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Spirometry and respiratory oscillometry: Feasibility and concordance in schoolchildren with asthma

Clara Domínguez-Martín BSN, MSN[1,2] | Alfredo Cano MD, PhD[1,2] | Nuria Díez-Monge MD, PhD[1,2] | Ana María Alonso-Rubio MD, PhD[2,3] | Isabel Pérez-García MD[2,3] | María Teresa Arroyo-Romo MD[3] | Irene Casares-Alonso MD, PhD[3] | Ana María Barbero-Rodríguez MD[2,3] | Reyes Grande-Alvarez MD[3] | María Teresa Martínez-Rivera MD[3] | Mónica Sanz-Fernández MD[3]

[1]Department of Pediatrics, Río Hortega University Hospital, Valladolid, Spain

[2]School of Medicine, University of Valladolid, Valladolid, Spain

[3]Primary Healh Care, West Valladolid Health Area, Valladolid, Spain

Correspondence
Alfredo Cano, MD, PhD, Hospital Universitario Río Hortega, Dulzaina 2, Valladolid 47012, Spain.
Email: alfredo.cano@uva.es

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Abstract

Objective: The purpose of this study was to describe the feasibility of respiratory oscillometry (RO) in schoolchildren with asthma, and the concordance of its results with those of spirometry, to determine its clinical usefulness.

Methods: RO and spirometry were performed in 154 children (6 to 14-year-old) with asthma, following strict quality criteria for the tests. Their feasibility (probability of valid test, time of execution, number of maneuvers needed to achieve a valid test, and perceived difficulty) was compared. The factors that influence feasibility were analyzed with multivariate methods. FEV1, FEV1/FVC, FVC and FEF25-75 for spirometry, and R5, AX and R5-19 for RO, were converted into *z*-scores and their concordance was investigated through intraclass correlation coefficients (ICC) and *kappa* indices for normal/abnormal values.

Results: There were no differences in the probability of obtaining a valid RO or spirometry (83.1% vs. 81.8%, $p = 0.868$). RO required a lower number of maneuvers [mean (SD) 4.2 (1.8) versus 6.0 (1.6), $p < 0.001$] and less execution time [5.1 (2.7) versus 7.6 (2.4) minutes, $p < 0.001$], and patients considered it less difficult. Age increased the probability of obtaining valid RO and spirometry. The concordance of results between RO and spirometry was low, and only between zFEV1 and zAX could it be considered moderate (ICC = 0.412, *kappa* = 0.427).

Conclusion: RO and spirometry are feasible in children with asthma. RO has some practical advantages, but the concordance of its results with spirometry is low.

KEYWORDS

asthma, child, cross-sectional studies, oscillometry, spirometry

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1 | INTRODUCTION

Spirometry is the most important lung function test in both adults and children, with a wide trajectory of use in the clinical setting,[1] and can be performed in children in Primary Care.[2] The recent guidelines of the European Respiratory Society (ERS) for the diagnosis of asthma in children[3] recommends against diagnosis based only on symptoms or response to treatment, and favors diagnosis supported by lung function tests (spirometry).

Other techniques for studying lung function in asthma have been incorporated into the clinical setting, as commercial equipment has become available for their execution. These include the growing use of respiratory oscillometry (RO), a technique that requires little cooperation from the patient[4] and use of which has been investigated not only in asthma, but also in neuromuscular diseases,[5] cystic fibrosis,[6] postinfectious bronchiolitis obliterans,[7] vocal cord dysfunction[8] or chronic lung disease of prematurity.[9]

RO is especially useful for studying changes in the small airways (SA).[10] In asthma, the involvement of the SA has clinical, therapeutic, and prognostic significance,[11] and therefore RO may be especially useful in the diagnosis and follow-up of children with asthma.[12]

However, the real usefulness of RO has not yet been established, and its use is not included in the recommendations of the current asthma clinical guidelines. Studies having evaluated the diagnostic performance of RO are few and small, and it is not clear what RO can contribute with respect to conventional methods of studying lung function.[13] A key question to decide whether RO can replace or supplement spirometry in the diagnosis of asthma or other diseases is knowing its degree of agreement in the normal/abnormal classification of the results obtained. Another important aspect for clarifying its position in the clinical setting is the feasibility of the test, that is, questions like the probability of obtaining a valid test, the execution time necessary or the difficulty perceived by the patient.

This study aims to determine the feasibility of RO in schoolchildren with asthma, compare it to that of spirometry, and measure the concordance of the results of both techniques.

2 | METHODS

2.1 | Sample

For this cross-sectional study, a convenience sample was recruited between October 2021 and April 2022 at primary care offices and at the pediatric pulmonology office of a tertiary hospital. It included children 6–14 years of age with a clinical diagnosis of asthma (typical episodic symptoms, reversible with antiasthma treatment, and with no symptoms, signs, or clinical history suggestive of other diagnoses), and who had experienced symptoms or received treatment for asthma in the previous 12 months. Patients from any spectrum of severity were included. Children with contraindications to spirometry were excluded.[14]

Through interviews with main caregivers and review of clinical records, data were collected on hospitalizations, asthma exacerbations with use of oral corticosteroids, unscheduled visits to Primary Care or the Emergency Room, recent use of rescue bronchodilators and level of treatment.[15]

2.2 | Measurements

Participants were instructed to avoid the use of any asthma medication for 18 h before undergoing the lung function studies, which were done between 4:00 p.m. and 8:00 p.m., and always in the same order (RO before spirometry).[16] All the tests were executed over the course of 7 consecutive months and by the same nurse. High-efficiency viral-bacterial filters were always used, and the devices were calibrated daily before each work session.

RO was done using a Tremoflo C-100 system (Thorasys Thoracic Medical Systems), following the procedures proposed by the ERS[16]; it was performed in a seated position, with a nose clip and manual holding of the cheeks. The system applies nonharmonic oscillation frequencies ("pseudo-random noise") in the range 5–37 Hz, superimposed on the pressure waves generated during breathing at tidal volume. Simultaneous changes in pressure, flow and volume over 20 s were recorded at each maneuver. At least three consecutive maneuvers were repeated, and each maneuver was considered acceptable if ≥16 s of the recording time was free of artifacts (swallowing, obstruction of the mouthpiece with the tongue, air leak or cough). If any of them was not acceptable, or if the coefficient of variation (CoV) of resistance at 5 Hz (R5) between the measures obtained was >15%, the maneuvers were repeated in search of a valid test, defined as three acceptable maneuvers with CoV-R5 ≤ 15%. The number of consecutive maneuvers was limited to 8, the same as recommended in spirometry. For the analysis, the mean of the values obtained from R5, the difference between the resistance at 5 and 19 Hz (R5-19) and the area under the reactance curve (AX), expressed as *Z*-scores or standard deviations (SD) were used, according to the reference equations of Ducharme et al.[17] Values were considered abnormal when the *Z*-score was >1.645 SD.[18]

The spirometry was performed with a Fleisch-type spirometer Pneumotrac with Spirotrac-5 software (Vitalograph Ltd), according to the recommendations of the American Thoracic Society (ATS) and the ERS,[14] in a seated position and with nasal clip. Up to eight consecutive maneuvers were performed, looking to obtain three acceptable maneuvers that met the ATS/ERS reproducibility criteria ("A" quality). "A" or "B" quality tests were considered valid, that is, when ≥2 acceptable maneuvers with a difference ≤0.15 L for both FEV1 and FVC were achieved.[14] The variables collected were the forced expiratory volume in the first second (FEV1), the forced expiratory volume (FVC), the ratio FEV1/FVC, and the forced expiratory flow between 25%–75% of the FVC (FEF25-75). The results were expressed as *Z*-scores according to the Global Lung

Initiative (GLI) reference equations.[19] Values were considered abnormal when the *Z*-score was <-1.645 SD.[18]

The degree of asthma control was measured using the validated CAN questionnaire[20] (caregiver version). The CAN evaluates asthma control in the 4 previous weeks using a 9-item Likert scale. Responses are scored 0 to 4 (total questionnaire score 0 to 36), a lower score corresponding to better control. A CAN ≥ 8 identifies poorly controlled asthma.

The feasibility of RO and spirometry was evaluated with these variables:

1. Proportion of children who achieved valid tests, as defined above.
2. Number of maneuvers necessary to achieve a valid test.
3. Time used to achieve a valid test, including the initial explanation of the procedure.
4. Difficulty of the test, assessed by the children on a numeric scale from 0 (very easy) to 10 (very hard).

2.3 | Analysis

The feasibility of the tests was studied by comparing the feasibility variables between RO and spirometry using McNemar or Student's *t*-tests for paired data, as appropriate.

Factors that might influence the feasibility were investigated through multiple logistic or linear regression models, including age, sex, previous experience in performing the test and degree of asthma control as explanatory variables, and likelihood of a valid test, execution time, number of maneuvers needed and difficulty score as dependent variables.

The concordance between the *z*-scores of the parameters of both tests was analyzed in three ways: (1) using two-way mixed, absolute agreement, single measure intraclass correlation coefficients (ICC); (2) the concordance of normal/abnormal results was analyzed using Cohen's κ coefficients; (3) agreement between some pairs of parameters were graphically analyzed through Bland–Altman plots. For a correct interpretation of ICC and Bland–Altman plots, an inversion of the signs of zR5, zAX, and zR5-10 (positive/negative) was made specifically for these analysis, as higher (worse) RO results should agree with lower spirometry results. κ coefficients and ICC were deemed moderate if >0.40, and strong if >0.75.

For a technical assessment, the correlation of CoV-R5 with age and with the number of RO maneuvers was analyzed through Pearson's correlation coefficients (r).

For all comparisons, an α error <0.05 was accepted as statistically significant.

2.4 | Sample size

Assuming that 10% of patients do not achieve valid tests, to make an estimate with a 95% confidence level and a precision of 5%, a sample of 139 patients would be necessary.

2.5 | Ethics

The study was approved by the Clinical Research Ethics Committee of the hospital. The children's parents received written information and signed a participation consent.

3 | RESULTS

One hundred sixty-four patients were reached, and 154 participated in the study. None had contraindications for spirometry. Table 1 shows clinical and demographic characteristics of the participants. Of them, 30.1% had poorly controlled asthma. Half of the patients had never had a spirometry, and none had previously had an RO. The most frequently abnormal parameters were FEF25-75 for spirometry and AX for RO.

Table 2 shows the analysis of the feasibility of spirometry and RO. There was no difference in the percentage of patients who achieved a valid test, but RO required a lower number of attempts, less execution time, and was evaluated as less difficult. There were 109 patients (70.8%)

TABLE 1 Clinical and demographic characteristics of study participants.

Age, years. Mean (SD)	9.9 (2.5)
Male sex, %	62.3
Any previous spirometry, %	49.4
Any previous RO, %	0.0
Hospitalization, any time, %	25.5
Hospitalization, previous 12 months, %	3.2
Hospitalization, previous 3 months, %	0.7
Exacerbation requiring oral corticosteroids, previous 3 months, %	14.1
Unscheduled consultation in Primary Care, previous 3 months, %	24.8
ER consultation, previous 3 months, %	11.7
Days with rescue bronchodilator use, previous 3 months. Mean (SD)	4.0 (9.4)
Current daily maintenance therapy, %	51.9
Uncontrolled asthma, %	30.1
Low FEV1, %	15.9
Low FVC, %	7.1
Low FEV1/FVC, %	24.6
Low FEF25-75, %	27.0
High R5, %	14.8
High AX, %	18.8
High R5-19, %	10.2

Abbreviations: ER, emergency room; RO, respiratory oscillometry; SD, standard deviation.

TABLE 2 Feasibility measures and comparison between spirometry and RO.

Variable	Spirometry	RO	*p* Value
Patients achieving a valid test, %	81.8	83.1	0.868[a]
Execution time, min. Mean (SD); range	7.6 (2.4); 3.3–14.1	5.1 (2.7); 2.0–16.3	<0.001[b]
Number of maneuvers until a valid test. Mean (SD)	6.0 (1.6)	4.2 (1.8)	<0.001[b]
Difficulty score. Mean (SD)	5.0 (2.8)	1.9 (2.1)	<0.001[b]

Abbreviations: RO, respiratory oscillomery; SD, standard deviation.

[a]McNemar test.

[b]Paired *T*-test.

TABLE 3 Multivariate models for the feasibility outcomes (RO).

	Likelihood of a valid test[a] OR (95% CI)	Execution time, min[b] Regression coefficient (95% CI)	Number of maneuvers[b] Regression coefficient (95% CI)	Difficulty score[b] Regression coefficient (95% CI)
Age (each year)	1.23 (1.01–1.50)	−0.22 (−0.41 to −0.03)	−0.12 (−0.25–0.00)	0.02 (−0.11–0.16)
	p = 0.038	*p* = 0.023	*p* = 0.058	*p* = 0.729
Male sex	0.54 (0.20–1.41)	−0.58 (−1.54–0.39)	−0.03 (−0.66–0.60)	−0.07 (−0.77–0.62)
	p = 0.205	*p* = 0.240	*p* = 0.918	*p* = 0.836
Uncontrolled asthma	1.53 (0.56–4.22)	−0.03 (−1.05–0.99)	0.07 (−0.59–0.74)	0.66 (−0.08–1.40)
	p = 0.407	*p* = 0.952	*p* = 0.826	*p* = 0.080

Note: Previous experience with oscillometry was not included, as any patient had previously performed RO.

Abbreviations: CI, confidence interval; OR, odds ratio; RO, respiratory oscillometry.

[a]Multivariate logistic regression.

[b]Multivariate linear regression.

TABLE 4 Multivariate models for the feasibility outcomes (spirometry).

	Likelihood of a valid test[a] OR (95% CI)	Execution time, min[b] Regression coefficient (95% CI)	Number of maneuvers[b] Regression coefficient (95% CI)	Difficulty score[b] Regression coefficient (95% CI)
Age (each year)	1.32 (1.07–1.62)	−0.04 (−0.21–0.13)	−0.02 (−0.13–0.09)	0.35 (0.14–0.55)
	p = 0.008	*p* = 0.635	*p* = 0.728	*p* = 0.001
Male sex	0.44 (0.17–1.17)	0.62 (−0.23–1.47)	0.42 (−0.15–0.99)	−0.59 (−1.58–0.40)
	p = 0.100	*p* = 0.150	*p* = 0.143	*p* = 0.242
Uncontrolled asthma	1.82 (0.66–5.05)	−0.11 (−1.00–0.78)	−0.19 (−0.79–0.41)	0.28 (−0.77–1.34)
	p = 0.251	*p* = 0.806	*p* = 0.526	*p* = 0.596
Previous spirometry	0.92 (0.38–2.23)	−0.96 (−1.80 to −0.11)	−0.81 (−1.37 to−0.24)	−0.68 (−1.66–0.31)
	p = 0.847	*p* = 0.026	*p* = 0.005	*p* = 0.178

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Multivariate logistic regression.

[b]Multivariate linear regression.

who achieved valid tests in both techniques. About 13.5% of patients with valid spirometry did not achieve valid RO, and about 14.8% of patients with valid RO did not achieve valid spirometry.

Of the valid spirometry tests, 97.6% were "A" quality, and the difference in FEV1 and FVC was ≤0.10 L in 89.7% and 87.3%, respectively. In the valid RO, the CoV-R5 had a mean of 6.8% (SD 3.3%), and in 84.4% of tests it was ≤10%. The CoV-R5 was not correlated with the patient's age ($r = -0.018$; $p = 0.839$) or with the number of maneuvers performed ($r = 0.098$; $p = 0.269$).

Tables 3 and 4 show the results of the multivariate models for the variables of feasibility of RO and spirometry, respectively. For both techniques, age increased the probability of obtaining a valid

TABLE 5 Concordance of RO and spirometry parameters and classification of results.

	zFEV1	zFEV1/FVC	zFVC	zFEF25-75
zR5	ICC = 0.221 (p = 0.007)	ICC = 0.178 (p = 0.024)	ICC = 0.104 (p = 0.142)	ICC = 0.196 (p = 0.008)
	K = 0.217 (p = 0.023)	K = 0.194 (p = 0.038)	K = 0.051 (p = 0.567)	K = 0.242 (p = 0.007)
zAX	ICC = 0.412 (p < 0.001)	ICC = 0.316 (p < 0.001)	ICC = 0.182 (p = 0.019)	ICC = 0.315 (p < 0.001)
	K = 0.427 (p < 0.001)	K = 0.261 (p = 0.006)	K = 0.261 (p = 0.003)	K = 0.348 (p < 0.001)
zR5-19	ICC = 0.380 (p < 0.001)	ICC = 0.368 (p < 0.001)	ICC = 0.118 (p = 0.109)	ICC = 0.272 (p < 0.001)
	K = 0.309 (p = 0.001)	K = 0.284 (p = 0.001)	K = −0.104 (p = 0.271)	K = 0.220 (p = 0.008)

Note: For a correct interpretation of ICC (agreement of higher RO results with lower spirometry results), an inversion of the signs of zR5, zAX and zR5-19 (positive/negative) was made.

Abbreviations: ICC, intraclass correlation coefficient; K, kappa coefficient for normal/abnormal values; RO, respiratory oscillometry.

test, and it also reduced the time necessary to achieve a valid RO. Likewise, prior experience reduced the number of maneuvers and the time necessary to obtain a valid spirometry.

Table 5 shows the analysis of concordance between the spirometry and RO parameters. zFEV1, zFEV1/FVC, and zFEF25-75 had statistically significant ICC and kappa with all RO parameters, but zFVC only had significant ICC and kappa with zAX. However, the only correlation with ICC > 0.4 was between zFEV1 and zAX. In respect to the concordance of normal/abnormal results, the only κ coefficient >0.4 was also between zFEV1 and zAX. The agreement between normal/abnormal results of zFEV1 and zAX is graphically represented in Figure 1A.

Figure 1B shows the Bland-Altman plot of the agreement between zFEV1 and zAX. zAX was usually less than zFEV1, at a similar magnitude throughout the range of results.

4 | DISCUSSION

4.1 | Findings

In schoolchildren with asthma, RO and spirometry are feasible tests, and most patients achieve valid tests with a brief execution time. Execution time, the number of maneuvers and perceived difficulty are lower with RO, but a valid test is achieved with the same frequency in both techniques. Age and prior experience seem to facilitate obtaining a valid test. RO and spirometry results have a significant, but low-moderate correlation, and the concordance of normal/abnormal results is generally weak.

4.2 | Interpretation

There are still several aspects related to the execution and interpretation of RO that are not adequately resolved. There are two different procedures for generating pressure waves in RO: with square wave pulses with harmonic frequencies (IOS systems) or with nonharmonic sine waves ("pseudo-random noise," sometimes called AOS).[16] Some commercial equipment (IOS or AOS) can give inexact measurements, with significant errors.[21] Moreover, in adults, higher resistance and lower reactance values are obtained with IOS than with AOS, and the difference depends on the type of airway disease.[22] As a result, it is unclear whether the reference values should be specific for different RO systems or even for each commercial equipment, or if they should be different for different ethnic groups. Most of the reference values published to date, in children and adults, have been obtained with IOS systems, and are rather conflicting.[23] One study in children compared different reference values obtained by IOS to identify poorly controlled asthma,[24] and concluded that the most suitable for it would be the Mexican ones of Gochicoa-Rangel.[25] But the same authors also found a good concordance in normal/abnormal classification of RO results using various reference values, and regardless of the ethnic origin of the population from which those values were obtained.[26] We have selected reference values obtained with the same equipment that we used (Tremoflo C-100), from a western population of mixed ethnic origin.[17] Only one other prior study has provided reference values for children obtained with the same equipment, in a Middle Eastern population.[27] The problems of selecting a suitable reference standard become clear when we consider that two different types of commercial AOS equipment, used in children from the same population, produce results that are sufficiently different as to create their own reference values for each type of equipment.[17]

Validity criteria for RO are poorly defined. We have used a limit of ≤15% of CoV-R5 to determine the validity, which is the limit recommended for children by the ERS.[16] In adults, the ERS recommends a limit of ≤10%; however, it also recognizes that there is no evidence supporting those cut-off points and does not identify the age at which a patient should be considered an adult. Recently, a limit of 10% in adults has been supported as a quality criterion for RO,[28] but there have been no developments in this regard for children. In our sample, CoV-R5 was usually less than 10% and did not vary with age, so it is possible that the CoV-R5 limit may be reduced to 10% for children >5 years as well. Other measurements obtained with RO are much more variable than R5,[29] and no test validity criteria have been formulated based on them.

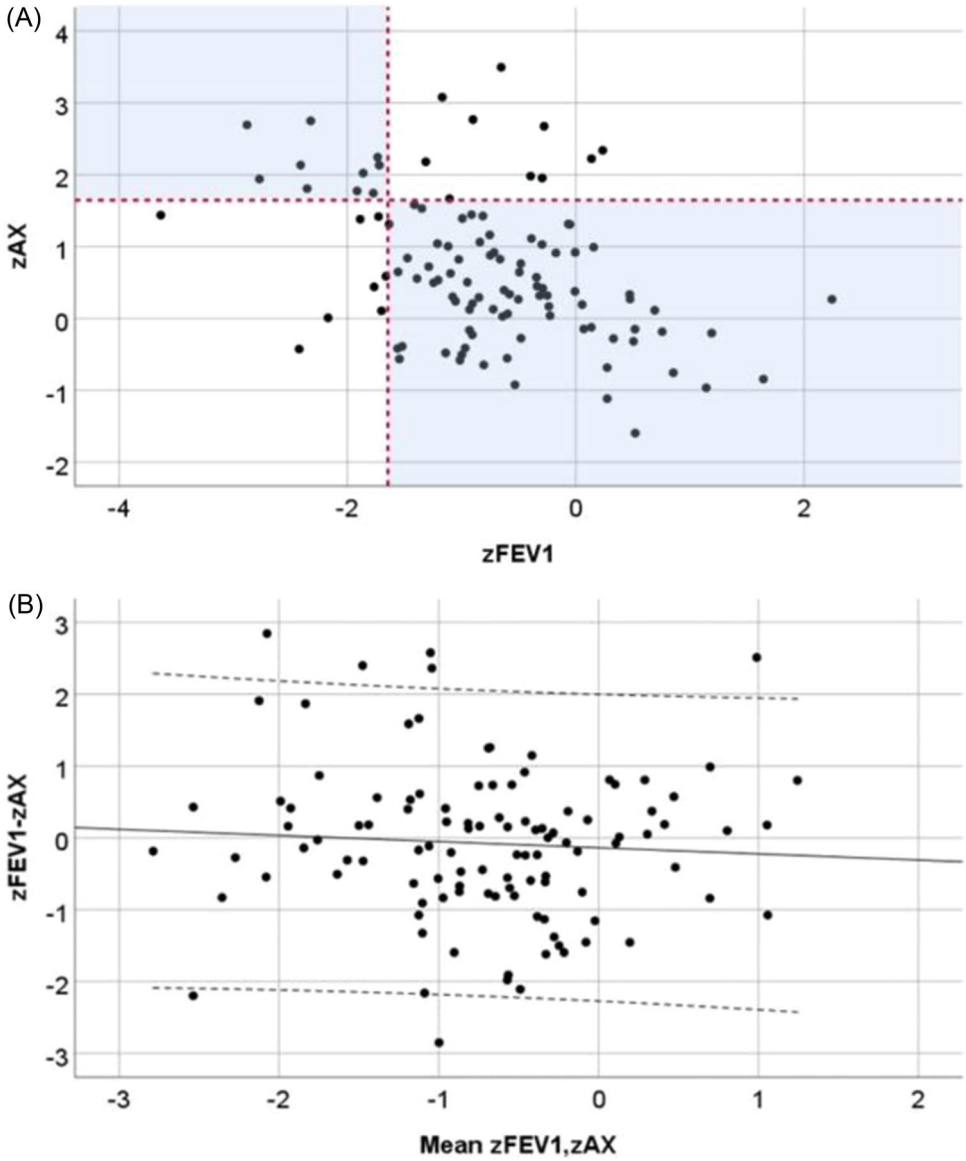

FIGURE 1 Graphic representation of the concordance between zFEV1 and zAX. (A) Scatter plot of zFEV1 and zAX, showing the limits of normal values (dashed lines). Dark areas represent concordance in normal/abnormal results. (B) Bland–Altman plot showing the (continuous) regression line between mean zFEV1, zAX and difference zFEV1-zAX, and their 95% confidence interval (dashed) lines. For a correct interpretation (agreement of higher AX results with lower FEV1 results), an inversion of the sign of zAX (positive/negative) was made. [Color figure can be viewed at wileyonlinelibrary.com]

Likewise, the number of maneuvers necessary to obtain a valid RO in children has not been well studied. In a small study (20 children 4–18 years of age with an asthma attack), which considered a recording time of only 13 s acceptable, 75% of children achieved a valid test (3 maneuvers with CoV-R7 < 15%) after performing a mean of 3.9 (SD 0.8) maneuvers.[30] This is a figure only slightly lower than what we obtained (Table 2). In adults, it has been observed that the repetition of maneuvers beyond 3 does not manage to improve CoV-R5,[28] and we found no correlation between the number of maneuvers and CoV-R5.

As for the feasibility of RO and spirometry, there are two studies that can be directly compared with our own. Gunawardana et al,[31] in a small sample (47 patients), clinically similar to ours (children 5 to 16-year-old with wheezing or asthma) and using the same RO commercial equipment, found a much greater difference (98% vs. 68%) in the probability of obtaining valid RO and spirometry, and also a somewhat greater correlation between RO and oscillometry results (but linear correlation, not ICC). Like us, they found that age was associated with a greater probability of achieving valid spirometry. Other feasibility aspects were not investigated in that study. Lundberg et al.[32] in 6-year-old children (88 born at <27 weeks gestational age and 84 healthy controls) also found a greater probability of obtaining a valid RO than a valid spirometry (93% vs. 60%). They used an IOS system, and as criterion for RO validity they only mention a criterion of coherence, which the most recent

recommendations do not consider suitable.[16] In spirometry, they measured FEV0.75 rather than FEV1, requiring reproducibility (not quantified) of FEV0.75 and FVC. They found correlations (not ICC) between the RO and spirometry results somewhat greater than ours, and they also identified AX as the only RO parameter with a significant correlation with FVC. They used GLI reference values for spirometry and Gochicoa-Rangel (IOS) for RO, and they provided sufficient data to calculate the concordance in normal/abnormal results, which is similar (κ between 0.10 and 0.26) to what we found.

The greater probability of achieving a valid spirometry in our patients, compared to the studies by Gunawardana and Lundberg, could be explained by the difference in age of participants. On the other hand, the results of these two studies and our own contrast with a large study in preschool children (3 to 5-year-old) that, unexpectedly, found a greater probability of obtaining a valid spirometry than a valid RO, even though the spirometry only attempted to obtain FEV0.5 and current ATS/ERS quality criteria were not applied.[33]

4.3 | Limitations

We have used a convenience sample, that could potentially not be representative of the population of children with asthma. However, our patients came from both primary and specialized health care, so they are not a sample biased towards the most severe disease.

The lower age limit of our study (6 years) prevented us from drawing conclusions applicable to preschoolers, a population in which the difficulty of performing spirometry is greater, and in which the use of RO may therefore be of greater benefit. However, greater feasibility of RO in preschoolers has not been demonstrated,[33] and that aspect should be studied further.

The tests were always performed by the same nurse, so we cannot know the effect that variability between technicians with different experience levels could generate.

We have included patients with a clinical diagnosis of asthma, without requiring the stricter criteria currently required. However, this should not affect the feasibility results nor the concordance of results.

We have used the same statistical criterion of normality for both tests, which is the one proposed by the ERS/ATS.[18] Although this criterion has statistical sense, it is not known whether it has clinical meaning with regard to RO.

About the reproducibility of spirometry maneuvers, we have used the current ATS/ERS recommendations. Stricter criteria have recently been proposed,[34] but most of our patients would have met them. If these criteria are included in future standardizations of spirometry, the number of valid spirometry tests could be lower, or the number of maneuvers to achieve a valid spirometry could be higher.

4.4 | Conclusions

In schoolchildren with asthma, valid RO results can be obtained with the same frequency as with spirometry, in less time and with a lower number of maneuvers. However, the concordance of results between RO and spirometry is moderate-to-low, so it is necessary to expand knowledge of the clinical usefulness of RO before proposing it as a general alternative to spirometry in this age group.

AUTHOR CONTRIBUTIONS

Clara Domínguez-Martín: Conceptualization; data gathering; statistical analysis; writing; review and editing. **Alfredo Cano**: conceptualization; data gathering; statistical analysis; writing; review and editing. **Nuria Díez-Monge**: Conceptualization; data gathering; writing; review and editing. **Ana María Alonso-Rubio**: Conceptualization; data gathering; and review. **Isabel Pérez-García**: Conceptualization; data gathering; and review. María Teresa **Arroyo-Romo**: Conceptualization; data gathering; and review. **Irene Casares-Alonso**: Conceptualization; data gathering; and review. **Ana María Barbero-Rodríguez**: Conceptualization; data gathering; and review. **Reyes Grande-Alvarez**: Conceptualization; data gathering; and review. **María Teresa Martínez-Rivera**: Conceptualization; data gathering; and review. **Mónica Sanz-Fernández**: Conceptualization; data gathering; and review.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Clara Domínguez-Martín http://orcid.org/0000-0001-7794-4104
Alfredo Cano http://orcid.org/0000-0001-9831-4136
Nuria Díez-Monge http://orcid.org/0000-0002-3934-819X
Ana María Alonso-Rubio http://orcid.org/0000-0003-1615-4330
Irene Casares-Alonso http://orcid.org/0000-0003-3032-1297
Mónica Sanz-Fernández http://orcid.org/0000-0002-8466-4685

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