Investigation of Primary Ciliary Dyskinesia in Children with Bronchiectasis in Iran

sohila alyasin¹, Behjat Maneshian^{2,*} and Shadi Niliyeh²

¹Allergy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

²Department of Pediatrics, Namazi Teaching Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

*Corresponding author: Department of Pediatrics, Namazi Teaching Hospital, Shiraz University of Medical Sciences, Shiraz, Iran. Email: maneshianb@gmail.com

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Abstract

Background: Primary ciliary dyskinesia (PCD) is a rare genetic disorder with signs and symptoms of recurrent chronic sinusitis, otitis media, pneumonia, bronchiectasis, male infertility, and situs inversus. The diagnosis of PCD has always been one of the challenging issues that is mostly made through screening tests. These include the saccharin test and measurements of exhaled and nasal nitric oxide (NO) level, transmission electron microscopy (TEM) for evaluating ultrastructure of the cilia, high-speed video microscopy for evaluating ciliary beat patterns, immunofluorescent staining of the cilia in the biopsies, and genetic studies. As there had not been any epidemiological studies in Iran to detect the prevalence of PCD in the general population, the current research has been undertaken for the first time using screening tests of saccharin and measurement of the level of exhaled NO (fractional exhaled NO) to investigate the prevalence of PCD.

Objectives: Primary ciliary dyskinesia (PCD) is a rare genetic disorder with the basis of an abnormal ciliary movement that causes chronic respiratory infections, bronchiectasis, infertility in males, and situs inversus. The significance of earlier diagnosis is for better care and prevention of complications. In this regard, we studied the PCD in children with bronchiectasis by saccharin test and measurement of exhaled nitric oxide.

Methods: In this cross-sectional study, 31 patients with a definite diagnosis of bronchiectasis were evaluated regarding nitric oxide exhalatory measurement (FeNO) and a saccharin test for the confirmation of PCD diagnosis. The cut-off point of 20 ppb was considered as the normal level for FeNO test and the sensation of fewer than 60 minutes for the normal range of the saccharin test. Age, gender, and cardioposition were recorded for the patients.

Results: Unlike the saccharine test, the measurement of exhaled nitric oxide had a high sensitivity (90.3% versus 54.8%) for the diagnosis of PCD. Cardioposition and gender did not have significant effects on the outcomes of exhaled NO and saccharin test (P -value > 0.05). Besides, the patients' age did not affect FeNO measurement but was significantly higher among those with abnormal saccharin test (P -value = 0.028).

Conclusions: The FeNO test had a remarkable sensitivity of 90.3% for the diagnosis of PCD, and its outcomes were not affected by age, gender, and cardioposition. The saccharin test had a sensitivity of 54.8% and was influenced by age, while not by gender or cardioposition. Although there are more accurate tests for diagnosis of PCD such as TEM and genetic studies, we decided to investigate PCD in children with bronchiectasis by performing two screening tests, NO and saccharin, because of several issues.

Keywords: Saccharine Test, Primary Ciliary Dyskinesia, Exhaled Nitric Oxide Test, Bronchiectasis

1. Background

Primary ciliary dyskinesia (PCD) is a rare genetic disorder that mostly has an autosomal recessive pattern of inheritance (1). Previous studies have declared a variety of prevalences from 1 per 30000 persons to the prevalence of 1 per 15000 in different communities (2-4).

Abnormal ciliary movements in a variety of organs are the principal basis of this disorder, among which the respiratory tract is the most important one. This is because of the critical rule of coordinated ciliary beats, known as

mucociliary clearance leading to a mucosal defense of the respiratory tract. Thus, any impairment in mucociliary clearance can cause recurrent chronic sinusitis, otitis media, pneumonia, and bronchiectasis. As mentioned above, other manifestations of this disorder in other organs include male infertility and situs inversus (Kartagener's syndrome), the results of the abnormal and ineffective movement of spermatozoa and abnormal ciliary movements in the embryonic era, respectively (3, 5).

The diagnosis of PCD has always been one of the chal-

lenging issues in pediatric sciences that is mostly made through screening tests. These tests include the saccharin test and measurements of exhaled and nasal nitric oxide (NO) level, transmission electron microscopy (TEM) for evaluating ultrastructure of the cilia, high speed video microscopy for evaluating ciliary beat patterns, immunofluorescent staining of the cilia in the biopsies, and genetic studies (6).

The saccharin test is an easy, simple, useful, accessible, and noninvasive screening test that can be used not only for screening of PCD but also for following the children up during treatment courses (7).

The next screening test is the measurement of exhaled NO level as an inflammatory mediator produced by the lungs of human beings and animals (8, 9). The pathophysiologic role of this compound has been well-documented in asthma as a chronic pulmonary disease, while information about it in PCD is still a question (10). Previous studies (11, 12) have shown a considerable change in exhaled NO levels in chronic pulmonary diseases such as PCD, cystic fibrosis (CF), and allergic rhinitis (AR); therefore, this means that it has been recommended as another screening test of the above-mentioned disorders. Official American Thoracic Society Clinical Practice Guidelines have declared the exhaled NO level of less than 25 ppb among adults and less than 20 ppb in children as a cut-off in 2011 (10).

Based on our research, no epidemiological study has been previously conducted in Iran to detect the prevalence of PCD in the general population. As mentioned before, the diagnosis of PCD has mostly been made through screening tests, including saccharin test and measurements of exhaled and nasal NO level, TEM, high-speed video microscopy, and genetic studies (6). Although TEM, high-speed video microscopy, and genetic studies are more accurate for the diagnosis of PCD, we encountered certain problems using them. Based on our research from several centers in Iran, there was a lack of equipment for TEM and high-speed video microscopy, lack of experienced personnel for diagnosis of PCD by TEM and high-speed video microscopy, high cost for importing equipment, and high cost and unavailability of genetic studies. On the other hand, it is worth considering that although bronchiectasis in children is not rare, there is not any available screening as well as more accurate diagnostic tests for these patients in Iran.

2. Objectives

Thus, the current research has been undertaken using screening tests of saccharin and measurement of the level of exhaled NO (FeNO, fractional exhaled NO) to investigate the prevalence of PCD and providing the ground for

making two screening tests available, expanding studies on PCD by performing more accurate tests, and convincing the authorities to allocate funds for the diagnosis of PCD.

3. Methods

3.1. Sampling

As no previous studies in the community of Iran had investigated the prevalence of PCD, all the patients who met the criteria for the diagnosis of bronchiectasis and were referred to the Asthma, Allergy, and Immunology department of the Emam Reza Clinic of Shiraz were assessed through simple sampling.

All 7-18-year-old children who had bronchiectasis and met one of the following criteria were included:

- 1) Complete or incomplete situs inversus or
- 2) Presence of a first-degree family member with a documented diagnosis of PCD (3, 13).

The excluding criteria were:

- 1) Documented diagnosis of cystic fibrosis achieved through sweat chloride assessment,
- 2) Diagnosis of bronchiolitis obliterans detected through CT scan,
- 3) Documented diagnosis of immune deficiency through laboratory tests, and
- 4) Documented diagnosis of tuberculosis by positive tests.

Following the study protocol approved by the Ethics Committee of Shiraz University of Medical Sciences (code: IR.sums.med.rec.1395.s208), the entire study procedure was explained for our participants, and they were requested to present their written consent form of participation in the study.

3.2. Saccharin Test

To perform the saccharin test, a small particle of saccharin, 1 millimeter in diameter, was placed directly on the nasal mucosa at the inferior turbinate, with a 1-centimeter of distance from the anterior nasal passage. Patients were requested not to sneeze, cough, or sniff as much as possible during the test. Then, the time for a child to feel the taste of saccharin was recorded in minutes.

In cases who did not feel the saccharin taste, their ability of taste sensation was evaluated by placing the particle of saccharin on their tongue (13) to make sure that they could feel the sweet taste of saccharin through the sense of taste.

In patients with severe rhinorrhea or nasal congestion, the saccharin test was performed following the improvement of their symptoms.

3.3. Measurement of Level of Exhaled NO

For measuring the level of exhaled NO (FeNO), a NO-breath device from Bedfont Scientific Ltd in the United Kingdom was prepared. According to the device protocol, following a tight connection of a new mouthpiece to the NObreathFlo, it was connected to the device. Then, the child was asked to exhale after regular inspiration through the mouthpiece for 10 seconds. After a minute, the exhaled NO was shown on the monitor of the device. Due to the Official American Thoracic Society Clinical Practice Guidelines in 2011, the low level of exhaled NO is recommended to be less than 25 ppb in adults and less than 20 ppb in children (10). Thus, 20 ppb was considered as a cut-off level.

3.4. Statistical Analysis

The collected data, including age, gender, the time of sweet taste of saccharin sensation, the level of exhaled NO, and the presence or the absence of dextrocardia, were entered into SPSS version-21 (IBM; the United States). Descriptive data were presented in mean and percentages while Chi-square test and Mann-Whitney U test were utilized for inferential analysis. A P-value of less than 0.05 was considered as a significant level.

4. Results

In this study, we evaluated the results of two screening tests of saccharin and measurement of exhaled NO test for diagnosis of PCD in children with bronchiectasis who were referred to the Asthma, Allergy, and Immunology department of Emam Reza Clinic in Shiraz. By simple sampling, the total numbers of 31 patients with a most probable diagnosis of PCD were included.

The analysis consisted of two parts: descriptive and inferential. In the descriptive section, the mean, standard deviation, frequency, percentage, and minimum and maximum numbers were explained. In the inferential part, the chi-square test was used to examine the relationship between the qualitative factors. To compare the mean of the quantitative factor among other factor classes, the Mann-Whitney U-test and independent t-test were used. The significance level was considered 0.05 at all of them.

Of the total number of 31 patients, 28 (90.3%) had the FeNO measurements of less than 20 ppb, while only 17 (54.8%) presented no sensation of the saccharin within 60 minutes that was in favor of PCD. These findings were 92.3% and 53.8% for those patients who probably had Kartagener's syndrome (dextrocardia and bronchiectasis) for FeNO measurements and saccharin test, respectively. Comparison of FeNO measurements in favor of PCD with

those against this diagnosis showed a significant difference (90.3% vs. 9.7%; P-value < 0.001) while saccharin test showed no statistical differences (54.8% vs. 45.2%; P-value = 0.59, Table 1).

The gender distribution of tests in favor of PCD, neither in the saccharin test (P-value > 0.99) nor in FeNO measurements (P-value = 0.87) presented statistical differences (Table 2), while positive saccharin tests were significantly affected with age (P-value = 0.028), but the FeNO tests were not affected (P-value = 0.864, Table 3).

The presence of dextrocardia, as the determinant of the Kartagener's syndrome, and the results of the FeNO measurements and saccharin test have been presented in Table 4. Based on this table, the cardioposition did not statistically affect the outcomes of FeNO and saccharin tests (P-value > 0.05).

The average of FeNO measurements among those with probable Kartagener's syndrome was 9.5 ± 4.96 ppb (range: 1 - 17) and for those with mere bronchiectasis was 5.75 ± 3.04 ppb (range: 2 - 12) with statistically higher amounts among those with concurrent bronchiectasis and dextrocardia (P-value = 0.02).

5. Discussion

The diagnosis of PCD has always been one of the challenging issues in pediatric sciences. Although TEM, high speed video microscopy, and genetic studies are more accurate for diagnosis of PCD, there had been several problems such as lack of equipment, lack of experienced personnel, high cost for importing equipment, and high cost and unavailability of genetic studies. On the other hand, it is worth considering that although bronchiectasis in children is not rare, there is not any available screening or more accurate diagnostic tests for these patients in Iran. Therefore, we decided to investigate PCD in children with bronchiectasis by performing two screening tests, NO and saccharin.

In the current study, 31 patients with the diagnosis of bronchiectasis were assessed regarding the presence of PCD. Therefore, two tests of FeNO measurement and saccharin test were performed. The findings of this study represented that the FeNO measurements in favor of PCD were found in 92.3% of the patients, while only 54.8% of saccharin tests revealed results consistent with the diagnosis of PCD. In other words, the sensitivity of FeNO measurement was remarkably higher in diagnosing PCD as compared to the other routine test, the saccharin test. Among the assessed factors in this study, gender and presence of dextrocardia (the probable diagnosis of Kartagener's syndrome) were neither associated with the FeNO measurement out-

Table 1. Assessment of FeNO and Saccharin Tests' Sensitivity for the Diagnosis of Primary Ciliary Dyskinesia

Test Results	FeNO Measurement		Saccharin Test	
	< 20 ppb	> 20 ppb	Not sensing in 60 Minutes	Sensing in 60 Minutes
Total number of the patients	28	3	17	14
Sensitivity, %	90.3		54.8	
Kartagener's syndrome	12	1	7	6
Total	13		13	
Sensitivity, %	92.3		53.8	

Table 2. Gender Distribution of the Patients with a Positive Test in Favor of Primary Ciliary Dyskinesia

Prevalence	Frequency (%)	Frequency of Each Sex		P-Value
		Male	Female	
PCD due to FeNO	28 (90.2)	14 (50)	14 (50)	1
PCD due to saccharin test	17 (54.8)	9 (53)	8 (47)	0.87

Table 3. Relationship Between FeNO and Saccharin Test and the Patient's Mean Age

Tests	Numbers	Mean Age $\pm$ SD	P-Value
FeNO measurement, ppb			0.864
< 20	28	15.18 $\pm$ 2.30	
> 20	3	14 $\pm$ 4.34	
Saccharin test			0.028
Not sensing in 60 minutes	17	16 $\pm$ 1.48	
Sensing in 60 minutes	14	13.93 $\pm$ 2.67	

comes nor saccharin test, while those who had a saccharin test in favor of PCD were remarkably older.

In the current study, we considered the cut-off point of 60 minutes for the saccharin test in order to make the diagnosis of PCD, while Adde et al. (cited in Vergani) presented that the cut-off point of 30 minutes has the sensitivity of 95%, and this rate decreases to 75% by the consideration of 60 minutes as the cut-off time for positive saccharin test outcomes (14). The notable low sensitivity of this test in the current study may be attributed to the used cut-off value. Furthermore, despite previous studies, recent guidelines such as the European Respiratory Society Guidelines do not support the diagnostic value of the saccharin test now, and they prefer the selection of novel techniques (15).

Therefore, exhaled NO level was considered for further assessments in the diagnosis of PCD. Previous studies have shown that exhaled NO level is lower among patients with PCD and cystic fibrosis than the general population (16). Also, the Official American Thoracic Society Clinical Prac-

tice Guidelines presented the cut-off point of 25 ppb and 20 ppb as the normal range of exhaled NO for adults and children, respectively (10).

You et al. (17), in a study regarding the amount of exhaled NO in the general population of China, presented that FeNO is affected by gender (male to female, 2: 1) but not age, height, and weight. These findings are different from the current presentation (17) as we found no association between FeNO findings with gender, age, and cardioposition. These differences may be attributed to the racial differences between the Iranian and Chinese.

Boon et al. (16) conducted their study using this technique for the diagnosis of PCD and presented the sensitivity of 89.5% and specificity of 58.3% at the cut-off point of 10 ppb for the FeNO test in the diagnosis of PCD. Although outcomes of this study are in favor of FeNO administration for the diagnosis of PCD, other studies have presented the more excellent values of nasal FeNO measurements for the more accurate diagnosis of PCD. It is a limitation in our study that we performed our study using oral FeNO measurements.

The other study carried out by Corbelli et al. (18) presented the cut-off point of 105 ppb for the diagnosis of PCD. They declared that FeNO levels lower than 105 ppb had a specificity of 88% for the detection of PCD among the patients who had been confirmed with the diagnosis of PCD through the histological study. They also reported that FeNO levels upper than 105 ppb could exclude the diagnosis of PCD with 100% confidence (18).

Other studies have presented different cut-offs for the nasal FeNO among the patients with PCD. For instance, Mateos-Corral et al. (19) presented the cut-off of 60.8 ppb with specificity and sensitivity of 100%; Marthin and Nielsen (20) mentioned the oral cut-off of 72.6 ppb with a sensitivity of 94.3% and specificity of 100%. Harris et al. (21) declared the nasal exhaled NO of 38 ppb with the sensitivity and specificity of 100% and 95%, respectively, and Narang et al. (22) in a similar amount to this study, presented the cut-off of 25 ppb with the specificity of 96% and sensitivity of 75%. This considerable diversity in the cut-off presentations

Table 4. The Association of Cardioposition with the Results of FeNO and Saccharin Tests

Test	Position of the Heart		P-Value
	Dextrocardia (Kartagener's Syndrome)	Without Dextrocardia (Idiopathic Bronchiectasis)	
FeNO measurements, ppb			0.76
< 20	12	16	
> 20	1	2	
Saccharin test			0.78
Not sensing in 60 minutes	7	10	
Sensing in 60 minutes	6	8	

may be attributed to the racial, physical, and genetic differences of the communities. As this study is the first one in the population of Iran, further studies are strongly recommended.

5.1. Conclusions

Based on the current study, the FeNO test had the remarkable sensitivity of 90.3% for the diagnosis of PCD, and its outcomes were not affected by age, gender, and cardioposition. This is while the saccharin test had a sensitivity of 54.8% and was affected by age but not by gender or cardioposition. Although there are more accurate tests for the diagnosis of PCD, such as TEM and genetic studies, we decided to investigate PCD in children with bronchiectasis by performing two screening tests, NO and saccharin, because there had been several problems in the availability and performance of more accurate tests.

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Footnotes

Authors' Contribution: Sohila Alyasin designed the study. Behjat Maneshian carried out the experiment and prepared the manuscript. Shadi Niliyeh had contributed to manuscript preparation.

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References

- Bush A, Cole P, Hariri M, Mackay I, Phillips G, O'Callaghan C, et al. Primary ciliary dyskinesia: diagnosis and standards of care. *Eur Respir J*. 1998;**12**(4):982-8. doi: [10.1183/09031936.98.12040982](https://doi.org/10.1183/09031936.98.12040982). [PubMed: [9817179](https://pubmed.ncbi.nlm.nih.gov/9817179/)].
- Hogg C. Primary ciliary dyskinesia: when to suspect the diagnosis and how to confirm it. *Paediatr Respir Rev*. 2009;**10**(2):44-50. doi: [10.1016/j.prpv.2008.10.001](https://doi.org/10.1016/j.prpv.2008.10.001). [PubMed: [19410200](https://pubmed.ncbi.nlm.nih.gov/19410200/)].
- Knowles MR, Daniels LA, Davis SD, Zariwala MA, Leigh MW. Primary ciliary dyskinesia. Recent advances in diagnostics, genetics, and characterization of clinical disease. *Am J Respir Crit Care Med*. 2013;**188**(8):913-22. doi: [10.1164/rccm.201301-0059CI](https://doi.org/10.1164/rccm.201301-0059CI). [PubMed: [23796196](https://pubmed.ncbi.nlm.nih.gov/23796196/)]. [PubMed Central: [PMC3826280](https://pubmed.ncbi.nlm.nih.gov/PMC3826280/)].
- Boon M, Jorissen M, Proesmans M, De Boeck K. Primary ciliary dyskinesia, an orphan disease. *Eur J Pediatr*. 2013;**172**(2):151-62. doi: [10.1007/s00431-012-1785-6](https://doi.org/10.1007/s00431-012-1785-6). [PubMed: [22777640](https://pubmed.ncbi.nlm.nih.gov/22777640/)].
- Bush A, Chodhari R, Collins N, Copeland F, Hall P, Harcourt J, et al. Primary ciliary dyskinesia: current state of the art. *Arch Dis Child*. 2007;**92**(12):1136-40. doi: [10.1136/adc.2006.096958](https://doi.org/10.1136/adc.2006.096958). [PubMed: [17634184](https://pubmed.ncbi.nlm.nih.gov/17634184/)]. [PubMed Central: [PMC2066071](https://pubmed.ncbi.nlm.nih.gov/PMC2066071/)].
- Lucas JS, Burgess A, Mitchison HM, Moya E, Williamson M, Hogg C, et al. Diagnosis and management of primary ciliary dyskinesia. *Arch Dis Child*. 2014;**99**(9):850-6. doi: [10.1136/archdischild-2013-304831](https://doi.org/10.1136/archdischild-2013-304831). [PubMed: [24771309](https://pubmed.ncbi.nlm.nih.gov/24771309/)]. [PubMed Central: [PMC4145427](https://pubmed.ncbi.nlm.nih.gov/PMC4145427/)].
- Deborah S, Prathibha KM. Measurement of nasal mucociliary clearance. *Clin Res Pulmonol*. 2014;**2**(2):1019.
- Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A*. 1987;**84**(24):9265-9. doi: [10.1073/pnas.84.24.9265](https://doi.org/10.1073/pnas.84.24.9265). [PubMed: [2827174](https://pubmed.ncbi.nlm.nih.gov/2827174/)]. [PubMed Central: [PMC299734](https://pubmed.ncbi.nlm.nih.gov/PMC299734/)].
- Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*. 1988;**333**(6174):664-6. doi: [10.1038/333664a0](https://doi.org/10.1038/333664a0). [PubMed: [3131684](https://pubmed.ncbi.nlm.nih.gov/3131684/)].
- Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med*. 2011;**184**(5):602-15. doi: [10.1164/rccm.9120-11ST](https://doi.org/10.1164/rccm.9120-11ST). [PubMed: [21885636](https://pubmed.ncbi.nlm.nih.gov/21885636/)]. [PubMed Central: [PMC4408724](https://pubmed.ncbi.nlm.nih.gov/PMC4408724/)].
- Arnal JF, Didier A, Rami J, M'Rini C, Charlet JP, Serrano E, et al. Nasal nitric oxide is increased in allergic rhinitis. *Clin Exp Allergy*. 1997;**27**(4):358-62. [PubMed: [9146927](https://pubmed.ncbi.nlm.nih.gov/9146927/)].

12. Kharitonov SA, Rajakulasingam K, O'Connor B, Durham SR, Barnes PJ. Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *J Allergy Clin Immunol*. 1997;**99**(1 Pt 1):58–64. doi: [10.1016/s0091-6749\(97\)70301-4](#). [PubMed: [9003212](#)].
13. Corbo GM, Foresi A, Bonfitto P, Mugnano A, Agabiti N, Cole PJ. Measurement of nasal mucociliary clearance. *Arch Dis Child*. 1989;**64**(4):546–50. doi: [10.1136/adc.64.4.546](#). [PubMed: [2751328](#)]. [PubMed Central: [PMC1791962](#)].
14. Vergani KP. *Asma grave em crianças e adolescentes: análise da variação fenótica e fatores relacionados ao não controle clínico*. Universidade de São Paulo; 2017.
15. Lucas JS, Barbato A, Collins SA, Goutaki M, Behan L, Caudri D, et al. European Respiratory Society guidelines for the diagnosis of primary ciliary dyskinesia. *Eur Respir J*. 2017;**49**(1). doi: [10.1183/13993003.01090-2016](#). [PubMed: [27836958](#)]. [PubMed Central: [PMC6054534](#)].
16. Boon M, Meyts I, Proesmans M, Vermeulen FL, Jorissen M, De Boeck K. Diagnostic accuracy of nitric oxide measurements to detect primary ciliary dyskinesia. *Eur J Clin Invest*. 2014;**44**(5):477–85. doi: [10.1111/eci.12254](#). [PubMed: [24597492](#)].
17. You S, Zhang J, Bai Y, Ji L, Wang H. Normal values of nasal NO and exhaled NO in young Chinese people aged 9 - 22 years. *World J Otorhinolaryngol Head Neck Surg*. 2016;**2**(1):22–7. doi: [10.1016/j.wjorl.2016.02.004](#). [PubMed: [29204545](#)]. [PubMed Central: [PMC5698517](#)].
18. Corbelli R, Bringolf-Isler B, Amacher A, Sasse B, Spycher M, Hammer J. Nasal nitric oxide measurements to screen children for primary ciliary dyskinesia. *Chest*. 2004;**126**(4):1054–9. doi: [10.1378/chest.126.4.1054](#). [PubMed: [15486363](#)].
19. Mateos-Corral D, Coombs R, Grasemann H, Ratjen F, Dell SD. Diagnostic value of nasal nitric oxide measured with non-velum closure techniques for children with primary ciliary dyskinesia. *J Pediatr*. 2011;**159**(3):420–4. doi: [10.1016/j.jpeds.2011.03.007](#). [PubMed: [21514598](#)].
20. Marthin JK, Nielsen KG. Choice of nasal nitric oxide technique as first-line test for primary ciliary dyskinesia. *Eur Respir J*. 2011;**37**(3):559–65. doi: [10.1183/09031936.00032610](#). [PubMed: [20525709](#)].
21. Harris A, Bhullar E, Gove K, Joslin R, Pelling J, Evans HJ, et al. Validation of a portable nitric oxide analyzer for screening in primary ciliary dyskinesias. *BMC Pulm Med*. 2014;**14**:18. doi: [10.1186/1471-2466-14-18](#). [PubMed: [24507708](#)]. [PubMed Central: [PMC3929562](#)].
22. Narang I, Ersu R, Wilson NM, Bush A. Nitric oxide in chronic airway inflammation in children: diagnostic use and pathophysiological significance. *Thorax*. 2002;**57**(7):586–9. doi: [10.1136/thorax.57.7.586](#). [PubMed: [12096200](#)]. [PubMed Central: [PMC1746369](#)].