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Cut-off points defining normal and asthmatic bronchial reactivity to exercise and inhalation challenges in children and young adults

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ABSTRACT: An analysis was undertaken to determine the optimal cut-off separating an asthmatic from a normal response to a bronchial provocation challenge by exercise and the inhalation of methacholine or histamine in children and young adults.

Data were extracted, after appropriate correction, from published studies available in Medline of large random populations that complied with preset criteria of suitability for analysis, and the distribution of bronchial reactivity in the healthy population for exercise and inhalation challenges were derived. Studies on the response to exercise and methacholine inhalation in 232 young asthmatics of varying severity were carried out by the authors and the distribution of bronchial reactivity of a young asthmatic population obtained. Comparisons of the sensitivity and specificity of the challenges were aided by the construction of receiver operating characteristic curves.

The optimal cut-off point of the fall in forced expiratory volume in one second (FEV1) after exercise was 13%, with a sensitivity (power) of 63% and specificity of 94%. For inhalation challenges, the optimal cut-off point for the dose of methacholine or histamine causing a 20% fall in FEV1 was 6.6 μmol, with a sensitivity of 92% and a specificity of 89%.

The cut-off values were not materially affected by the severity of the asthma and provide objective data with which to evaluate the results of bronchial provocation challenges in children and young adults.

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The definition of asthma has always been problematic but in recent years a better understanding of the pathology and pathophysiology of the disease has enabled it to move beyond a collection of symptoms and physical signs. The structural changes are associated with a greater than normal variation in airway calibre, and this bronchial hyperreactivity has been accepted as a cardinal feature of the disease in the various guidelines for the diagnosis and management of asthma which have been published [1]. Although this bronchial hyperreactivity may be apparent from spontaneous variation in lung function or response to medication, it can also be demonstrated by deliberately provoking bronchoconstriction by the inhalation of various chemical agents and by the cooling and drying of the airways caused by exercise or hyperventilation. As recently reviewed by PATTEMORE and HOLGATE [2], there is some doubt as to whether this type of bronchial reactivity documented by provocation tests is an essential feature of bronchial asthma since various studies have apparently shown that normal subjects can be hyperreactive and asthmatics may sometimes be unresponsive [3]. However, children with current asthma are likely to show bronchial hyperreactivity on provocation, have spontaneous variation in lung function and require medications to control symptoms [4]. Part of the difficulty in defining the relationship between asthma and bronchial hyperreactivity is due to the fact that patients with certain other pulmonary diseases such as cystic fibrosis or chronic obstructive pulmonary disease (COPD) of various types can also respond abnormally to the inhalation of methacholine or histamine [5–7]. Nevertheless, as far as children and young adults are concerned, the great majority of symptomatic and hyperreactive subjects in any population will be asthmatic, and tests of bronchial reactivity are used chiefly to identify such patients in cases in which the diagnosis is in doubt.

Reviewing the literature on this topic reveals that many investigators have found that although the specificity of bronchial provocation challenges by exercise or inhalation (normal subjects with negative test/total normal subjects tested) is generally of the order of ≥80–90%, the sensitivity of the challenges for detecting asthma (asthmatics with positive test/total asthmatics tested) is generally only of the order of 40–70% [8–13]. Even more striking is the poor positive predictive value of challenges (asthmatics with positive test/total subjects with a positive test), which is of the order of 10–50% [9, 12, 13]. These indices depend, of course, upon what is defined as normal and in many studies the choice of cut-off has been made on a purely arbitrary basis. The positive or negative predictive values also depend on the proportion of asthmatics in the population studied.

The present study was undertaken using the best available data as normal subjects from the literature and the results of studies by the authors on asthmatics to define the range of bronchial reactivity in healthy and asthmatic children and young adults. Using the results of the analyses of exercise and inhalation challenges, the definition of the optimal cut-off points, sensitivity and specificity of these challenges in the detection of asthmatic bronchial hyperreactivity have been attempted.

Subjects and methods

Selection of data on normal subjects from the literature

In order to define the response of normal children and adolescents to inhalation and exercise challenges, a search was conducted of the authors′ database and the Medline database between 1962 and 1998. Every relevant paper was studied in its full format and those providing adequate data were used in the present analysis. Adequate data were defined by the following criteria: 1) subjects selected by random population survey; 2) total number of normal subjects >100; 3) age range of subjects 5–25 yrs; 4) absolute numbers of subjects in all groups and subgroups detailed; 5) classification of subjects as healthy or symptomatic clearly defined; 6) response measured in terms of forced expiratory volume in one second (FEV_1); 7) inhalation challenge end point defined as a 20% fall in FEV_1; and 8) methods used to undertake challenges clearly defined in the present or previous publications of the investigators.

As a result of the search, eight studies of asymptomatic children and adolescents who were assumed to be healthy normal subjects were selected for inclusion (tables 1 and 2). Data on the response to exercise and/or the responses to inhalation of either histamine or methacholine were available from these studies [8, 9, 13–18]. The criterion for defining normality in most studies was the lack of respiratory symptoms or wheeze at any time (tables 1 and 2). Because of the small numbers of subjects studied or the widely differing techniques used, studies of bronchial reactivity evaluated by means of hyperventilation or the inhalation of nonisotonic aerosols and other agents such as adenosine 5′ monophosphate were not considered.

Exercise challenges in asymptomatic subjects from the literature

In all the studies selected, the exercise challenges were undertaken similarly, namely 6 min of relatively hard running or cycling in an environment of moderate temperature and humidity (table 1). The results of exercise challenges were expressed in terms of the fall in FEV_1 after exercise as a percentage of the pre-exercise, baseline value. Rises in FEV_1 were taken as negative falls and hence there were no censored values.

Inhalation challenges in asymptomatic subjects from the literature

Unlike exercise challenges, there were major differences between the various inhalation challenges (table 2) in terms of the techniques of inhalation and the method of expressing the results, which required special treatment in order to render them comparable. The studies that were judged suitable for further analysis employed either histamine or methacholine, one of three different inhalation techniques and one of two methods of expressing the results.

Yan technique. In this technique, originally described by YAN *et al.* [19], the subject inhaled one or more deep breaths from a hand-operated, calibrated nebulizer, after which lung function was measured. The number of breaths and the concentration of drug (histamine) was increased in a standardized fashion from 3.13 $mg \cdot mL^{-1}$ to 50 $mg \cdot mL^{-1}$ until a 20% fall in FEV_1 or the maximum dose had been reached. The exact end point was determined by interpolation. The total cumulative dose (expressed in μmol of histamine) causing this end point was taken as the result and termed the provocative dose of agent causing a 20% fall in FEV_1 (PD_{20}) to histamine.

Five-breath technique. In this technique, employed in the studies of SEARS *et al.* [16, 17], the subject inhaled five consecutive deep breaths from a regular compressed air jet nebulizer, which delivered the drug to a face mask. Lung function was measured and the concentration of drug (methacholine) was increased in 10-fold steps from 0.025 $mg \cdot mL^{-1}$ to 25 $mg \cdot mL^{-1}$ until a 20% fall in FEV_1 or the maximum dose had been reached. The exact end point was determined by interpolation. The concentration of methacholine in $mg \cdot mL^{-1}$ causing this end point was taken as the result and termed the provocative concentration of agent causing a 20% fall in FEV_1 (PC_{20}) to methacholine.

Cockcroft technique. In the tidal breathing technique originally described by COCKCROFT *et al.* [20], the subject inhaled the agent (either histamine or methacholine) delivered to a face mask or mouthpiece *via* a jet nebulizer during 2 min of quiet tidal breathing. Lung function was measured and the concentration of drug was increased, usually in doubling concentration, from ~0.03 $mg \cdot mL^{-1}$ to 8.0–32.0 $mg \cdot mL^{-1}$ until a 20% fall in FEV_1 or the maximum dose had been reached. The exact end point was determined by interpolation. The concentration of histamine or methacholine in $mg \cdot mL^{-1}$ causing this end point was taken as the PC_{20}.

Table 1. – Data on response to exercise in normal subjects from the literature

First author [Ref.]	Criteria	Age yrs	Subjects n	Type of exercise	Time min	Cardiac frequency beats·min^{-1}	Temp. °C	RH %
RIEDLER [8]	NW	13–15	152	Free run	6	180–190	23	50
HABY [14]	NW	8–11	435	Free run	6	180	16	55
BACKER [13]	NW	7–16	391	Treadmill	6	160–180	21	40–50

RH: relative humidity; NW: never wheezed; NRS: no respiratory symptoms.

Table 2. – Data on responses to inhalation of histamine or methacholine in normal subjects from the literature

First author [Ref.]	Criteria	Age yrs	Subjects n	Type of inhalation	Agent	Nebulizer output
PATTERMORE [11]	NW	7–10	1513	YAN [19]*	Histamine	0.003 mL·squeeze^{-1}
ASHER [15]	NRE	8–10	1890	YAN [19]*	Histamine	0.003 mL·squeeze^{-1}
SEARS [16]	NRE	9	466	5-breath^{+}	Methacholine	0.2 mL·min^{-1}
SEARS [17]	NACW	11	570	5-breath^{+}	Methacholine	0.2 mL·min^{-1}
BACKER [18]	NRS	7–16	367	COCKCROFT [20]$^{\#}$	Histamine	0.14 mL·min^{-1}

*: single-breath cumulative dose method; $^{+}$: five deep breaths noncumulative concentration; $^{\#}$: 2-min tidal breathing noncumulative concentration method. NW: newer wheezed; NRE: no respiratory symptoms ever; NACW: no asthma or current wheezing; NRS: no respiratory symptoms.

In order to render the results obtained by these different techniques comparable, it was decided to standardize them all so that they approximated as closely as possible to the cumulative dose expressed in µmol. The data of PATTEMORE *et al.* [11] and ASHER *et al.* [15] who employed the Yan technique were used unaltered. The data of the studies of SEARS *et al.* [16, 17] using the five-breath technique were converted as follows. The dose of methacholine inhaled at any concentration step was assumed to be the product of the concentration of the drug, the nebulizer output (0.2 mL·min^{-1}), the time (assuming that five deep breaths take 30 s) and the duty cycle of deep breathing (assumed to be 0.5). This dose of methacholine expressed in mg was converted to µmol (using the factor 5.109, based on the molecular weight of the drug). A graph was then plotted of the cumulative dose against the corresponding concentration step for the four dose steps used by SEARS *et al.* [16, 17] and the PD_{20} corresponding to the PC_{20} in the studies were derived from the regression: $PD_{20}=1.533 \times PC_{20} - 0.024$. A similar approach was used for the study of BACKER *et al.* [18] using the Cockcroft technique comprising 2 min of tidal breathing. The dose of histamine inhaled at any concentration step was assumed to be the product of the concentration of the drug, the nebulizer output (0.14 mL·min^{-1}), the time (2 min) and the duty cycle of tidal breathing (assumed to be 0.3). This dose of histamine in mg·mL^{-1} was converted to µmol (using the factor 0.271, based on the molecular weight of the drug). A graph was then plotted of the cumulative dose against the corresponding concentration step for the doubling dose steps from 0.03 mg·mL^{-1} to 8.0 mg·mL^{-1} used by BACKER *et al.* [18] and the PD_{20} corresponding to the PC_{20} in the studies were derived from the regression: $PD_{20}=0.542 \times PC_{20} - 0.008$.

Exercise and inhalation challenges on asthmatic subjects

The data from all patients undergoing bronchial challenges in the authors′ department, by means of exercise or the inhalation of methacholine, as an aid to the diagnosis and evaluation of lung disease are stored on a computerized database which includes pertinent clinical information. From this database, all complete records of children and young people aged 5–25 yrs who had undergone both exercise and methacholine challenges within 1 week and whose clinical diagnosis was bronchial asthma were extracted. This diagnosis was based on the typical clinical picture of episodic reversible attacks of airway obstruction with symptom-free intervals, and the severity was determined on the basis of the medication needed to control the asthma during a follow-up of ≥3 months in the authors′ clinics. Asthma severity was termed mild if it was controlled by bronchodilators on an as-needed basis only, as moderate if it was controlled by low-dose inhaled corticosteroid prophylaxis (≤400 µg·day^{-1}) or nonsteroidal prophylaxis (cromolyn sodium or theophylline) and severe if it was only controlled by high-dose inhaled corticosteroid prophylaxis (>400 µg·day^{-1}). Many of these studies had been undertaken before the various national and international guidelines were published, but this classification is similar to those now utilized in the guidelines [1, 21]. The challenges were undertaken by the staff of the pulmonary function laboratory of the Institute of Pulmonology who were unaware of the classification of asthma severity of the patient. The details of the patients included in the study are given in table 3.

Bronchial challenges were only undertaken if the prechallenge FEV_1 was ≥60% of the predicted value. All short-acting bronchodilator drugs and cromolyn sodium were omitted for 12 h before challenges and long-acting bronchodilators for 24 h, but inhaled corticosteroids were continued in their usual doses in those patients who took them for prophylaxis. If both challenges were performed on the same day, enough time was allowed (>3 h) after the first challenge for the FEV_1 to return to within 10% of the baseline value before commencing the second challenge.

Bronchial challenge by exercise. The exercise challenge was carried out using 6 min of treadmill running with the treadmill set at a slope of 10°C and a speed of 5 km·h^{-1}. This produced a cardiac frequency of 160–180 beats·min^{-1}. The FEV_1 was measured before and 1, 3, 5, 10 and 15 min after exercise. The challenge was performed in an air-conditioned laboratory and the subjects breathed room air with a temperature of 22–26°C and a relative humidity of 48–56%. The result of the exercise challenge was calculated as the greatest fall in FEV_1 after exercise expressed as a percentage of the pre-exercise value.

Bronchial challenge by methacholine. Fresh solutions of methacholine were made up in phosphate buffer at a range of concentrations, 0.03–32 mg·mL^{-1}. Inhalation challenges were performed by means of the Cockcroft tidal breathing method [20]. The nebulizer chamber (Respirgard II nebulizer system; Marquest Medical Products, Inc., Englewood, NJ, USA) was filled with 2.0 mL of test solution and an airflow of 5 L·min^{-1} resulted in a mean rate of nebulization of 0.34 mL·min^{-1} over 2 min. The nebulizer was connected through

Table 3. – Asthmatics from Hadassah University Hospital

	Mild	Moderate	Severe	Total
Patients n	121	65	46	232
Males n	78	47	32	157
Females n	43	18	14	75
Age yrs	12.23 (6.05–18.40)	11.56 (5.62–17.50)	12.21 (6.71–17.70)	12.04 (6.04–18.04)
FEV_1 % pred	87.4 (89.0–85.8)	86.4 (89.2–83.7)	80.4 (83.0–77.7)	85.7 (87.0–84.2)

*: mean with 5th and 95th percentiles in parentheses; +: mean with 95% confidence interval in parentheses. FEV_1: forced expiratory volume in one second.

a one-way valve system to a mouthpiece through which the child breathed normally while wearing a nose clip. The first inhalation comprised phosphate buffer and subsequent inhalations methacholine in doubling concentrations. Each solution was inhaled for 2 min during tidal breathing, with measurement of lung function 1 min after each inhalation until the FEV_1 had fallen by ≥20% from the post-buffer value or until the maximum concentration was reached. Lung function was measured in duplicate using a pneumotachograph-based system (Vitalograph Compact; Vitalograph, Buckingham, UK) and the highest value of FEV_1 at each interval was recorded. The PC_{20} was derived from a plot of fall in FEV_1 against log methacholine concentration. For the purposes of the present study, the PC_{20} in $mg \cdot mL^{-1}$ was converted to the PD_{20} in µmol using the same technique as described above for the Cockcroft method but a measured nebulizer output of 0.34 $mL \cdot min^{-1}$. This gave the relationship $PD_{20}=PC_{20} \times 2.084 - 0.033$.

Derivation and statistical analysis of the distribution of bronchial reactivity to exercise

In order to define the response of children to exercise, it was assumed that the fall in FEV_1 after exercise (ΔFEV_1) was normally distributed. The weighted mean ΔFEV_1 and the weighted mean SD of the ΔFEV_1 ($SD\Delta FEV_1$) based on the numbers of subjects in the three studies of normal children included in the survey were calculated. The mean ΔFEV_1 and SD of the ΔFEV_1 for the asthmatics in the authors' studies were also calculated. Using a computer graphics program (Prism 2; GraphPad Software Inc., San Diego, CA, USA), the normal distribution curves of exercise-induced bronchial reactivity were constructed for the normal and asthmatic children. For any given value of ΔFEV_1 it is possible to calculate the proportion of asthmatics with a positive response (*i.e.* sensitivity) and the proportion of normal children with a negative response (*i.e.* specificity). This was done by expressing the selected value of ΔFEV_1 as a "Z-score", where Z=(mean - ΔFEV_1)/SD for the distributions of normal and asthmatic subjects respectively. From a table of the area of the tail of a normal distribution curve ("Z-score" table), the proportion of normal or asthmatic subjects expected to respond at the chosen level of ΔFEV_1 and hence sensitivity or specificity was determined.

It can also be shown that the value of Z, where sensitivity and specificity are equal (Z_s), is given by the relationship:

$$\text{Mean } \Delta FEV_1(n) + Z_s \times SD\Delta FEV_1(n) = \text{Mean } \Delta FEV_1(a) - Z_s \times SD\Delta FEV_1(a)$$

where n denotes normal children and a asthmatics. This allows the optimal balanced ΔFEV_1 corresponding to Z_s to be calculated.

Derivation and statistical analysis of the distribution of bronchial reactivity to inhalation

In order to define the response of children to inhalation challenges by either methacholine or histamine, it was assumed that the response to either agent expressed in molar terms was similar, as suggested by HIGGINS *et al.* [22] or shown by replotting the data of SEKEREL *et al.* [23]. It was assumed that the distribution of bronchial reactivity in normal subjects was log-normal, which seems a reasonable approximation from other published studies [11, 24]. From each of the studies of inhalation challenges in normal children, the proportion responding at a given cumulative dose in µmol derived as described above was noted and the equivalent value of Z for that proportion was read from a "Z-score" table. By definition, this gives the relationship:

$$Z = \frac{\log PD_{20} - \log \text{Mean } PD_{20}}{SD\log PD_{20}}$$

where $SD\log PD_{20}$ is the SD of $\log PD_{20}$. Rearranging this gives:

$$\log PD_{20} = SD\log PD_{20} \times Z + \log \text{Mean } PD_{20}$$

and, thus, if $\log PD_{20}$ is plotted against Z, the intercept on the $\log PD_{20}$ axis is log Mean PD_{20} and the slope is the $SD\log PD_{20}$. The data from the five studies in normal children were used to calculate the regression of $\log PD_{20}$ on Z (see results), and hence log Mean PD_{20} and $SD\log PD_{20}$ for the normal population were determined. Once these parameters had been calculated for the normal population they were used together with data from the asthmatic population to construct normal distribution curves of inhalation-induced bronchial reactivity in the same fashion as for the exercise studies. Graphs relating sensitivity and specificity to PD_{20} and the balanced optimal PD_{20} were also determined as for the exercise data.

Results

Exercise challenges

From the three studies of normal children included in the survey [8, 13, 14], there were a total of 978 results, which gave a weighted mean ΔFEV_1 of 5.28% and a weighted mean SD of 5.55% (table 4). For the total group in the

Table 4. – Exercise responses in normal children from the literature and asthmatic children from Hadassah University Hospital

	Subjects n	ΔFEV1 Mean %	ΔFEV1 SD
Normal children			
RIEDLER [8]	152	5.90	6.90
HABY [14]	435	6.10	4.69
BACKER [13]	391	4.14	5.83
Total	978	5.28*	5.55*
Asthmatics			
Mild	121	15.84	13.71
Moderate	65	22.75	16.22
Severe	46	21.12	13.87
Total	232	18.82	14.83

*: weighted. FEV1: forced expiratory volume in one second.

authors' studies of 232 asthmatics, the mean ΔFEV1 was 18.82% with an SD of 14.83% (table 4). The normal distribution curves for the normal and asthmatic children are shown in figure 1, which was drawn assuming that the total number of asthmatics (the area under the curve for asthmatics) was 10% of the total population [25]. The range of responses of asthmatics is wide (as indicated by the relatively large SD). Nevertheless, there was a good separation between the asthmatics and the normal children at an ~10% ΔFEV1, with most of the normal children having a <10% fall in FEV1 and most of the asthmatic having a >10% fall. The sensitivity and specificity of different values of ΔFEV1 in separating the asthmatic from the normal response is shown in figure 2. It can be seen from figure 2 (or calculated on the basis of equal Z-scores) that the optimal balanced ΔFEV1, was 8.95%, with an equal sensitivity and specificity of 74.7%. Choosing a higher cut-off point for ΔFEV1 of say 15% would yield a specificity of 96% but a sensitivity of only 60%.

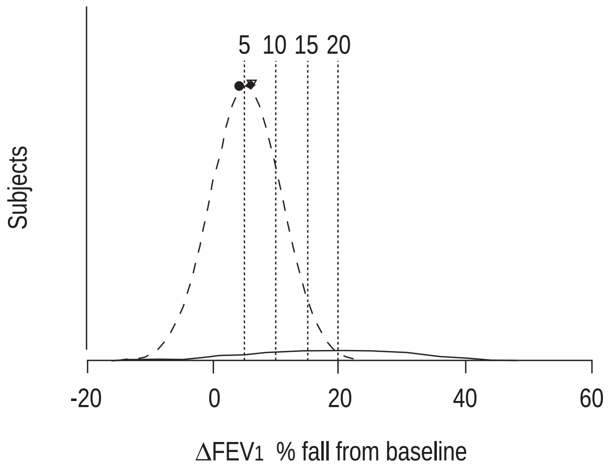

Fig. 1. – Theoretical population distribution of bronchial reactivity to exercise expressed as fall in forced expiratory volume in one second (ΔFEV1) based on data for normal children from studies in the literature (– – –) and in asthmatics from studies at Hadassah University Hospital (–). The area under the curve represents the total size of the population and has been drawn as 90% of the total for the normal children and 10% for the asthmatics. - - -: cut-off points at various values of ΔFEV1 ◆; ▽; ●: mean values for the studies in normal children: [8]; [14]; [13].

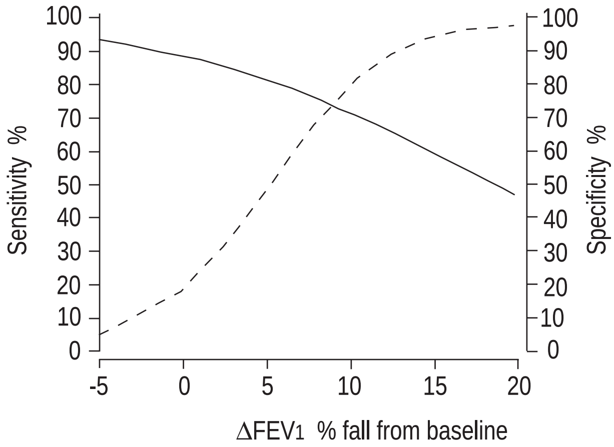

Fig. 2. – Exercise reactivity: sensitivity (–) and specificity (– – –) for various cut-off values fo fall in forced expiratory volume in one second (ΔFEV1) for distinguishing normal children from asthmatics. The balanced optimal value of ΔFEV1 (8.95%) occurs where sensitivity and specificity are equal, at 74.7%.

On the other hand, choosing a lower cut-off point for ΔFEV1 of say 5% would yield a sensitivity of 82.5% but a specificity of only 48.0%.

Inhalation challenges

The data from the inhalation studies on normal children extracted from the literature are summarized in table 5. The exact numbers of normal children included in the analysis are difficult to define since some of the children in two pairs of studies may have been included in both ([11] and [15], [16] and [17]), and this is not entirely clear from the original publications. However, the number of subjects is not needed for the calculations described above, and is only used as a guide to the size of the population studied. To avoid the creation of possibly spurious extra data points in the analysis, the data on normal children of PATTEMORE *et al.* [11] and ASHER *et al.* [15] were combined and averaged as they involved identical methods and virtually identical results. Likewise, the data on normal children of SEARS *et al.* [16, 17] were combined and

Table 5. – Standard deviation score (Z-value) corresponding to the percentage of a normal distribution at or below the chosen cut-off for inhalation challenges in normal children

First author [Ref.]	Subj. n	Cut-off PD20 µmol	log cut-off PD20 mmol	Subjects with ≥20% ΔFEV1 at ≤PD20 %	Z-value
PATTEMORE [11], ASHER [15]	3403	7.80	0.8921	9.67	1.30
SEARS [16, 17]	1036	7.10	0.8510	11.00	1.23
SEARS [16]	570	2.27	0.3560	3.68	1.79
SEARS [17]	466	0.71	-0.1494	1.28	2.24
BACKER [18]	367	4.33	0.6362	10.3	1.26

Subj.: subjects; PD20: provocative dose of agent (histamine or methacholine) causing a 20% fall in forced expiratory volume in one second (FEV1); ΔFEV1: fall in FEV1.

averaged for the analysis of responsiveness to <25 mg·mL^{-1}, which was common to both studies, whereas the responsiveness to <2.5 mg·mL^{-1} and <8.0 mg·mL^{-1} was used individually as each was available from only one of the two studies.

For each of the five studies of normal children listed in table 5, the values for log PD_{20} and the Z-values for the proportion of normal subjects responding at this dose are plotted in figure 3. The regression of logPD_{20} on Z had a correlation coefficient of 0.97 ($p<0.01$) with an intercept on the y-axis (log Mean PD_{20}) of 1.9851, which is equivalent to a mean PD_{20} of 97 µmol. The slope of the regression line yielded a value for SDlog PD_{20} of 0.9386 (there is no single numerical equivalent of the SD of a logarithm). Using only the data of SEARS *et al.* [16, 17], the results of this analysis were virtually identical, as can be surmised from the closeness of the points to the regression line in figure 3. Omitting the data of BACKER *et al.* [18], who used histamine with the Cockcroft technique (see *Discussion*), from the regression yielded a somewhat higher value for the mean PD_{20} of normal subjects (152 µmol)

For the total group in the authors′ studies of 232 asthmatics, simple statistical analysis yielded a log Mean PD_{20} of -0.2563, equivalent to a mean PD_{20} of 0.55 µmol with an SDlog PD_{20} of 0.7515. Using the data for the normal and asthmatic children, normal distribution curves for an inhalation challenge were constructed (fig. 4) in a similar fashion to those for exercise. The result shows that there was a good separation between asthmatics and normal children at a PD_{20} of approximately 5–6 µmol. The sensitivity and specificity of inhalation challenge were calculated in the same way as for exercise for different values of PD_{20}, and the results are shown in figure 5. The optimal balanced value of PD_{20} for separating asthmatics from normal subjects, as seen in the graph and calculated as for exercise, was 5.5 µmol, with a sensitivity and specificity of 90.7%. Choosing a higher cut-off point for PD_{20} of say 8 µmol would yield a sensitivity of 96.3% but a specificity of only 87.5%. On the other hand, choosing a lower cut-off point for PD_{20} of say 3 µmol would yield a specificity of 94.6% but a sensitivity of only 83.5%.

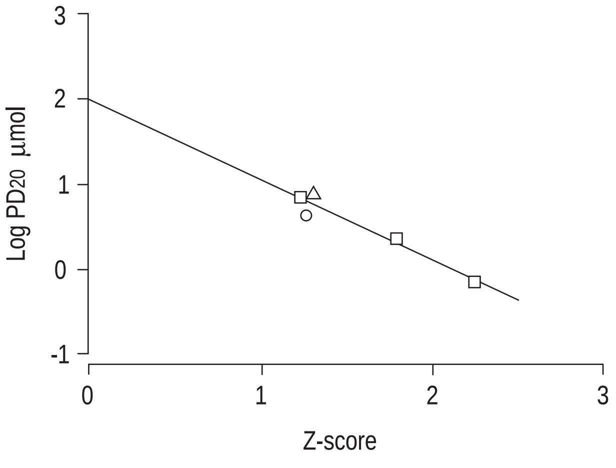

Fig. 3. – Plott of cut-off values of provocative dose of agent (histamine or methacholine) causing a 20% fall in forced expiratory volume in one second (PD_{20}) on a log scale against number of SDs from the mean of a normal distribution (Z-score) for studies on normal children taken from the literature. See text for derivation and corrections applied to the data. ○: histamine, tidal breathing [18]; □: methacholine, five-breaths [16, 17]; △: histamine, single breath [11, 15].

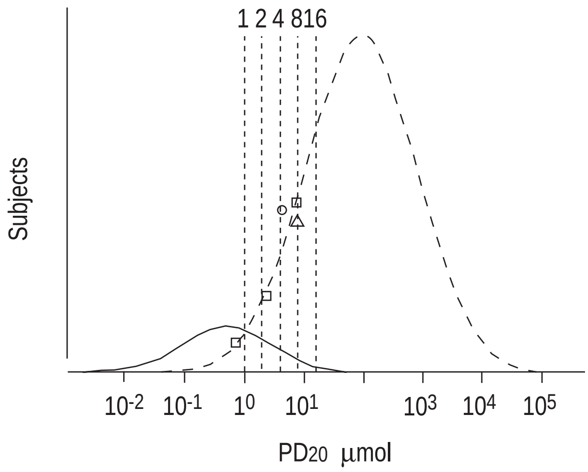

Fig. 4. – Theoretical population distribution of bronchial reactivity to the inhalation of histamine or methacholine expressed as provocative dose of agent causing a 20% fall in forced expiratory volume in one second (PD_{20}) based on data for normal children from studies in the literature (– – –) and in asthmatics from studies at Hadassah University Hospital (–). The area under the curve represents the total size of the population and has been drawn as 90% of the total for the normal children and 10% for the asthmatics. - - -: cut-off points at various values of PD_{20}. ○; □; △: mean values for the studies in normal children. For definitions of symbols, see figure 3.

Sensitivity and specificity in relation to asthma severity

Similar calculations of sensitivity, specificity and optimal balanced cut-off values for exercise and inhalation challenges were undertaken for each of the three groups of asthmatic children. These results together with those for the total group are given in tables 6 and 7, which show that

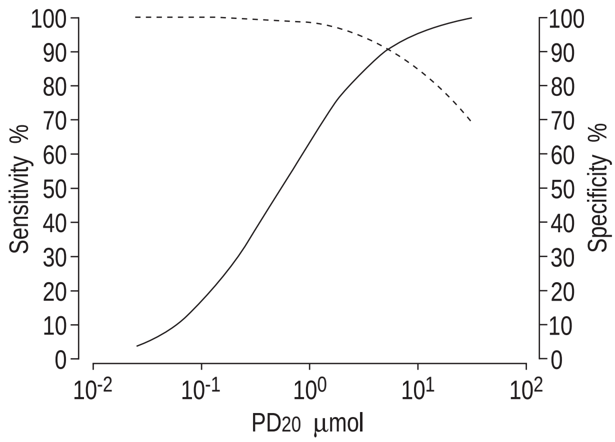

Fig. 5. – Histamine/methacholine reactivity: sensitivity (——) and speci-ficity (– – –) for various cut-off values of provocative dose of agent causing a 20% fall in forced expiratory volume in one second (PD_{20}) for distinguishing normal children from asthmatics. The balanced optimal value of PD_{20} (5.50 µmol) occurs where sensitivity and specificity are equal, at 90.7%.

Table 6. – Parameters for each group of asthmatics and for the total group relating to the derivation of the balanced optimal fall in forced expiratory volume in one second (ΔFEV_1) after exercise challenge

	Mean ΔFEV_1 %	SD ΔFEV_1	Z-value	Sensitivity= specificity %	Balanced optimal ΔFEV_1	PPV %
Mild	15.84	13.71	0.56	71.3	8.11	21.6
Mod.	22.75	16.22	0.82	79.5	9.38	30.1
Severe	21.12	13.87	0.84	80.0	9.46	30.8
Total	18.82	14.83	0.67	74.7	8.95	24.7

Mod.: moderate. SDΔFEV_1: SD of ΔFEV_1; Z-value: (mean - ΔFEV_1)/SD; PPV: positive predictive value of cut-off points assuming that 10% of the total population are asthmatics.

the optimal balanced ΔFEV_1 was higher and PD_{20} lower with more severe asthma, whereas the specificity and sensitivity higher for both types of challenge with more severe asthma.

Comparison of exercise and inhalation challenges, receiver operating characteristic curves

The above analysis using sensitivity, specificity and the optimal balanced cut-off values does not give a complete picture of the relative value of the two types of challenge nor does it necessarily define the best optimal cut-off point for distinguishing asthma from normality unless the two populations have distributions with identical SDs. To further explore this point, receiver operating characteristic (ROC) curves [26–28], shown in figure 6, were constructed. In an ROC plot, the sensitivity (percentage of true positive results) is plotted against the percentage of false positive results (100 - specificity). The line with a slope of -45° represents points on the plot at which sensitivity and specificity are equal. The nearer a curve approaches the top left of the ROC plot, the greater the sum of the sensitivity and specificity (100 - false positives) and hence the lines with slopes of 45° drawn as tangents to the PD_{20} and ΔFEV_1 curves represent points on those curves at which the sum of their individual sensitivity and specificity are greatest. The solid curved lines are theoretical ROC plots for normally distributed populations of identical SDs whose means are separated by the index of detectability (d′) of 1, 2 and 3 SD; the higher this index, the better the test. From these plots, the following information was obtained about the inhalation and exercise challenges. 1) The similarity in shape of the inhalation ROC curve to the theoretical SD plots indicates that the SDs of normal and asthmatic children were similar, whereas that for exercise indicates that they were quite different. 2) For any chosen level of false positive results (type I error), the sensitivity (power) of an inhalation challenge was considerably greater than that of an exercise challenge in differentiating between asthmatic and normal responses. The separation intervals for inhalation and exercise (d′) along the -45° line also indicate the greater discriminatory ability of the inhalation challenge. 3) The optimal (greatest combination) sensitivity and specificity for inhalation occurred at a sensitivity of 92.4% and specificity of 89.3%, whereas that for exercise occurred at a sensitivity of 62.8% and specificity of 94.2%. 4) From the data on the optimal (greatest combination) sensitivity and specificity and the relationships shown in figures 2 and 5, the optimal (greatest combination) cut-off for an inhalation challenge occurred at a PD_{20} of 6.6 µmol and for an exercise challenge at a ΔFEV_1 of 13%.

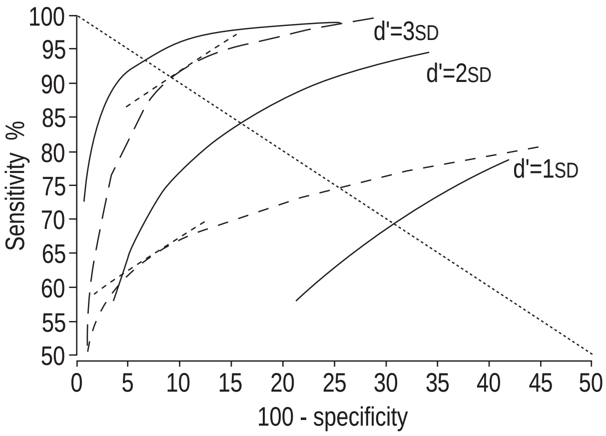

Fig. 6. – Receiver operating characteristic curves for exercise challenges (- - - - -) and inhalation challenges (– – –) together with theoretical curves for populations with mean separations (d′) of 1, 2 and 3 SDs. Sensitivity and specificity are equal at the points at which the curves cross the line with a slope of -45° (· · ·), and the points at which the tangents with slopes of 45° (– – – -) touch the curves indicate the optimal (greatest combination) values, at which sensitivity plus specificity is maximal. For fuller explanation, see text.

Discussion

In this analysis, the authors′ data on asthmatic children and young adults were compared with the best available published data on healthy children in order to define the ranges of bronchial reactivity to exercise and inhalation challenges in these groups.

Studies of large populations of randomly selected children and adolescents are very difficult to perform for a variety of reasons, and, since it was also necessary to know the exact details of the methods used in the studies, it is hardly surprising that only a relatively small number of studies could be used in the analysis. It is important to emphasize that although the validity of the data in the published studies is the responsibility of the authors of those studies, the present use of their data and the interpretation of the results is entirely the authors′ responsibility. It was attempted to select those studies for which it was possible to be reasonably certain that the investigators were indeed studying healthy children by methods which were adequately described. The definition of asthma or respiratory disease in these studies was based on the answers of the children or their parents to standardized questionnaires and this inevitably introduces an uncertainty as to the accuracy of the diagnosis. However, for the present study only concerned those children and adolescents who apparently had never had any respiratory symptoms at any time in their life (table 1), and it seems likely that they were indeed healthy subjects.

This study has inevitably involved a number of assumptions regarding the normal populations studied, the techniques used (especially for the inhalation challenges),

Table 7. – Parameters for each group of asthmatics and for the total group relating to the derivation of the balanced optimal provocative dose of agent (histamine or methacholine) causing a 20% fall in forced expiratory volume in one second (ΔFEV1) after inhalation challenge*

	log Mean PD20	SDlog PD20	Z-value	Sensitivity = specificity %	Balanced optimal PD20 µmol	PPV%
Mild	-0.0369	0.7175	1.22	88.9	6.90	47.1
Moderate	-0.4424	0.7817	1.41	92.1	4.58	54.4
Severe	-0.5876	0.6314	1.64	94.9	2.80	67.4
Total	-0.2563	0.7515	1.33	90.7	5.50	52.0

*: where sensitivity and specificity are equal. SDlog PD20: SD of log PD20; 2-value: (mean - log PD20)/SD; PPV: positive predictive value of cut-off points assuming that 10% of the total population are asthmatics.

the method of deriving the normal distribution curve of normal subjects for inhalation based on those few subjects who responded and, finally, the validity of comparing the asthmatics studied in Israel with the normal subjects from Australia, New Zealand and Denmark. All of the present authors have extensive experience of working outside Israel and know of no evidence to suggest that Israeli children differ materially from those in the countries from which the normal data were drawn. The major assumptions in the present analysis relate to the derivation of PD20 values in inhalation studies on normal children using different inhalation techniques. The most straightforward studies used the Yan technique in which the dose of drug has been shown to be cumulative [19]. The studies of SEARS *et al.* [16, 17] used the five-breath technique, which is similar in many ways to the Yan technique, except that the drug is inhaled from a regular nebulizer rather than some type of dosimeter. Since the duration of the five-breath challenge is also short and since methacholine was used, it was felt to be very likely that the dose was cumulative and, therefore, that the assumptions made about conversion to PD20 were valid. The studies using the Cockcroft technique are more problematic. It is fairly certain, from the study of JUNIPER *et al.* [29], that when histamine is used with this technique the dose is unlikely to be cumulative and hence the inclusion of the data of BACKER *et al.* [18] must cast some doubt on the results using the regression shown in figure 3. If these data are excluded from the regression, the mean PD20 of the normal subjects is higher (152 instead of 97 µmol) with a very small effect on the calculated optimal balanced PD20 (5.9 instead of 5.5 µmol) and the calculated optimal (greatest combination) PD20 (7.2 instead of 6.6 mmol). As far as the authors′ studies are concerned, the Cockcroft technique was used but with methacholine, and the study of JUNIPER *et al.* [29] showed a "small but significant" cumulative effect with this agent. From the data in figure 3 of their paper, it looks as if the effect on the dose using the Cockcroft method was approximately double that of a single dose, which is almost exactly what would be expected if it were cumulative. The only data on normal subjects using the Cockcroft technique with methacholine which answered our preset criteria were from the study of ENARSON *et al.* [12], but this was in adults aged 39–45 yrs. Even so, using these data instead of those of BACKER *et al.* [18] in the regression in figure 3 yielded a value for the mean PD20 of the normal population of 119 µmol and hence an optimal balanced PD20 only marginally above 5.5 µmol. It is felt that these data support the assumptions made and that the optimal balanced PD20 lies between 5.5 and 5.9 µmol, whereas the optimal (greatest combination) PD20 lies between 6.6 and 7.2 µmol. As far as the back conversion of an end-point dose (PD20) to an end-point concentration (PC20) is concerned, this would appear to be possible for methacholine but probably not for histamine using the Cockcroft method. From the present results, the methacholine concentration for the optimal balanced PC20 is ~2.8 mg·mL^{-1} and that for the optimal (greatest combination) PC20 ~3.3 mg·mL^{-1} (provided the nebulizer output is ~0.34 mL·min^{-1}).

A problem with all studies of bronchial reactivity by inhalation is how to handle the data on those who fail to develop a 20% fall in FEV1 even at the highest dose of agent administered. O′CONNOR *et al.* [30] calculated the dose/response slope (DRS) and showed that it was useful in differentiating between different reactivities when they compared nine asthmatic with 10 normal children. Although the DRS method extends the range of usable data somewhat in subjects with low reactivity, it does not help as far as those with no response or even a rise in FEV1 up to the maximum dose administered are concerned. In such subjects, the DRS is either zero or negative and cannot be transformed logarithmically. PEAT *et al.* [24] arbitrarily added 3.0 to the DRS in such cases, but this is exactly equivalent to arbitrarily saying that the PD20 was 6.7 µmol in all subjects in whom the uncorrected DRS was zero. Using the present approach, arbitrary values are not assigned to censored data and the calculations are based on the proportion of the population achieving a fall in FEV1 of 20% in response to the inhalation. From these data, the population curve was derived on the assumption that bronchial reactivity to inhalation is log-normally distributed. This assumption seems reasonable within the limitations of the inhalation methods [11, 24], and certainly approximates closely to values obtained for bronchial reactivity to exercise, for which there is no problem regarding censored values [14, 31].

It has been common practice in epidemiological studies to define bronchial hyperreactivity in terms of an arbitrary cut-off point such as a ΔFEV1 of 10 or 15%, a PD20 of 7.8 µmol or a PC20 of 8 mg·mL^{-1} [8, 9, 15, 18]. Looking at figures 1 and 4, it is obvious that choosing an arbitrary cut-off is unreliable since the proportion of healthy and asthmatic responders is critically dependent upon the cut-off chosen. However, if it is important to increase sensitivity at the expense of specificity or vice versa then a suitable cut-off point can be chosen from figure 1 or 4, and the effects on sensitivity and specificity predicted.

For example, in a clinical situation, if it is important to avoid misclassifying a normal child as having an asthmatic response then a low false positive rate may be accepted at the expense of a lower sensitivity. By increasing the cut-off point of ΔFEV_1 from 9% (optimal balanced cut-off) to 13% (optimal greatest combination cut-off) the sensitivity (power) of the challenge falls from 75% to 63%, but the chance of misclassifying a normal child falls from 25% to 6%.

These optimal cut-off values are of course dependent upon the results of the challenges in the population of asthmatics studied in the authors' laboratory and these may not represent the true spectrum of asthma in the community since they were referred to a specialist paediatric pulmonary clinic for investigation. The population of asthmatics comprised 52% with mild asthma, 28% with moderate asthma and 20% with severe asthma, as defined above. In a total population survey of >35,000 17-yr-old army recruits in Israel [25], it was noted that, of the asthmatics in the population, there were 62% with mild, 37% with moderate and 1% with severe asthma, defined in similar terms to those used in the current study. Thus the present hospital-based population contained a significantly greater proportion of severe asthmatics than did the general community ($p<0.0001$). Therefore, the optimal balanced cut-off points for exercise and inhalation challenges were calculated for each asthma severity grade and it was found that there were small differences between the groups (tables 6 and 7). However, in absolute terms, the variation in sensitivity, specificity and cut-off between the groups was trivial for exercise and within one doubling dose for PD_{20}. Thus, the composition of the present population of asthmatics is unlikely to have materially biased the result. The small effect of asthma severity on the optimal cut-off points also makes it very unlikely that national differences could have biased the results.

The usefulness of a test is sometimes evaluated by its positive predictive value (PPV), *i.e.* the percentage of positive tests occurring in subjects with the disease in question. In the present study, it was assumed that 10% of the population were asthmatics. For the optimal 13% ΔFEV_1 cut-off, this would yield a PPV for exercise of 55% but, if the balanced sensitivity and specificity cut-off of 9% were used, the PPV for exercise would be only 25%. In the study of BACKER *et al.* [9] using exercise, the PPV was also 25%. The PPV for inhalation in the present study using the optimal (greatest combination) PD_{20} would be approximately 53% and in published studies using inhalation the PPV ranges 20–60% depending upon the cut-off chosen [12, 13]. If the proportion of asthmatics in the population were greater then the PPV would also be greater since more of the responsive subjects would be true asthmatics. Similar considerations affect the overall shape of the distribution of bronchial reactivity in the total population, which has been described as unimodal [11]. However, the relatively small number of asthmatics in a total population survey means that it may be very difficult to visualize the distribution as bimodal, as shown in figures 1 and 4.

The implications of a challenge indicating bronchial reactivity above or below the cut-off values depends upon the purpose of the challenge. If the child is randomly selected from the general population for the purpose of some type of epidemiological survey then, to the extent that the overall incidence of asthma in the community is 10%, a positive inhalation challenge means that the child has an ~50:50 chance (PPV) of being asthmatic. However, the present study was based on truly symptomatic asthmatics and totally healthy children. Thus, if the challenge is carried out on a patient with symptoms which suggest asthma, a positive or negative result for an inhalation challenge has an ~90% chance of supporting or refuting the diagnosis.

In conclusion, studies of normal children and adolescents published in the literature provide enough data to enable the construction of a theoretical population distribution of the bronchial reactivity of normal subjects to exercise and the inhalation of histamine or methacholine. When these results are compared with the results from a large number of asthmatics of similar age, it is possible to define the optimal cut-off points for both exercise (fall in forced expiratory volume in one second of 13%) and inhalation challenges (provocative dose of agent (histamine or methacholine) causing a 20% fall in forced expiratory volume in one second between 6.6 and 7.2 µmol), which yield the best combination of sensitivity and specificity or answer specific questions. It is these cut-off points rather than arbitrarily chosen values that should be used in judging the results of a bronchial provocation test.

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