233/412 (56.6)#	354/551 (64.2)*°#	0.017
Smokers	132/212 (62.3)	143/215 (66.5)	112/170 (65.9)	148/234 (63.2)	0.768
Non-smokers	505/889 (56.8)	507/897 (56.5)	358/694 (51.6)*	526/843 (62.4)*	0.001

BHR - bronchial hyper-responsiveness. The subjects who obtained a 20% fall in FEV_1 with a methacholine dose of 2 400 μg were considered as hyper-responsive. evaluated on 4,154 patients. *°#statistically significant differences between groups when they were compared. *P* value calculated with Chi-square test whereas post-hoc comparisons were made by χ^2 test with Bonferroni correction

Table 3: Seasonal values of $LogPD_{20}$ obtained in 2 845 subjects with BHR subdivided into various sub-groups

	January to March (Winter)	April to June (Spring)	July to September (Summer)	October to December (Autumn)	*P*
All BHR subjects (PD_{20}<2 400 μg)	2.53 ± 0.51	2.48 ± 0.48*	2.59 ± 0.49*	2.51 ± 0.52	0.050
Males	2.53 ± 0.49	2.51 ± 0.48	2.53 ± 0.49	2.48 ± 0.51	0.580
Females	2.53 ± 0.53	2.49 ± 0.50*	2.6 ± 0.49*	2.53 ± 0.53	0.042
Age <20 years old	2.56 ± 0.47*	2.46 ± 0.46	2.47 ± 0.52	2.42 ± 0.53*	0.048
Age between 20 and 35 years old	2.51 ± 0.52	2.48 ± 0.51	2.57 ± 0.51	2.51 ± 0.52	0.316
Age between 36 and 51 years old	2.51 ± 0.53	2.54 ± 0.49	2.62 ± 0.48	2.53 ± 0.53	0.140
Age>/=52 years	2.57 ± 0.54	2.55 ± 0.49	2.62 ± 0.41	2.59 ± 0.49	0.786
Normal weight or underweight	2.56 ± 0.50	2.49 ± 0.50	2.56 ± 0.51	2.53 ± 0.53	0.163
Overweight or obese	2.49 ± 0.52	2.51 ± 0.47	2.59 ± 0.46	2.48 ± 0.51	0.058
Smokers	2.53 ± 0.51	2.51 ± 0.49	2.58 ± 0.50	2.49 ± 0.53	0.080
Non-smokers	2.58 ± 0.50	2.48 ± 0.48*	2.65 ± 0.46*	2.61 ± 0.51	0.030
Moderate to Severe BHR	1.71 ± 0.22	1.74 ± 0.21	1.73 ± 0.23	1.71 ± 0.22	0.687
Mild BHR	2.34 ± 0.17	2.34 ± 0.17	2.33 ± 0.17	2.35 ± 0.17	0.555
Borderline BHR	2.99 ± 0.22	2.96 ± 0.21	2.99 ± 0.21	3 ± 0.22	0.171

BHR = Bronchial hyper-responsiveness. Subjects with $PD_{20} \leq 100$, $100<PD_{20} \leq 400$, and $PD_{20}>400$ were considered to be affected by moderate-to-severe, mild, and borderline BHR; see Table 2 for statistical analysis. *Statistically significant differences between groups when they were compared. Data were expressed as mean±standard deviation; *P* was calculated by using ANOVA test; post-hoc multiple comparisons among groups were calculated by using Bonferroni test

(2.36±0.47 μg) if compared with summer (2.70±0.50 μg). In addition, the $LogPD_{20}$ value was lower in non-smoking overweight/obese subjects in spring (2.49±0.47 μg) and autumn (2.47±0.48 μg) in comparison with summer (2.64±0.51 μg; *P*=0.005) (data not present in tables).

The month-to-month comparisons of the $LogPD_{20}$ values and the prevalence of hyper-responsiveness according to severity did not show any significant differences [Figure 2].

The logistic regression model [Table 4] showed a significantly higher BHR risk in females (Odd Ratio [OR]: 1.536; *P*=0.0001), smokers (OR: 1.279; *P*=0.003), overweight or obese subjects (OR: 1.219; *P*=0.005), whereas age and FEV_1% were protective factors (increasing their values, the risk of being hyper-responsive decreased). Among the seasons, autumn showed a significantly higher BHR risk (OR: 1.378; *P*=0.001) compared with summer. Spring (OR: 1.330; *P*=0.021) and autumn (OR: 1.331; *P*=0.020) were also risk factors for a higher degree of severity in BHR (using a PD_{20}<400 μg as BHR limit; [Table 4]), when summer was used as a reference season.

Table 4: Adjusted odd ratios (logistic regression) for risk factors and 95% confidence interval associated to the presence of bronchial hyper-responsiveness established by using 2 400 μg or 400 μg PD_{20} cut-offs

	PD_{20}<2 400 μg			PD_{20}<400 μg		
	OR	95% CI	*P*	OR	95% CI	*P*
Females	1.536	1.341-1.761	0.0001	1.030	0.865-1.227	0.742
Age	0.985	0.980-0.989	0.0001	0.989	0.983-0.996	0.001
FEV_1%	0.968	0.963-0.973	0.0001	0.974	0.967-0.980	0.0001
Smoking	1.279	1.087-1.505	0.003	0.899	0.753-1.073	0.095
Overweight or obese	1.219	1.061-1.400	0.005	1.433	1.201-1.711	0.0001
Winter	1.136	0.944-1.367	0.176	1.186	0.930-1.512	0.169
Spring	1.197	0.995-1.440	0.056	1.330	1.043-1.697	0.021
Autumn	1.378	1.142-1.662	0.001	1.331	1.046-1.693	0.020

FEV_1 = Forced expiratory volume in one second; OR=Odd ratios. Females, smokers, overweight/obese, and seasons (winter, spring, and autumn) were compared with males, non-smokers, underweight/normal weight subjects, and summer, respectively. This logistic regression assessed only 4154 subjects whose smoking history was known

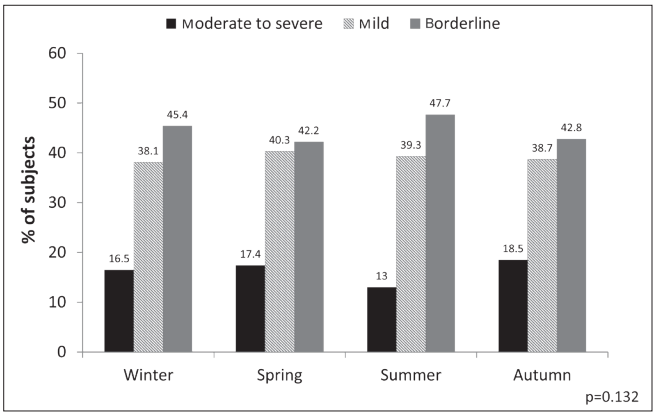

Figure 1: Prevalence of bronchial hyper-responsiveness obtained in the different seasons in subjects with moderate-to-severe, mild, and borderline BHR

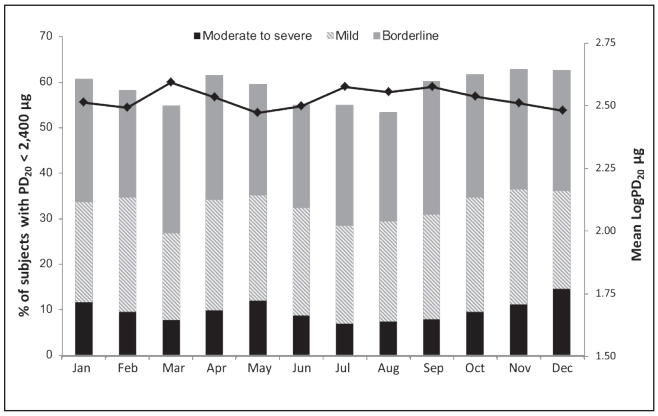

Figure 2: Monthly mean $LogPD_{20}$ (points) and prevalence of BHR according to severity (bars) obtained in 2845 subjects with PD_{20}<2400 µg

Discussion

This study, carried out on a large number of subjects, highlights that some seasons may influence the results of methacholine challenge tests. In fact, a lower number of hyper-responsive subjects were found in summer compared with autumn. Furthermore, the logistic regression models showed a higher (+39.3%) bronchial risk of hyper-responsiveness in autumn in comparison with summer when we chose a 2400 µg cut-off value to divide hyper-responsive subjects from normal subjects. There was also a higher risk (+34.7%) for a higher degree of severity in airway hyper-responsiveness (using a cut-off of 400 µg) both in spring and in autumn, again compared with summer. This result is certainly due to a greater allergen exposure in these seasons in comparison with summer. This major exposure may cause an increase in airway inflammation with a consequent increase in BHR. Blood eosinophil numbers were indeed higher in BHR subjects with seasonal allergic rhinitis during pollen season than those without BHR, whereas there was no difference in the number of these cells during off-season in these two groups.[21] After a nasal challenge with grass pollen in rhinitis, BHR and both eosinophil numbers and eosinophil-cationic protein (ECP) concentration in induced sputum had increased and the latter two were correlated to methacholine responsiveness.[22] In addition, eosinophils and ECP, both in peripheral blood and sputum, increased in mite-sensitized asthmatics after *Dermatophagoides pteronyssinus* exposure and this rise was associated to a PD_{20} decrease.[23]

Fruchter and Yigla[14], in a similar study, partially confirmed our results as they found a lower number of positive methacholine tests in summer compared with winter and spring, due to a lower allergy burden in mites and in pollens in that season. Another study also showed that the sensitivity of exercise testing for asthma in adolescents is halved in summer due to a decreased asthma activity for a lower exposure to allergens, to air pollutants such as nitrogen oxides and sulfur dioxide and to respiratory viruses such as respiratory syncytial virus and influenza.[24]

Other authors have found, in agreement with our study, a greater autumnal airway hyper-responsiveness risk. In fact, some of them had already observed how airway hyper-responsiveness increased in autumn in multiple-sensitized allergic asthmatic patients.[3,7,8] The higher prevalence of BHR in autumn is probably due to an increased exposure to house dust mite in this season. In fact, a more severe airway hyper-responsiveness was found in mite-sensitized patients and this was strongly related to an increase of Der p 1 concentrations in floor dust, particularly at the beginning of autumn.[7] Other authors have also found that sensitization to house dust mite, as opposed to sensitization to pollen allergens alone, is associated with BHR, thus confirming that mite allergy plays the leading role in BHR.[6,25] Unfortunately, in our study, the allergic status of the subjects recruited is not known. However, it seems that 95.4% of young adults, with positive methacholine tests, resulted positive to aeroallergen skin prick tests.[24] We found a positive skin prick test in approximately 64% of a group of 811 subjects recruited in the same Tuscan area for suspected rhinitis and/or asthma. We found a higher sensitization to mites (61.8%), followed by cypress (56%), grasses (44%), olea (36.4%), and pellitory (19.6%) in these subjects.[26] Therefore, among the 4 826 subjects considered for our study, a large number of them might be allergic to house dust mites, thus influencing the variations in autumnal airway hyper-responsiveness. However, also atopic asthmatic subjects—who are exposed to high levels of dust mites (as it may happen in autumn) but not sensitized to this allergen—show evidence of increased airway reactivity.[27]

The increased mite-induced airway inflammation may also explain the increased obstruction of the airways in autumn demonstrated by a reduced FEV_1/FVC ratio found in this season compared with summer. Consequently, this autumnal increased obstruction may be responsible for the higher prevalence of BHR found in this season. In fact, it is known that the level of BHR is negatively correlated to pulmonary function.[18,27]

The autumnal drop in temperature, with the increase of upper or lower respiratory tract infections, might be another reason for the higher prevalence of hyper-responsiveness in autumn. In fact, some studies correlated bronchial infections with an increased airway hyper-responsiveness.[2,9-11] However, the absence of any BHR increase in winter, when bronchial infections were more frequent, does not explain this increase in hyper-responsiveness in autumn. On the contrary, Fruchter and Yigla found a higher prevalence of BHR in winter and not in autumn.[14] It is known that in Israel, in particular in Haifa where the study was performed, the climate is usually

milder if compared with that of Northern Italy, which makes the Israeli winter similar to the Italian autumn. A very high humidity rate and very cold air may also influence airway hyper-responsiveness,[12,13] but humidity and the temperature in autumn do not usually reach extreme values in our area. Therefore, any direct influence of temperature and/or humidity on this BHR variability is unlikely.

Another important result of this study was the observation of a lower PD_{20} value in spring compared with summer. Furthermore, we found that there was a higher probability of having a PD_{20} lower than 400 μg in spring compared with the summer. This means that the higher pollen concentrations in spring, due to blooming of grasses, olea, and pellitory in our area, can determine a more severe BHR in subjects sensitized to these pollens. This is confirmed by some studies that have shown an increase in bronchial reactivity in spring in hyper-responsive subjects with rhinitis and/or asthma sensitized to Parietaria, Olea, and Gramineae.[2-6] It seems that Parietaria is more important than Olea and Gramineae as a risk factor for non-specific BHR.[28]

Our study highlighted that seasonal variability concerns particularly females and overweight/obese subjects. The prevalence of hyper-responsiveness in these subjects was higher in autumn in comparison with what was obtained in the other seasons, which was unexpected. Furthermore, the PD_{20} value was lower in females and in non-smoker overweight/obese subjects in spring compared with that in summer (eliminating the confounding effect of smoking). We have not found any explanations in literature for this greater prevalence of hyper-responsiveness in autumn and this greater hyper-reactivity in spring, in particular in these categories of subjects. Several studies have shown that the female sex has a higher BHR risk factor[18,29,30] as confirmed also in our study (+53.6% of risk increase). It has been suggested that the higher prevalence of airway hyper-responsiveness in females could be caused by their smaller lung volumes.[27] On the other hand, there are conflicting studies on a possible existence of a relation between BMI and hyper-responsiveness.[31-34] However, we found that an overweight/obese status is an independent high BHR risk (+21.9%). The obesity-related changes in TNF-alpha, leptin, and adiponectin may contribute to this BHR increase.[35] Therefore, for this particular predisposition to airway hyper-responsiveness, females and obese subjects probably develop a greater sensitivity to house dust mites and pollens compared with other categories of subjects (for example, males or subjects with normal weight). In autumn, when house dust mites increase, and in spring, when the pollen exposure is high, these subjects may show a higher reactivity to these allergens. This may suggest a different asthma phenotype in female and overweight/obese subjects. Children-adolescents also show a lower PD_{20} in autumn compared with winter. This season variability is probably due to a greater susceptibility to mites or pollen in young subjects with a small airway calibre where the allergic response already tends to be higher itself. Furthermore, in non-smokers aged between 20 and 35 years old (eliminating the confounding effect of smoking), a lower PD_{20} was found in spring compared with summer, probably due to a stronger bronchial reactivity to pollens in these young subjects rather than in those who were older where this reactivity is known to be lower.[16]

This study also confirmed how smoking can be a BHR risk factor (+27.9%) in subjects with typical asthma respiratory symptoms. Other studies had already observed how asymptomatic smokers showed an increased airway responsiveness compared with non-smokers.[36,37] Furthermore, allergic rhinitis (+/−asthma) hyper-responsive subjects showed an improvement in methacholine-induced BHR 12 months after they had stopped smoking, thus confirming the importance of such habit in BHR.[38] Smoking can cause chronic airway neutrophilic inflammation and oxidative epithelium damage, thus leading to the development of airway hyper-responsiveness;[39] in fact, a dose-dependent relationship between the number of cigarettes smoked and the degree of hyper-responsiveness has been demonstrated.[40] A large number of non-smokers showed a BHR in autumn compared with what was found in summer, whereas no seasonal variations were observed in smokers. Smoking probably induces a persistent inflammation or the worsening of the pre-existent inflammation induced by asthma which cancels the seasonal changes in BHR over the course of the year. The absence of smoke-induced inflammation makes this airway phlogosis susceptible to allergen-related seasonal changes in non-smokers.

We also analyzed if there were any seasonal variations in the different levels of BHR. We did not find any differences neither in prevalence nor in PD_{20} among the seasons in moderate-to-severe, mild, and borderline BHR. However, according to the guidelines,[1] the PC_{20} cut-off value for a positive methacholine test is 4 mg/ml, comparable with our PD_{20} dose of 400 μg. Using a cut-off value of 400 μg to define BHR in the logistic regression model, we found a high BHR risk both in autumn and spring. Therefore, subjects with PD_{20}<400 μg (with a higher BHR) may show a worsening of BHR in these seasons.

We must also add that the results seem to be in contradiction where the prevalence of BHR was higher in autumn while the PD_{20} was lower in the spring. This may be due to a higher proportion of subjects with severe BHR in spring (although not significant) compared with summer, but in autumn, we observed that the distribution of subjects with different levels of BHR is the same as that observed in spring. Alternatively, this increased BHR in spring could be due to a greater activity on the airways by spring allergens; on the contrary, autumnal allergens may cause a reduced bronchial reaction in subjects with BHR.

In summary, this study has shown a seasonal variability of airway hyper-responsiveness; therefore, when methacholine tests are repeatedly performed over the course of time (for example, in clinical trials), this must be taken into account. In addition, considering that there is a lower BHR in summer, especially in comparison with spring and autumn, a reduced therapy level in this season may be hypothesized in multi-sensitized persistent asthmatics, whereas in autumn and spring—when their BHR is higher—treatments may be increased.

In conclusion, this study has shown a seasonal variability in the response to the methacholine challenge test in subjects with suggestive asthma symptoms, which is probably due to a different allergens exposure in the various seasons. A higher probability of finding a hyper-responsiveness may be detected

in autumn and in spring, whereas a lower one may be found in summer. Spring is the season when BHR may be more severe. This seasonal variability seems to concern in particular females and overweight-obese subjects.

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