

ORIGINAL ARTICLE

AIRWAY DISEASES

Daily exhaled nitric oxide measurements and asthma exacerbations in children

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Abstract

Background: Fractional exhaled Nitric Oxide (FeNO) is a biomarker for eosinophilic airway inflammation and can be measured at home on a daily basis. A short-term increase in FeNO may indicate a higher risk of future asthma exacerbations.

Objective: To assess changes in FeNO before and after asthma exacerbations compared to a stable control period.

Methods: A *post hoc* analysis was performed on daily FeNO measurements over 30 weeks in children with asthma ($n = 77$). Moderate exacerbations were defined by an increase in symptom scores and severe exacerbations by prescription of prednisone. Individual mean and maximum FeNO, the variability of FeNO assessed by the coefficient of variation (CV), and slopes of FeNO in time were all quantified in 3-week blocks. Cross-correlation of FeNO with symptoms and autocorrelation of FeNO were assessed in relation to exacerbations and examined as predictors for exacerbations compared to reference periods using logistic regression.

Results: Fractional exhaled nitric oxide could be assessed in relation to 25 moderate and 12 severe exacerbations. The CV, slope, cross-correlation, and autocorrelation of daily FeNO increased before moderate exacerbations. Increases in slope were also randomly seen in 19% of 2-week blocks of children without exacerbations. At least 3–5 FeNO measurements in the 3 weeks before an exacerbation were needed to calculate a slope that could predict moderate exacerbations. No specific pattern of FeNO was seen before severe exacerbations.

Conclusion: Fractional exhaled nitric oxide monitoring revealed changes in FeNO prior to moderate exacerbations. Whether this can be used to prevent loss of asthma control should be further explored.

Asthma monitoring is challenging in children with frequent exacerbations. Asthma is commonly monitored on the basis of symptoms, exacerbation frequency, need for rescue treatment, and lung function (1). This approach does not take airway inflammation into account. The fraction of nitric oxide in exhaled air (FeNO) is a noninvasive, feasible biomarker that reflects eosinophilic airway inflammation (2). Fractional exhaled nitric oxide has excellent reproducibility and immediate results and has been validated and studied in relation to asthma control (3, 4). Clinical studies on monitoring of airway inflammation by means of FeNO were until now inconsistent, but some evidence for limited benefits, including less exacerbations, lower steroid doses, reduced airway hyperresponsiveness, and improved lung function was found (5–12).

It has been suggested that the combination of spirometry and FeNO allows objective assessment of asthma control status (13). However, FeNO concentrations during asthma exacerbations did not correlate with other measures of acute severity, suggesting that they might provide additive information (14, 15). Using a handheld NO analyzer, it is possible to monitor FeNO at home on a daily basis (16, 17). Recently, the CHildhood Asthma Respiratory Inflammatory Status Monitoring (CHARISM) study examined 77 atopic asthmatic children with daily telemonitoring of symptoms and FeNO for 30 weeks (18). This follow-up provided the opportunity to study FeNO fluctuation. Similar to fluctuation in lung function over time, we have shown that fluctuations in FeNO are not a random process but show internal long-range

correlation (19). We have previously shown that these correlation properties of daily FeNO are different in a subgroup of children with a high risk of exacerbation (20). In the present study, we describe FeNO variability in relation to exacerbations of asthma. We hypothesized that exacerbations were preceded by an increase in FeNO.

Methods

We performed a *post hoc* analysis of FeNO data from the CHARISM study (18). Fractional exhaled nitric oxide was measured daily in 77 atopic asthmatic children using a handheld airway inflammation monitor (NIOX MINO, Aerocrine, Solna, Sweden), along with daily symptom scores for 30 weeks at home. The study protocol was approved by the medical ethics committee of the participating centers.

Exacerbations were defined by a prespecified increase in symptom scores during 1 or 2 days (moderate exacerbation) or by prescription of an oral prednisone course (severe exacerbation; Table 1) (21). Daily FeNO and symptom scores were taken from 3-week blocks before and after the onset of exacerbations and were included in the analyses if at least 18 FeNO values out of 21 were available. To avoid carryover effects, second moderate exacerbations were excluded when they occurred within 21 days of the previous exacerbation. If a severe exacerbation was preceded by a moderate exacerbation, both were included in our analysis to account for this clinically relevant situation. We related the changes in FeNO to a stable reference period in the same individual. Reference periods were selected from the same patient when stable and matched for the use of same dose of inhaled corticosteroid (ICS) and use of long-acting beta-agonist (LABA). Reference periods were taken at least 6 weeks before or 3 weeks after the exacerbation. We estimated the risk of false-negative observations by examining slopes of children without exacerbations. Fluctuation and correlation properties in daily FeNO were quantified by the coefficient of variation (CV), the slope of daily FeNO (best-fit line through FeNO data), cross-correlation (strength of correlation between FeNO and symptoms), and autocorrelation (strength of correlation of FeNO with itself shifted by one or more days) (20, 22, 23).

Statistical evaluation

Analyses of FeNO were performed after natural log transformation. Fractional exhaled nitric oxide percentage was calcu-

lated by dividing individual daily values of FeNO by the median of the reference period and averaged over all children who had an exacerbation. Individual mean and maximum FeNO, as well as CV and slope of FeNO were calculated for periods of 21, 14, 10, 7, and 4 days prior to exacerbations and in matched reference periods. These parameters were then examined as predictors for the outcome of an exacerbation compared to reference periods, using logistic regression to estimate the odds ratios (OR) and the 95% Confidence Intervals (CI). Cross-correlation and autocorrelation were determined in periods of 21, 14, 10, and 7 days. We performed sensitivity analysis to estimate the number of measurements needed to detect changes in FeNO before an exacerbation by looking at the predictive power of single FeNO values at 21, 14, 10, 7, 4 days, and 1 day before an exacerbation. In order to test how many data points were needed to detect an exacerbation, randomly picked days were dropped from the time series and we calculated best-fit slopes for the remaining data points. Analyses were performed using custom-written software in MATLAB (The Mathworks Inc., Natick, MA, USA), and SAS PROC GENMOD was used for logistic regression analyses in SAS 9.1 for Windows (SAS Institute, Inc., Cary, NC, USA).

Results

Five patients were excluded owing to missing data over the whole period ($n = 4$) or unknown date of prednisone use ($n = 1$). Twenty-four patients had one or more moderate exacerbations, for a total of 38. Thirteen of these were excluded owing to missing data ($n = 9$) or overlap with moderate ($n = 3$) or severe exacerbation ($n = 1$). After quality control, 25 moderate exacerbations could be included for 3-week period analysis. Eleven patients had one or more severe exacerbations, for a total of 15. Three of these were excluded from analysis owing to missing data. In total, 12 severe exacerbations were selected for 3-week period analysis and were analyzed qualitatively, as statistical interference of low numbers could be unreliable. Two patients had moderate exacerbations preceding a severe exacerbation by 3 and 9 days. Demographics and baseline characteristics of children with moderate and severe exacerbations are given in Table 2. Maintenance and reliever medication used in the periods before, during, and after exacerbations and in the reference periods are depicted in Fig. 1.

Table 1 Criteria for determining moderate and severe exacerbations

Exacerbation	Description	Days
Moderate	3 points above the average daily symptom score* of the 2 preceding weeks	2 or more
	5 points above the average daily symptom score of the 2 preceding weeks	1 or more
Severe	Prednisone prescription, hospitalization, or emergency visits because of asthma	1 or more
End	Less than 3 points above the average daily symptom score of the 2 preceding weeks	3 or more

*Daily symptom score = total sum of wheezing, shortness of breath, coughing, and sleep disturbances symptoms; Symptoms could have values between 0 and 3 where 0 = no symptoms, 1 = occasional symptoms, 2 = symptoms most of the day, and 3 = asthma very bad, unable to do normal activities.

Table 2 General characteristics of study population

		Moderate	Severe
Demographics			
Patients	<i>N</i>	18	9
Exacerbations	<i>N</i>	25	12
Gender (male)	<i>N</i> (%)	8 (44.4)	2 (22.2)
Age (year)	Mean (SD)	11.7 (2.5)	10.1 (2.1)
Weight (kg)	Mean (SD)	43.8 (11.8)	37.6 (11.1)
Height (cm)	Mean (SD)	150.8 (14.4)	142.2 (13.4)
Medication			
ICS dose (µg per day)	Median (IQR)	400 (200–1000)	400 (200–1200)
LABA use per day	%	44.0	63.4
Rescue medication* use per day	%	21.0	20.1
Lung function			
FeNO† (ppb)	Median (IQR)	21 (14–32)	19 (10–40)
FEV1‡ (% pred)	Mean (SD)	86.7 (16.6)	78.7 (24.3)
Postbronchodilator FEV1§ (% pred)	Mean (SD)	90.1 (16.3)	85.6 (19.5)

ICS, inhaled corticosteroid; LABA, long-acting bronchodilator.

*Rescue medication = short-acting bronchodilator; †Median FeNO of total study period; ‡Baseline forced expiratory volume in 1 second;

§Baseline reversibility of FEV.

Moderate exacerbations

On the average, FeNO started to increase approximately 10 days before the onset of a moderate exacerbation. The mean change of FeNO before and after a moderate exacerbation is displayed in Fig. 2, upper panel. There was a significant increase in FeNO 3 days prior until 4 days after the exacerbations. Figure 1 (upper panel) shows that short-acting bronchodilator use was higher during exacerbations compared to the period before the moderate exacerbations (64.9% vs 15.9%, $P < 0.001$).

Severe exacerbations

Fractional exhaled nitric oxide showed marked variability around severe exacerbations, but no clear rise preceding the onset of prednisone treatment (Fig. 2, lower panel). Individual plots did not show a clear trend of symptoms in relation to severe exacerbations. There was however a rise in the use of rescue medication (Fig. 1, lower panel).

Daily FeNO fluctuations and moderate exacerbations

The risk of moderate exacerbation was not associated with geometric mean FeNO or maximum FeNO. A higher CV of FeNO during 14 and 10 days before an exacerbation compared to the reference period was significantly associated with moderate exacerbations [standardized CV 14 and 10 days: OR 2.27 (1.16; 4.44) and 1.93 (1.02; 3.66)] (Table 3). The slope of FeNO between 14 and 4 days before moderate exacerbations was associated with the exacerbation [slope in 14, 10, 7, and 4 days before the onset of exacerbation: OR 2.98 (1.22; 7.28), 1.58 (0.98; 2.54), 1.25 (0.97; 1.63), and 1.16 (1.00; 1.34), respectively]. A higher cross-correlation of FeNO and symptoms during 14 and 10 days

before the onset of moderate exacerbations was associated with exacerbation [standardized cross-correlation 14 and 10 days: OR 2.35 (1.12; 4.90) and 1.84 (0.94; 3.59), respectively]. Higher autocorrelation of FeNO was observed during 21, 14 and 10 days before exacerbations [OR 1.97 (1.04; 3.74), 2.09 (1.08; 4.03), and 1.71 (0.93; 3.16), respectively]. Increases in slope, with a cutoff defined as mean slope before moderate exacerbations, were randomly seen in 19% of 2-week blocks of children without exacerbations. In a receiver operating curve analysis, we found an area under the curve of 0.71 for slopes of 2-week blocks to predict exacerbations (test cutoff slope = 0.385; sensitivity, 64.0%; specificity, 74.3%).

Single FeNO values were not predictive of exacerbations. At least 3–5 data points in 3 weeks were required to detect a moderate exacerbation [for 3, 4, and 5 data points: OR 1.56 (0.97; 2.50), 1.92 (1.08; 3.41), and 1.79 (1.00; 3.20), respectively].

Discussion

In this proof-of-concept study, we examined daily FeNO measurements in relation to exacerbations of childhood asthma, compared to reference periods of the same child when stable. We found an increase in FeNO starting approximately 10 days before moderate, but not before severe exacerbations. Moderate exacerbations were quantitatively analyzed with novel mathematical methods (23). The CV, slope, cross-correlation, and autocorrelation of the FeNO time series all showed significant changes prior to moderate exacerbations. Increases in slope were also randomly seen in 19% of 2-week blocks of children without exacerbations. Single FeNO values were not predictive of exacerbations, and at least 3–5 data points in 3 weeks were required to detect an upcoming exacerbation.

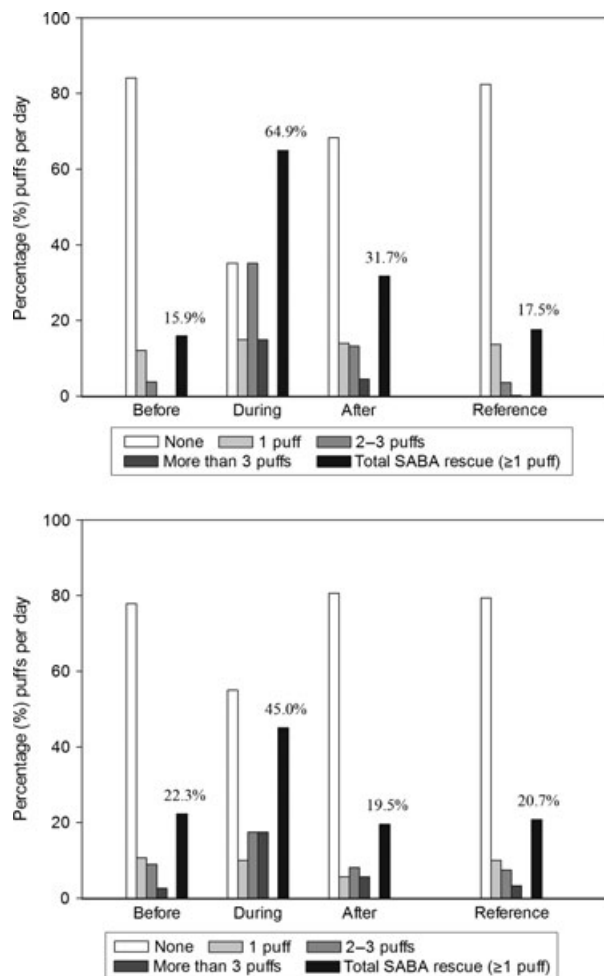


Figure 1 Short-acting bronchodilator (SABA) rescue medication use around exacerbations and in the reference periods. Percentage SABA rescue puffs per day in the periods before, during, and after exacerbations and in the reference period. Rescue medication use is depicted for moderate exacerbations (upper panel) and severe exacerbations (lower panel).

Previous studies on FeNO in relation to asthma management suggested that using FeNO to guide asthma treatment might reduce the risk of exacerbations (18). Unfortunately, most earlier studies on FeNO-guided asthma management were underpowered to demonstrate a significant effect on exacerbations (7–10). In the only study with sufficient power, FeNO monitoring significantly reduced the number of prednisone courses (11). However, design issues may have clouded the results (24). FeNO during acute severe exacerbations was studied previously and did not correlate with other measures of severity (14, 15). These studies concluded that FeNO is not informative for severe exacerbations. Longitudinal daily FeNO measurements in relation to exacerbations have not been studied before.

In our study, we found no evidence that daily FeNO measurements could predict severe exacerbations. However,

our study sample of children with severe exacerbations was relatively small. Surprisingly, daily symptoms were not strongly associated with severe exacerbations. We speculate that the symptom diaries might be insensitive for symptoms of severe exacerbations, e.g. because severe obstruction would not be recognized as wheeze or severe symptoms might have been misinterpreted and hence not properly labelled and scored. That a clinically relevant worsening of symptoms had occurred was evident from the increased use of rescue medication (Fig. 1). Unfortunately, diary questionnaires are not commonly validated for severe episodes. Spirometric measurements, which could have helped defining both moderate and severe exacerbations, were not obtained concurrently with FeNO. We did not measure PEF or FEV1 at home after ample consideration, because this would require the use of two different measurement devices with essentially different blowing techniques, and this was considered not feasible.

Asthma exacerbations are characterized by increases in airway inflammation, which can differ in type, depending on the pathogenesis that may involve infective or allergic stimuli (25). Fractional exhaled nitric oxide provides indirect information on eosinophilic airway inflammation (2). Respiratory viruses are associated with neutrophilic airway inflammation and are responsible for the majority of severe asthma exacerbations with emergency visits, hospitalization, and prednisone prescriptions (26, 27). This could explain why FeNO did not show an increase before such exacerbations in the present study. Indeed, experimental rhinovirus infection caused only a small increase in FeNO 1–2 days before an increase in symptoms (28, 29). In contrast, allergen exposure may cause elevated FeNO many days or even weeks before symptoms worsen (30). In our study, FeNO could detect moderate exacerbations 1–2 weeks before symptoms occurred. We, therefore, speculate that moderate exacerbations were preceded by increased eosinophilic airway inflammation.

Interestingly, the majority of children with exacerbations had a strong, positive cross-correlation between FeNO and symptoms and autocorrelation before exacerbations compared to the reference periods. We recently found that the level of cross-correlation between FeNO and symptoms in the whole study period was stronger in children with – than in those without exacerbations – and speculated that the level of cross-correlation may be useful to identify children at risk for exacerbations (20, 31). Treatment with inhaled corticosteroids may affect FeNO levels. The magnitude of the change in FeNO and its time course is dose-dependent (32). A point of consideration is, therefore, that our monitoring strategy was coupled to therapeutic intervention in the CHARISM study and might have modified the association between FeNO and exacerbations. Our study was not designed to evaluate individual FeNO changes as a result of steroid dose changes. As an increase in ICS would have reduced FeNO, we would have underestimated the effect (32).

We examined the possibility of false-negative episodes by looking at the number of periods in which we found a slope that was higher than the average 2-week blocks slope

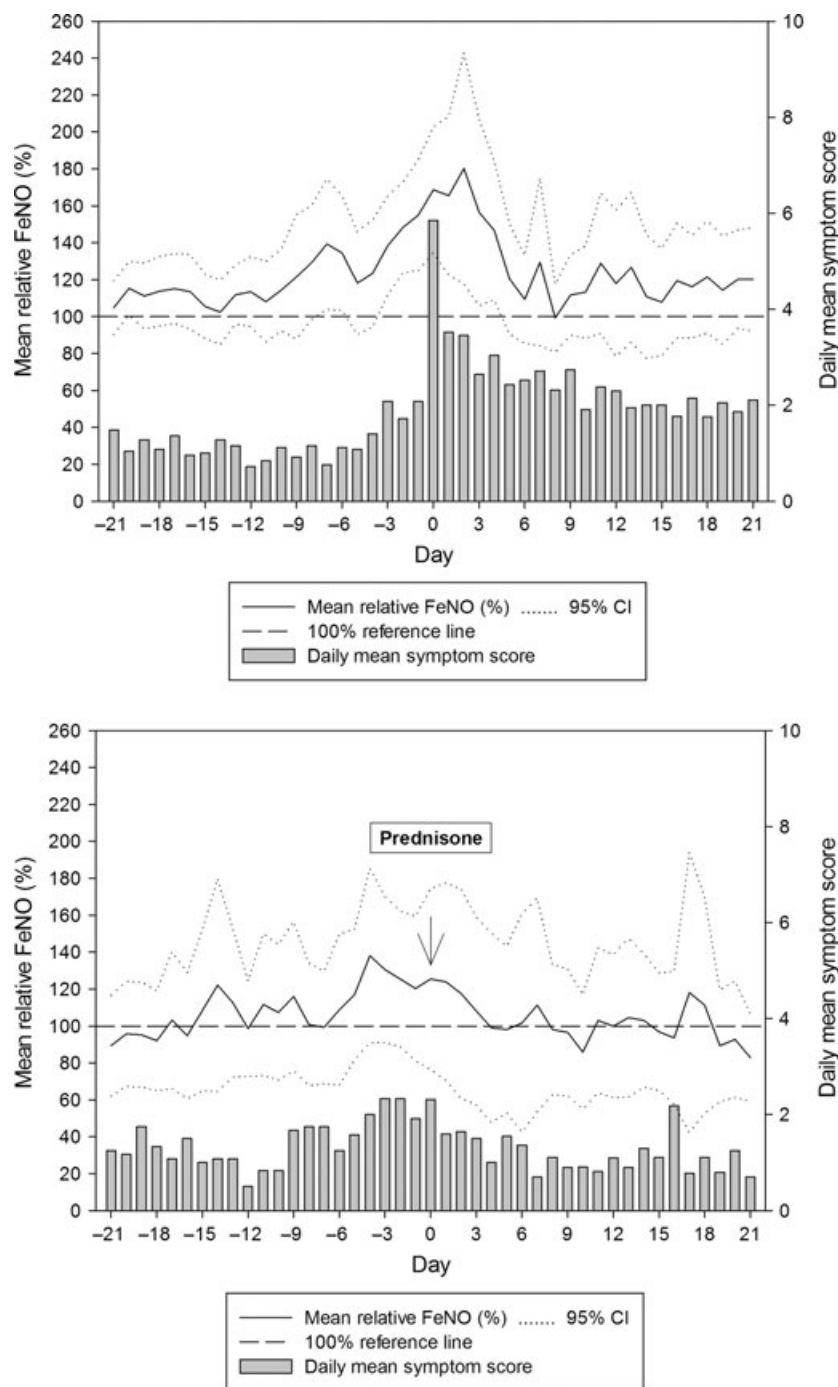


Figure 2 Percentage change in Fractional exhaled Nitric Oxide (FeNO) before and after exacerbations. Mean relative FeNO time series for 3-week periods centered around moderate exacerbations (upper panel) and severe exacerbations (lower panel) (onset exacerbation at day 0). Relative FeNO = FeNO divided by the median of the reference period. Bars show average daily symptom scores (sum of wheezing, shortness of breath, coughing, and sleep disturbances).

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preceding moderate exacerbations and found higher slopes in 19% of all 2-week blocks in children without exacerbations. However, daily FeNO slopes preceding moderate exacerbations were significantly higher compared to slopes throughout

the whole study period in children without exacerbations ($P < 0.001$). This observation shows that FeNO slope changes are not highly specific for children with imminent exacerbations, though indicative of an increased risk.

Table 3 Associations between moderate exacerbations and parameters derived from daily FeNO

Days before onset of exacerbation	21	14	10	7	4
Before moderate exacerbation vs. Reference period OR (95% CI)*					
Coefficient of variation†	1.52 (0.85;2.72)	2.27 (1.16;4.44)	1.93 (1.02;3.66)	1.54 (0.85;2.78)	1.07 (0.61;1.87)
Slope	1.74 (0.72;4.17)	2.98 (1.22;7.28)	1.58 (0.98;2.54)	1.25 (0.97;1.63)	1.16 (1.00;1.34)
Cross-correlation†‡	1.50 (0.79;2.83)	2.35 (1.12;4.90)	1.84 (0.94;3.59)	1.54 (0.79;3.00)	–
Autocorrelation†§	1.97 (1.04;3.74)	2.09 (1.08;4.03)	1.71 (0.93;3.16)	1.32 (0.74;2.34)	–

*Odds ratios (95% Confidence Interval) parameters derived from daily FeNO before moderate exacerbations were examined as predictors for the outcome of an exacerbation compared to parameters of daily FeNO derived from reference periods; †Coefficients were divided by the standard deviation to make ORs more directly interpretable(33); ‡Cross-correlation coefficient of daily FeNO with daily sum of symptoms on the same day; §Autocorrelation coefficient of FeNO with FeNO lagged by 1 day.

Clinical implications

This is a proof-of-concept study where different types of mathematical techniques were used in a dataset designed for another hypothesis. Long-term daily measurements of FeNO enabled us to look at periods of exacerbations relative to reference periods in the same subject. This analysis showed that there are indeed changes in FeNO before exacerbations compared to reference periods and quantifies some of these changes. The study sample size was small, 25 moderate exacerbations in 18 patients. There were only 12 severe exacerbations in nine patients, which may have decreased the power to detect significant associations between daily FeNO and severe exacerbations. We believe however that our sample size was sufficient for a proof-of-concept study for moderate exacerbations and think that our findings warrant further studies looking at changes in FeNO over time in selected populations, which may be better than looking at single and averaged values for monitoring and risk prediction in asthma (23). Our findings also suggest that regular FeNO measurements in the home setting could help to detect and perhaps even help prevent loss of asthma control. Such monitoring could be especially useful in a selected population with frequent moderate exacerbations.

In conclusion, daily FeNO monitoring revealed changes in FeNO prior to moderate exacerbations of asthma. Whether this can be used to prevent loss of asthma control should be further explored.

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Authors contributions

RV, EB, GS, UF, JJ: design, analysis, writing.

Conflict of interest statement

RvdV was supported by a junior research fellowship grant from Sophia Kinderziekenhuis Fonds, Rotterdam, the Netherlands. EB has received honoraria for nonpromotional CME activity lectures by Chiesi, MSD, Abbott, and Valeas. The original data collection was supported by a research grant from Aerocrine AG, Solna, Sweden.

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