Exhaled nitric oxide in stable adult cystic fibrosis patients, during exacerbation and following CFTR-modifying treatment

To the Editor:

In adult patients with cystic fibrosis (CF) fraction of exhaled nitric oxide (F_{ENO}) has been reported to be normal or abnormally low [1]. F_{ENO} has also been reported to correlate with spirometry [2–4], and to increase following treatment with ivacaftor [5, 6], suggesting that F_{ENO} could be used as a noninvasive, fast and easily available marker of CFTR-function. The aim of this study was to examine whether in adult CF patients, F_{ENO} was associated with patient characteristics and lung function, and whether F_{ENO} was affected by acute exacerbation. We also aimed to examine F_{ENO} evolution over a longer period after starting cystic fibrosis transmembrane conductance regulator (CFTR)-modifying treatment (ivacaftor and ivacaftor/lumacaftor).

We conducted a prospective observational study (December 2015–December 2017) at the CF Reference Centre of the University Hospital of Brussels, measuring F_{ENO} and standard lung function in adult CF patients in a stable condition (at ~3-monthly intervals) as well as during acute exacerbation, as defined by Bilton *et al.* [7]. We recorded sex, age, smoking status, genotype, pancreatic function, presence of atopy and/or coexistent asthma, and presence of colonisation. For the subgroup of CF patients without any factor known to confound F_{ENO} (atopy, coexistent asthma, active smoking, chronic treatment with corticosteroids), a control group of age- and sex-matched (1:1) healthy subjects was recruited from healthy never-smokers. In patients receiving CFTR-modifying treatment (ivacaftor or ivacaftor/lumacaftor) F_{ENO} was measured prior to the start of treatment, and at regular intervals during a follow-up period of 12 months. Log-transformed F_{ENO} values were used for comparisons between groups or subgroups (unpaired t-tests), or for comparison between a stable condition and acute exacerbation (paired t-test). Normality of the log-transformed F_{ENO} values as well as of the residuals (in comparative tests) was verified with the Chi-squared test. Rank correlations (Spearman) between F_{ENO} and lung function or body mass index (BMI) were also performed, using Medcalc (version 18; MedCalc Software, Ostend, Belgium).

In total, 62 adult CF patients were eligible, which included 17 patients without any factor known to confound F_{ENO} and seven patients on CFTR-modifying treatment (ivacaftor or ivacaftor/lumacaftor). A total of 264 F_{ENO} measurements were collected, in a stable condition as well as at onset of acute exacerbation (prior to antibiotic treatment). Median (95% CI) age was 30 (26–33) years and 68% were men, median stable forced expiratory volume in 1 s (FEV_1) was 2.50 L (71% predicted). 39% were homozygous for the F508del mutation. 48% were colonised with *Pseudomonas aeruginosa* and 82% were colonised with meticillin-sensitive *Staphylococcus aureus* (MSSA); only 5% were colonised with meticillin-resistant *S. aureus* (MRSA). From the total patient group of 62 patients, 55 showed pancreatic insufficiency (89%). Compared with healthy controls (geometric mean (95% CI) F_{ENO} 16.7 (11.8–23.7) ppb), the patient subgroup without F_{ENO} confounders showed a significantly lower F_{ENO} (10.7 (9.2–13.0) ppb; p=0.028). In the entire CF patient group, geometric mean F_{ENO} was 8.9 (7.8–10.3) ppb. In patients colonised with *P. aeruginosa,* F_{ENO} was lower (geometric mean 7.0 (5.6–8.8) ppb) compared to those who were not (11.1 (9.6–12.9) ppb; p<0.001). Receiver operating curve-analysis showed an area under the curve of 76%, with a specificity of 81% and a sensitivity of 70% for presence of *P. aeruginosa* (F_{ENO} cut-off: 8.8 ppb). In patients colonised with MSSA, F_{ENO} was higher (geometric mean (95% CI) 9.7 (8.4–11.2) ppb) compared to those who were not (6.2 (4.2–9.2) ppb; p=0.014). We found no association of

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F_{ENO} is low in stable adult patients with CF, and lower in patients colonised with *P. aeruginosa*. F_{ENO} cannot be used as a noninvasive marker of CFTR function, since prolonged follow-up showed only a transient rise in F_{ENO} after starting ivacaftor. http://ow.ly/PtfC30ojYFc

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*F*eNO with other clinical characteristics such as genotype F508del/F508del, pancreatic insufficiency or BMI. None of the lung function parameters (FEV_1, forced vital capacity, residual volume/total lung capacity, transfer coefficient of the lung for carbon monoxide) correlated with *F*eNO (p>0.1 for all). In the 52 patients with an acute exacerbation (geometric mean (95% CI) 10.1 (8.1–12.5) ppb), *F*eNO was similar to that in a stable condition (8.9 (7.6–10.4) ppb; p=0.16). In the three patients receiving ivacaftor, there was an initial rise in *F*eNO followed by a return towards pretreatment values 8–12 months after the start of CFTR-modifying treatment (figure 1a). In particular, *F*eNO increased from 6.7 to 16.3 ppb, from 10.4 to 18.6 ppb and from 15.5 to 26.9 ppb. In three out of the four patients receiving ivacaftor/lumacaftor, an initial increase could be observed in *F*eNO: from 8.9 to 15.4 ppb, from 12.2 to 14.0 ppb and from 16.6 to 19.9 ppb; yet, in one patient *F*eNO decreased from 15.5 to 12.3 ppb (figure 1b).

*F*eNO in CF patients has been described as either lower than, or not different from, that in healthy controls [1]. Differences in measuring methodology, small patient groups and confounders such as corticosteroid therapy may have contributed to these inconsistent results. In this prospective observational study on a substantial number of adult CF patients, in whom *F*eNO was measured multiple times over a 2-year period, we demonstrate that *F*eNO is consistently low in stable patients. Since CF is characterised by chronic neutrophilic airway inflammation, one could assume that *F*eNO, generally used as a marker of eosinophilic airway inflammation, is indeed not increased in CF patients. On the contrary, *F*eNO is decreased in stable CF patients. There are several possible explanations for this finding. Modelling of perturbed nitric oxide diffusion through a thickened mucus lining of the airways predicts a distinct *F*eNO decrease, even when nitric oxide production is unaltered [8]. Malnutrition/malabsorption may result in a lack of L-arginine, the substrate for nitric oxide production [1, 9, 10]. In patients with CF, increased levels of asymmetric dimethyl arginine [10], the endogenous inhibitor of cellular arginine uptake and nitric oxide synthase (NOS) activity, and the inability to upregulate inducible NOS (iNOS) despite the presence of chronic inflammation [1, 9, 10] may also be responsible. Finally, the consumption of nitric oxide by *P. aeruginosa* and other denitrifying organisms [1, 9, 10], as well as variants in the genes encoding constitutive NOS, which have been associated with low *F*eNO and colonisation with *P. aeruginosa* [11, 12], could explain the significantly lower *F*eNO we observed in patients colonised with *P. aeruginosa*. Hence, a low *F*eNO may signal *P. aeruginosa* colonisation, and as such, be prognostic of disease progression. This hypothesis would need to be confirmed by a prospective trial following up patients with *P. aeruginosa* colonisation. We also detected an association between the presence of MSSA colonisation and higher *F*eNO, but this observation may have been biased by the uneven distribution (51 with *versus* 11 patients without MSSA colonisation).

A subject of controversy is whether reduced iNOS expression is primarily related to reduced or absent CFTR function, or due to chronic inflammation. The hypothesis of a link between decreased CFTR function and lower iNOS expression is interesting, since it implies that *F*eNO could be used as a noninvasive marker of CFTR-function, as suggested by two studies [5, 6]. In 15 CF patients (eight adults and seven children) [5] and in five children with CF [6] these authors showed *F*eNO increases 4 weeks after the start of treatment with ivacaftor, a CFTR-modifying drug, and this effect was more pronounced in the

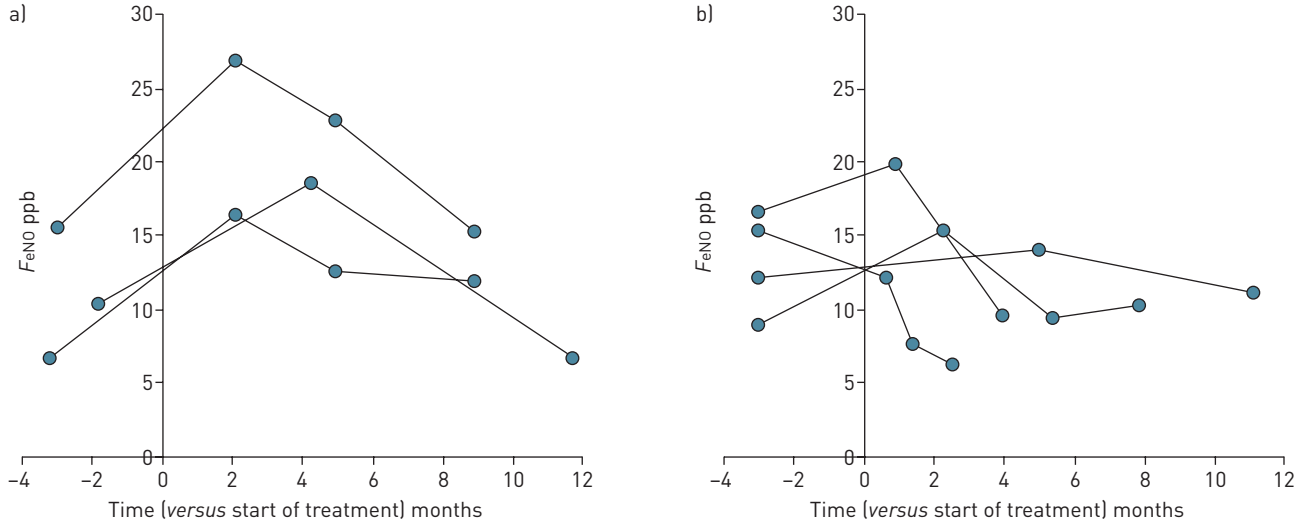

FIGURE 1 a) Evolution of fraction of exhaled nitric oxide (F_{eNO}) in the three patients started on treatment with ivacaftor during the study period. b) Evolution of F_{eNO} in the four patients started on treatment with ivacaftor/lumacaftor during the study period. Zero on the time axis indicates the start of treatment with a) ivacaftor or b) ivacaftor/lumacaftor.

paediatric cohort. We also observed an initial rise in F_{ENO} in each of the three adult patients in the first few months after starting treatment with ivacaftor. However, this was a transient effect, and F_{ENO} values were back to baseline values by 8–12 months after the start of treatment. Over this period, none of the three patients experienced an acute exacerbation, lung function decline (rather the opposite) or other health-related problems. Changes in F_{ENO} after starting ivacaftor/lumacaftor in patients homozygous for F508del were less consistent compared with the observed F_{ENO} changes in the patients receiving ivacaftor treatment. A possible explanation is that ivacaftor/lumacaftor only partially rescues CFTR function in these patients, as also supported by the more modest and variable clinical changes (in sweat chloride and FEV_1) seen in association with this treatment [13]. To our knowledge, our study is the first to follow up F_{ENO} for a period exceeding 1 month after the start of ivacaftor treatment, and the first study to monitor F_{ENO} during treatment with ivacaftor/lumacaftor over a comparable follow-up period of up to 11 months after the start of treatment.

In conclusion, in this prospective observational study spanning 2 years, we reported consistently low F_{ENO} values in stable adult patients with CF. F_{ENO} was even lower in patients with *P. aeruginosa* colonisation, suggesting that F_{ENO} monitoring could be used as a marker of *P. aeruginosa* colonisation and as such be prognostic for disease progression. Taking into account the limited patient numbers studied, and thus using caution interpreting these results, our observations do not support the hypothesis that F_{ENO} can be used as a noninvasive marker of CFTR function, since prolonged follow-up of F_{ENO} after the start of CFTR-modifying treatment showed a return of F_{ENO} towards pretreatment values after a transient rise in the patients treated with ivacaftor, and no consistent changes in the patients treated with ivacaftor/lumacaftor.

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