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ORIGINAL RESEARCH

Effect of high-dose N-acetylcysteine on airway geometry, inflammation, and oxidative stress in COPD patients

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Background: Previous studies have demonstrated the potential beneficial effect of N-acetylcysteine (NAC) in chronic obstructive pulmonary disease (COPD). However, the required dose and responder phenotype remain unclear. The current study investigated the effect of high-dose NAC on airway geometry, inflammation, and oxidative stress in COPD patients. Novel functional respiratory imaging methods combining multislice computed tomography images and computer-based flow simulations were used with high sensitivity for detecting changes induced by the therapy.

Methods: Twelve patients with Global Initiative for Chronic Obstructive Lung Disease stage II COPD were randomized to receive NAC 1800 mg or placebo daily for 3 months and were then crossed over to the alternative treatment for a further 3 months.

Results: Significant correlations were found between image-based resistance values and glutathione levels after treatment with NAC ($P = 0.011$) and glutathione peroxidase at baseline ($P = 0.036$). Image-based resistance values appeared to be a good predictor for glutathione peroxidase levels after NAC ($P = 0.02$), changes in glutathione peroxidase levels ($P = 0.035$), and reduction in lobar functional residual capacity levels ($P = 0.00084$). In the limited set of responders to NAC therapy, the changes in airway resistance were in the same order as changes induced by budesonide/formoterol.

Conclusion: A combination of glutathione, glutathione peroxidase, and imaging parameters could potentially be used to phenotype COPD patients who would benefit from addition of NAC to their current therapy. The findings of this small pilot study need to be confirmed in a larger pivotal trial.

Keywords: functional respiratory imaging, computational fluid dynamics, computed tomography, chronic obstructive pulmonary disease, N-acetylcysteine

Introduction

Chronic obstructive pulmonary disease (COPD) is a heterogeneous disorder characterized by dysfunction of the small and large airways, as well as destruction of the lung parenchyma and its vasculature in highly variable combinations. The hallmark of COPD is expiratory flow limitation, which is slowly progressive and irreversible.[1–3] There is also increasing evidence that the inflammatory processes of COPD are closely associated with oxidative stress. Proinflammatory cytokines and growth factors stimulate production of reactive oxygen species. The resulting imbalance between oxidants and antioxidants triggers signaling cascades for a variety of transduction pathways and gene expression, leading in turn to altered expression of proinflammatory factors. Neutrophils are key cells in the inflammatory response in COPD.

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Excessive transmigration into the lung tissues and abnormal activation are largely responsible for overproduction of reactive oxygen species and release of proteolytic enzymes in the lungs.

N-acetylcysteine (NAC) is a glutathione precursor with beneficial properties. It is a source of sulfhydryl groups in cells and increases the level of reduced glutathione. Reduced glutathione serves as a reducing agent by forming intermolecular disulfide nonradical end product-oxidized glutathione. NAC is also a scavenger of free radicals as it interacts with reactive oxygen species, such as the hydroxyl radical (OH-) and hydrogen peroxide (H_2O_2).[4–7]

In COPD patients, NAC has been used as a mucolytic and for its antioxidant properties. In a large-scale study, it was not possible to demonstrate that NAC decreases the decline in forced expiratory volume in one second (FEV_1) in COPD patients after a period of 3 years.[8] In the same study, however, there were some preliminary indications that NAC reduce hyperinflation, since a decrease in functional residual capacity was observed over time. However, the effects of NAC are certainly dose-dependent.[9] It might well be that the lack of efficacy is partially explained by use of dosages that are too low.[9]

Considering all the above, it would be worthwhile to study the effect of higher dosages of NAC on the small airways, inflammation, and oxidative stress in COPD patients. In this randomized crossover study, patients with Global initiative for chronic Obstructive Lung Disease (GOLD) stage II COPD received placebo for 3 months and NAC at a dosage of 600 mg 3 times daily for 3 months in addition to their usual medication. For this trial, we used novel advanced imaging tools that have been demonstrated in the past to yield more sensitive outcome parameters compared with classical lung function tests.[10] This functional respiratory imaging method was validated in previous studies using gamma scintigraphy,[11,12] single photon emission computed tomography (CT),[13] and hyperpolarized 3He magnetic resonance imaging.[14] Subsequently, this method was used to assess the effect of a long-acting β_2-agonist,[15] a combination of a long-acting β_2-agonist and inhaled corticosteroid,[10] and a short-acting anticholinergic and short-acting β_2-agonist.[16] In the current study, we hypothesized that this method could be used to gain additional insight into the mode of action of NAC in COPD patients, particularly the relationship between upregulation of glutathione/glutathione peroxidase and changes in airways volume and resistance. Further, this pilot study aimed to provide a basis for identifying patient phenotypes that would benefit most from NAC therapy.

Materials and methods

The study was conducted according to all ethical principles. Approval was obtained from the ethics committee and all patients gave their informed consent (NCT00969904). The study started in August 2009 and ended in June 2012.

Patient population

Twelve COPD patients (nine men and three women) were included. To be eligible for the study, patients had to have documented COPD and meet the following inclusion criteria: a smoking history of at least 10 pack-years; compatible symptoms, including dyspnea, cough, and sputum production; a decreased Tiffeneau index (FEV_1/forced vital capacity [FVC]) < 0.7; age $\geq$40 years; cessation of smoking for at least one month; moderate to severe COPD with an FEV_1 30%–80% of predicted [GOLD stages II and III]; and treatment according to GOLD guidelines. The following exclusion criteria were applied: exacerbation during the last 8 weeks; allergy to acetylcysteine or to another element of the product; phenylketonuria; untreated active peptic ulcer; renal and/or cardiac insufficiency; previous treatment with NAC for more than 6 months or during the last 3 months; ongoing treatment with oral, intravenous, or intramuscular corticosteroids; pregnancy or breast-feeding; and treatment with an orally administered cephalosporin.

Study design

At all visits, patients received full lung function testing yielding the following parameters: FEV_1, FEV_1/FVC, peak expiratory flow from spirometry, and airway resistance (Raw) and specific airway resistance from body plethysmography. A low-dose multislice CT scan was taken at total lung capacity and functional residual capacity. From these images of the airways, lung and lobar volumes could be obtained as well as airway resistance values by means of computational fluid dynamics. In addition, a blood sample was taken to measure levels of glutathione, glutathione peroxidase, superoxide dismutase, and interleukin-8. Patients completed the Saint George's Respiratory Questionnaire (SGRQ, Table 1). At the first visit, patients were randomized according to a computer-generated randomization list to treatment with NAC (Fluimucil®, Zambon SpA, Bresso, Italy) at a dose of 600 mg three times daily or placebo in addition to their usual treatment. After 3 months of treatment, the patients returned to the hospital for further tests and were crossed over to NAC or placebo. After a further 3 months, all measurements were repeated.

To limit exposure to ionizing radiation, a dose reduction protocol was used for the CT scans. A VCT light speed scanner

Table 1 Flowchart describing visits and tests

Weeks	–2	0	12	24
Visit	1	2	5	8
	Screening	Baseline start treatment A	Stop treatment A, start treatment B	End of treatment
Informed consent	X			
Inclusion/exclusion criteria	X			
Physical examination	X			X
Monitoring current therapy	X			
Medical history	X			
Spirometry	X	X	X	X
NO		X	X	X
Body plethysmography		X	X	X
Diffusion		X	X	X
ABG		X	X	X
EBC		X	X	X
Low-dose multislice CT thorax		X	X	X
Blood collection and vital signs		X	X	X
SGRQ		X	X	X

Abbreviations: SGRQ, St George's Respiratory Questionnaire; ABG, arterial blood gas; EBC, exhaled breath condensate; CT, computed tomography; NO, nitric oxide.

with 64 detector rows (General Electric, Fairfield, CT, USA) was used. The multislice CT settings were as follows: tube voltage, 120 kV; tube current,10–100 mA; noise factor, 28; collimation, 0.625 mm; rotation time, 0.6 seconds; and pitch factor, 1.375. The resulting radiation dose was in the order of 1–2 mSv per scan. Images were reconstructed to a slice thickness of 0.6 mm. Digital Imaging and Communications in Medicine (DICOM) images were assessed using a commercially available software package approved by the US Food and Drug Administration (Mimics, Materialise, Leuven, Belgium). The tracheobronchial tree was subsequently segmented using a semiautomatic approach. A total of three airway models were obtained per patient, ie, a baseline model, a model after 3 months of treatment with NAC or placebo, and a repeat model 3 months after crossover from NAC or placebo. After segmentation, all models for the same patient were superimposed using a least squares method to ensure that a comparison was possible between the different geometries. Segmentation yields the airway volumes at the central ($iVaw_{cent}$) and distal ($iVaw_{dist}$) levels. In addition to airway volumes, it was also possible to extract the lung and lobar volumes from the CT images at functional residual capacity and total lung capacity. Using computational fluid dynamics, it is possible to describe the flow patterns inside the airways based on computer simulation. Computational fluid dynamics numerically solves the Navier–Stokes equations inside the prescribed flow domain, in this case the airway tree structures at different measurement points. The main outcome parameter of computational fluid dynamic calculations is airway resistance in the central ($iRaw_{cent}$) and distal airways ($iRaw_{dist}$).

Statistical analysis

Statistical analysis was performed using Statistica 9.1 software (StatSoft Inc, Tulsa, OK, USA). Differences were assessed using the Wilcoxon matched-pairs test for equal sample sizes that are related or Mann–Whitney *U* test for independent samples. Correlations were assessed using the Spearman's rank test. A P-value < 0.05 was considered to be statistically significant.

Results

Patients

All patients were categorized by GOLD guidelines as stage II with an average post-bronchodilation FEV_1 of 65.38% ± 7.12% predicted. The average age of the patients was 65.00 ± 9.63 years, average height was 171.17 ± 8.27 cm, and the average smoking history was 56.16 ± 33.12 pack-years (Table 2).

Image-based resistance and volume

Figure 1 shows image-based resistance (iRaw) and volume (iVaw) measurements for the individual patients. The left hand side depicts iRaw, while the right hand side shows iVaw. The left upper quadrant indicates an increase in iRaw, hence nonresponders. The left lower quadrant indicates a decline in iRaw, hence responders. For iVaw it is reversed, whereby the right upper quadrant indicates an increase in iVaw, therefore identifying responders, and the right lower quadrant indicates an decline in iVaw, hence nonresponders. From Figure 1, it can be seen that there was a relatively even distribution between patients who responded in terms of iRaw and iVaw measurements. There were six patients with a reported

Table 2 Patient characteristics at baseline

	Age, years	Height (cm)	Pack-years	FEV_1 (L)	FEV_1 (% predicted)	FEV_1/FVC (%)	TLC (% predicted)	RV (% predicted)
Males (n = 9)	66.0 ± 10.0	172.7 ± 8.8	62.5 ± 35.2	2.0 ± 0.3	66.0 ± 8.0	57.0 ± 6.2	97.8 ± 13.3	118.8 ± 30.7
Females (n = 3)	62.0 ± 9.5	166.7 ± 4.7	37.1 ± 18.4	1.6 ± 0.3	63.5 ± 4.1	56.4 ± 11.0	114.0 ± 14.5	147.3 ± 22.1

Abbreviations: TLC, total lung capacity; FEV, forced expiratory volume in one second; FVC, forced vital capacity; RV, residual volume.

reduction in $iRaw_{cent}$ and four with a reported reduction in $iRaw_{dist}$. In total, five patients demonstrated an increase in $iVaw_{dist}$ and three patients showed an increase in $iVaw_{cent}$. In general, the changes could be considered small. However, the differences are considerable for individual patients on a local scale. Figure 2 shows the changes in airway resistance after placebo and NAC treatment for a patient who could be considered an iRaw or NAC responder. It could be observed that the changes are not homogeneously distributed throughout the airway system with a general decline in airway resistance up to 50% after NAC treatment and an increase in airway resistance up to 20% after placebo. Figure 3 shows the results for a nonresponder in terms of iRaw after NAC treatment. It could be observed that local resistance tended to increase up to 20% both after placebo and after NAC treatment.

Glutathione peroxidase and glutathione

The study showed a significant correlation ($R = -0.7$, $P = 0.011$) between the level of glutathione after NAC treatment and the change in resistance of the central airways ($iRaw_{cent}$) measured using imaging and computational fluid dynamics (Figure 4). No correlation was found between

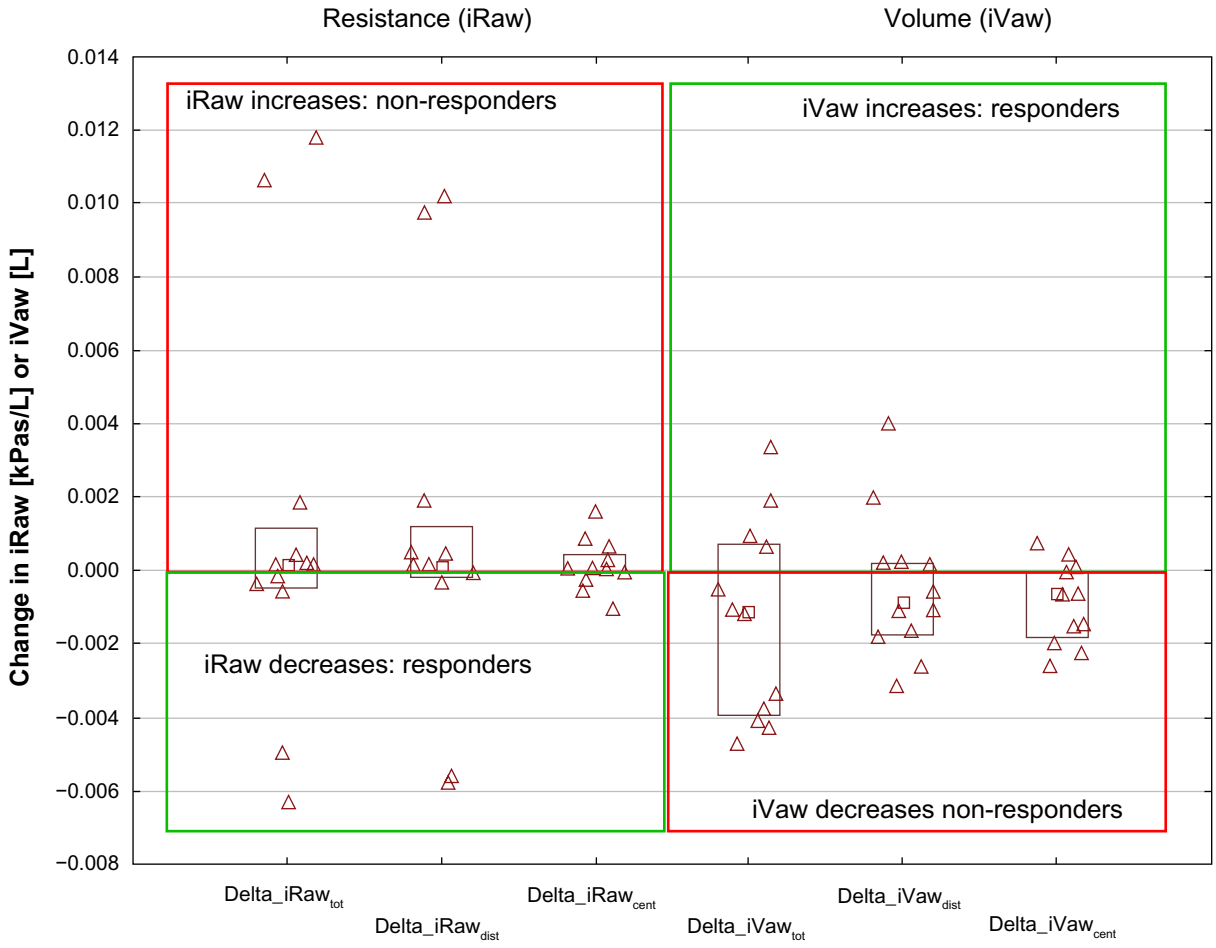

Figure 1 Changes in iRaw and iVaw for all patients.
Abbreviations: iRaw, image-based resistance; iVaw, image-based volume.

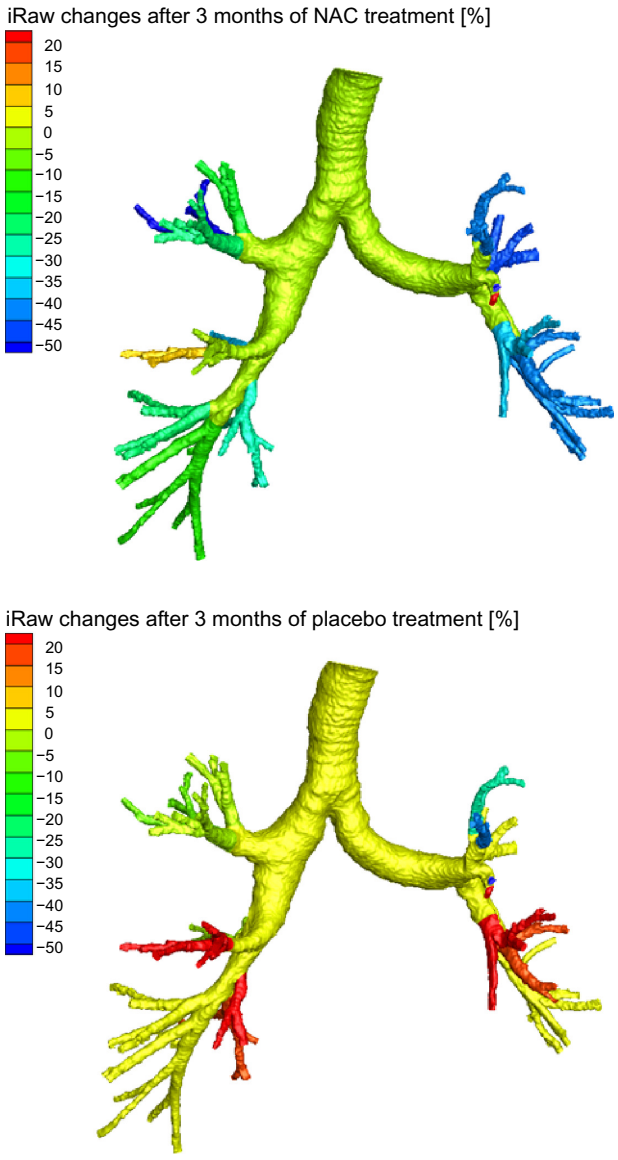

Figure 2 Changes in iRaw after 3 months of treatment with NAC (top) and placebo (bottom) in iRaw responders.
Abbreviations: NAC, N-acetylcysteine; iRaw, image-based resistance.

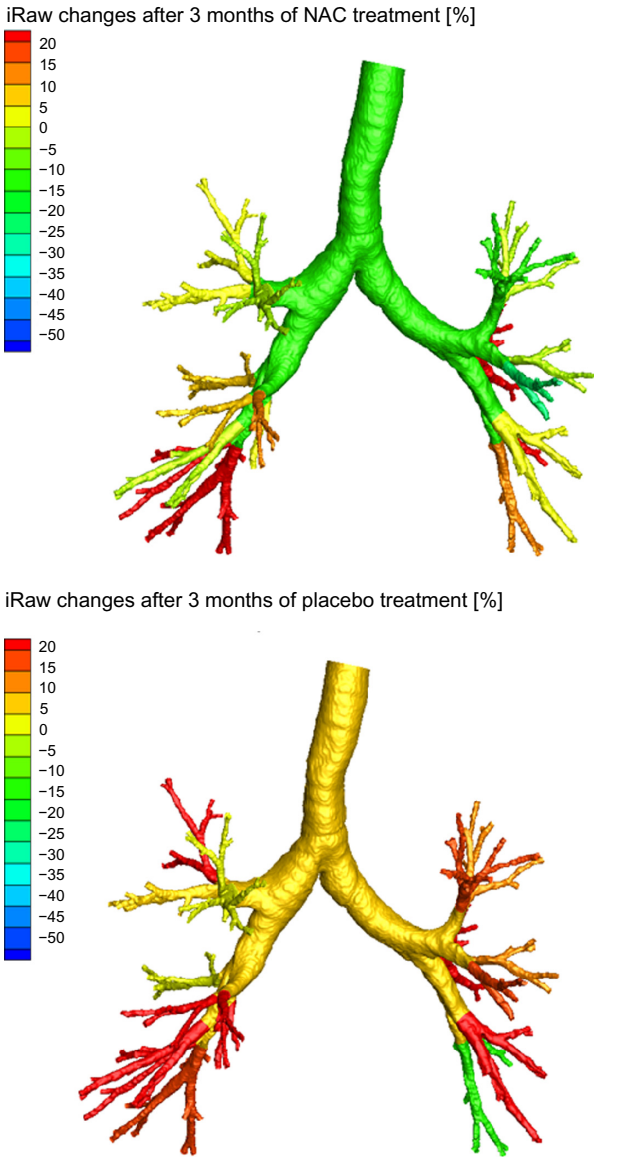

Figure 3 Changes in iRaw after 3 months of treatment with NAC (top) and placebo (bottom) in an iRaw nonresponder.
Abbreviations: NAC, N-acetylcysteine; iRaw, image-based resistance.

glutathione after placebo and $iRaw_{cent}$ after placebo. The level of glutathione peroxidase at baseline ($R = 0.61$, $P = 0.036$) and after placebo treatment ($R = 0.67$, $P = 0.017$) correlated with the change in distal airway resistance ($iRaw_{dist}$) after NAC treatment (Figure 5). No significant correlation was found between the baseline and placebo values for glutathione peroxidase and the change in $iRaw_{dist}$ after placebo. Using the Mann–Whitney *U* test, it could be observed that the level of glutathione after treatment was significantly higher ($P = 0.020$) in patients who demonstrated a reduction in $iRaw_{cent}$ compared with patients in whom $iRaw_{cent}$ stayed constant or increased (Figure 6). A similar result was found based on the segmented airway volume of the central airways ($iVaw_{cent}$). Glutathione after NAC treatment was significantly higher ($P = 0.042$) in patients with an increase in $iVaw_{cent}$. In patients who demonstrated a reduction in total (central + distal) image-based airway resistance ($iRaw_{tot}$), the change in glutathione peroxidase was significantly ($P = 0.035$) higher compared with patients in whom $iRaw_{tot}$ did increase. On average, glutathione peroxidase levels increased in the group with reduced $iRaw_{tot}$ and decreased in the group with elevated $iRaw_{tot}$ after treatment. FEV_1 also increased slightly in the group with reduced $iRaw_{tot}$ and decreased in the group with higher $iRaw_{tot}$ after NAC treatment, although the difference between the two groups was not significant (Figure 7).

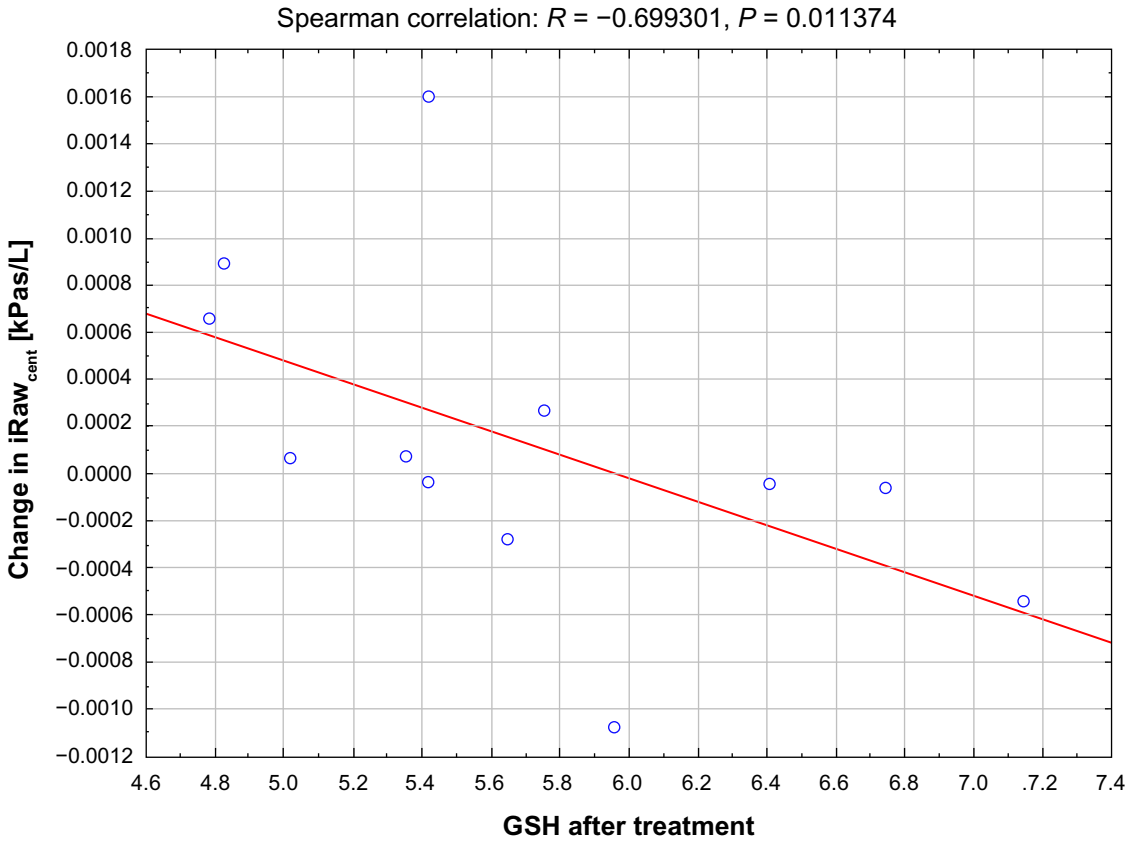

Figure 4 Significant correlation between change in computational fluid dynamics-based resistance of the central airways, $iRaw_{cent}$, and glutathione levels after NAC treatment.
Abbreviations: iRaw, image-based resistance; GSH, glutathione; NAC, N-acetylcysteine.

Hyperinflation

For patients in whom $iRaw_{dist}$ decreased, hyperinflation in terms of lobar functional residual capacity level also decreased (Figure 8). For patients in whom $iRaw_{dist}$ did not decrease, the median lobar functional residual capacity level increased. The difference between the two groups was significant ($P = 0.00084$). Two patients experienced a reduction in $iRaw_{dist}$ larger than 0.002 kPa/L. The Wilcoxon matched-pairs test showed a significant improvement in lobar inspiratory capacity (lobar total lung capacity – lobar functional residual capacity) in these patients compared with baseline ($P = 0.028$) and placebo ($P = 0.011$). A significant decline in lobar inspiratory capacity was observed between baseline and placebo ($P = 0.011$, Table 3). The SGRQ symptom score also improved in these two patients.

Discussion

This study aimed to assess the effect of high-dose NAC on image-based airway and lung geometry, inflammation, and oxidative stress in patients with moderate COPD. Previous studies[10,16] have demonstrated that functional respiratory imaging can detect changes that are not reflected in conventional pulmonary function tests. Significant correlations were found between imaging parameters and markers related to oxidative stress. The initial results of this placebo-controlled, crossover trial could assist in gaining further insight into the effect of NAC and selection of potential responders.

The effect of NAC in COPD patients has been the subject of many clinical trials in the past. The capability of NAC to increase levels of reduced glutathione and hence to enhance protection against oxidative stress has been clearly demonstrated in preclinical studies.[17–19] Nevertheless, it has been difficult to demonstrate consistently the added clinical value of NAC in COPD patients. Reasons often cited for this are the relatively small size of the clinical trials and variation in the dose of NAC used in the trials.[20] Positive effects of NAC demonstrated in previous trials include a reduction in air trapping and subsequent improvement in inspiratory capacity.[21] Also, a reduction in the hazard ratio for exacerbations was reported in BRONCUS (Bronchitis Randomized on N-acetylcysteine (NAC) Cost-Utility Study),[8] potentially due to enhanced protection against viral infections.[20] The use of lung function, and especially FEV_1, to establish the effect of NAC, which is not a bronchodilator, has been challenging because FEV_1

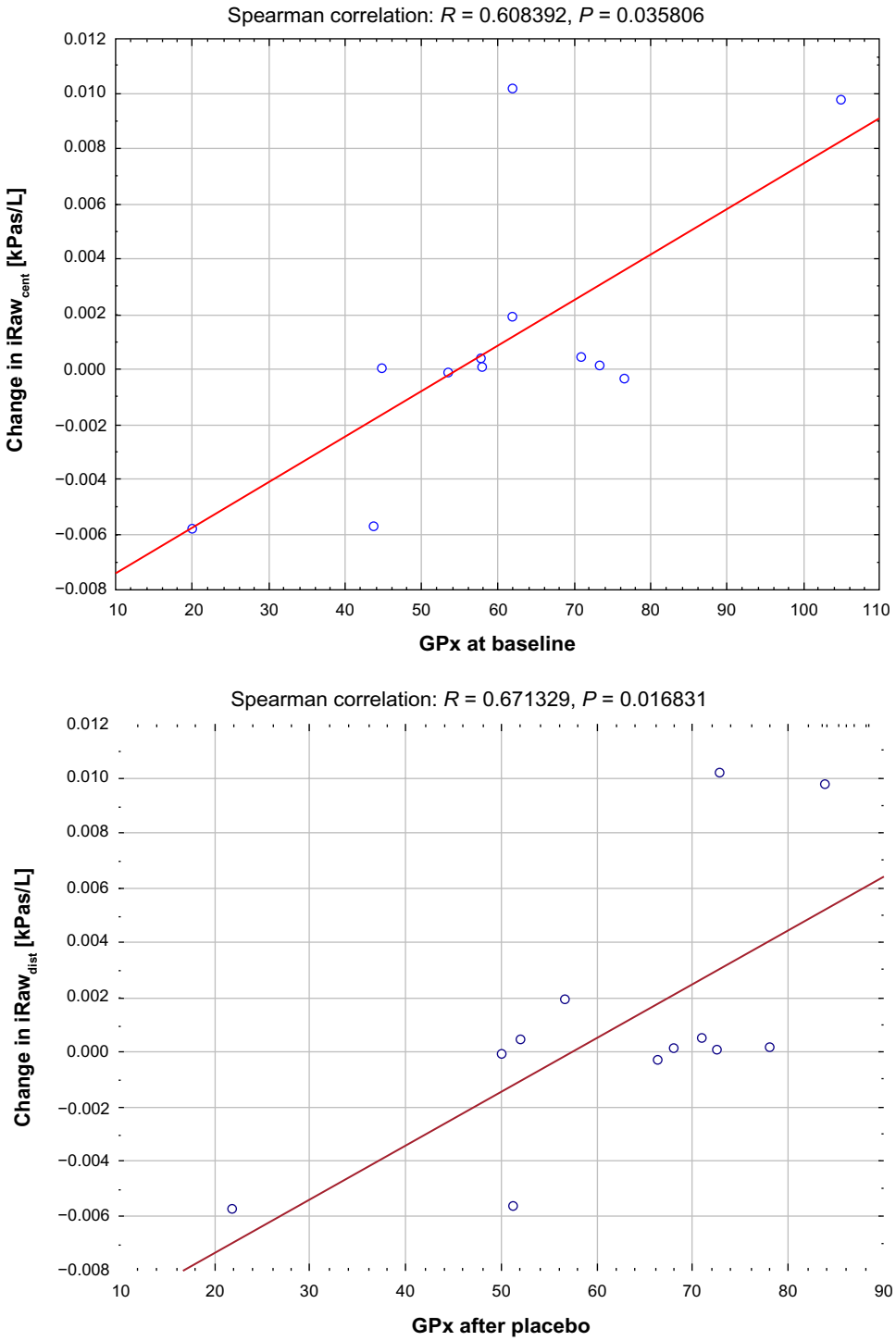

Figure 5 Significant correlation between change in computational fluid dynamics-based resistance of the distal airways $iRaw_{dist}$ and level of GPx at baseline (top) and after placebo (bottom).
Abbreviations: iRaw, image-based resistance; GPx, glutathione peroxidase.

is a poor predictor of clinical symptoms, exercise tolerance, and response to bronchodilators in COPD.[21–23]

Our placebo-controlled, crossover study, for the first time, uses novel imaging tools with a higher sensitivity to detect changes in the respiratory system induced by high-dose NAC. Previous studies have validated and demonstrated the capabilities of these new methods to show changes induced by short-acting and long-acting bronchodilators and anti-inflammatory compounds. Interestingly, even despite the small sample size, the current study was

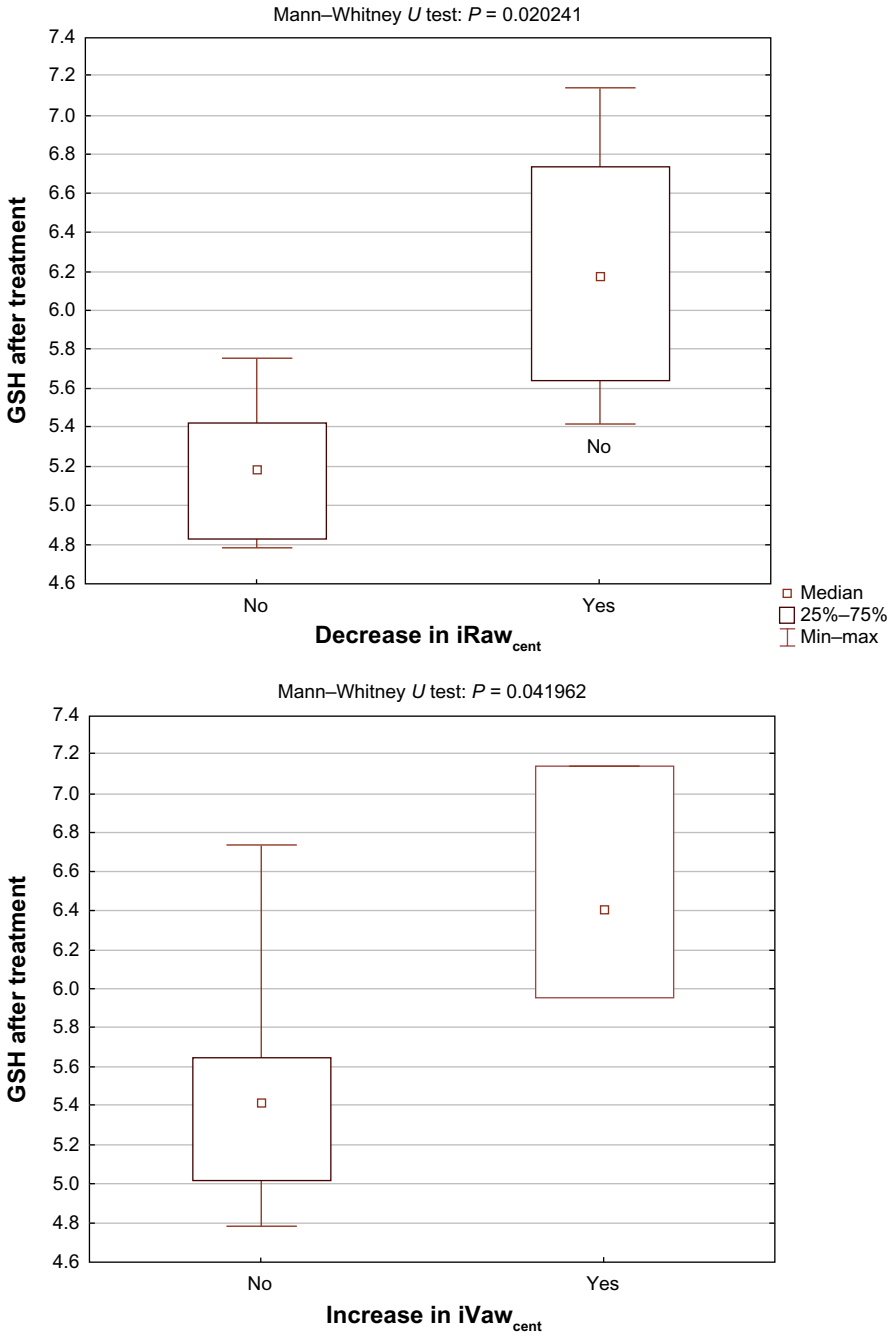

Figure 6 Glutathione after NAC treatment was significantly different for patients who experienced a decrease in $iRaw_{cent}$ (top) and an increase in $iVaw_{cent}$ (bottom).
Abbreviations: iRaw, image-based resistance; GSH, glutathione; iVaw, image-based volume.

able to show correlations between image-based parameters (ie, computational fluid dynamics-based resistance) and the enzyme glutathione peroxidase, which protects against oxidative stress, and the antioxidant glutathione. Both the enzyme and the antioxidant are known to be affected by NAC. To put these results into perspective, it is useful to compare the changes in central and distal airway resistance as measured by the CT/computational fluid dynamics combination with changes previously reported in our other imaging trials. It could be observed that for patients responding well to NAC, the magnitude of the reduction in airway resistance was in the same order as the effect induced by 320/90 µg budesonide/formoterol combination[10] and just below the maximum effect of 400 µg of salbutamol and 80 µg of ipratropium bromide.[16] As in previous studies, the placebo group also demonstrated in general an increase in airway resistance which indicates that, despite the lack of clear evidence, COPD patients need to use their

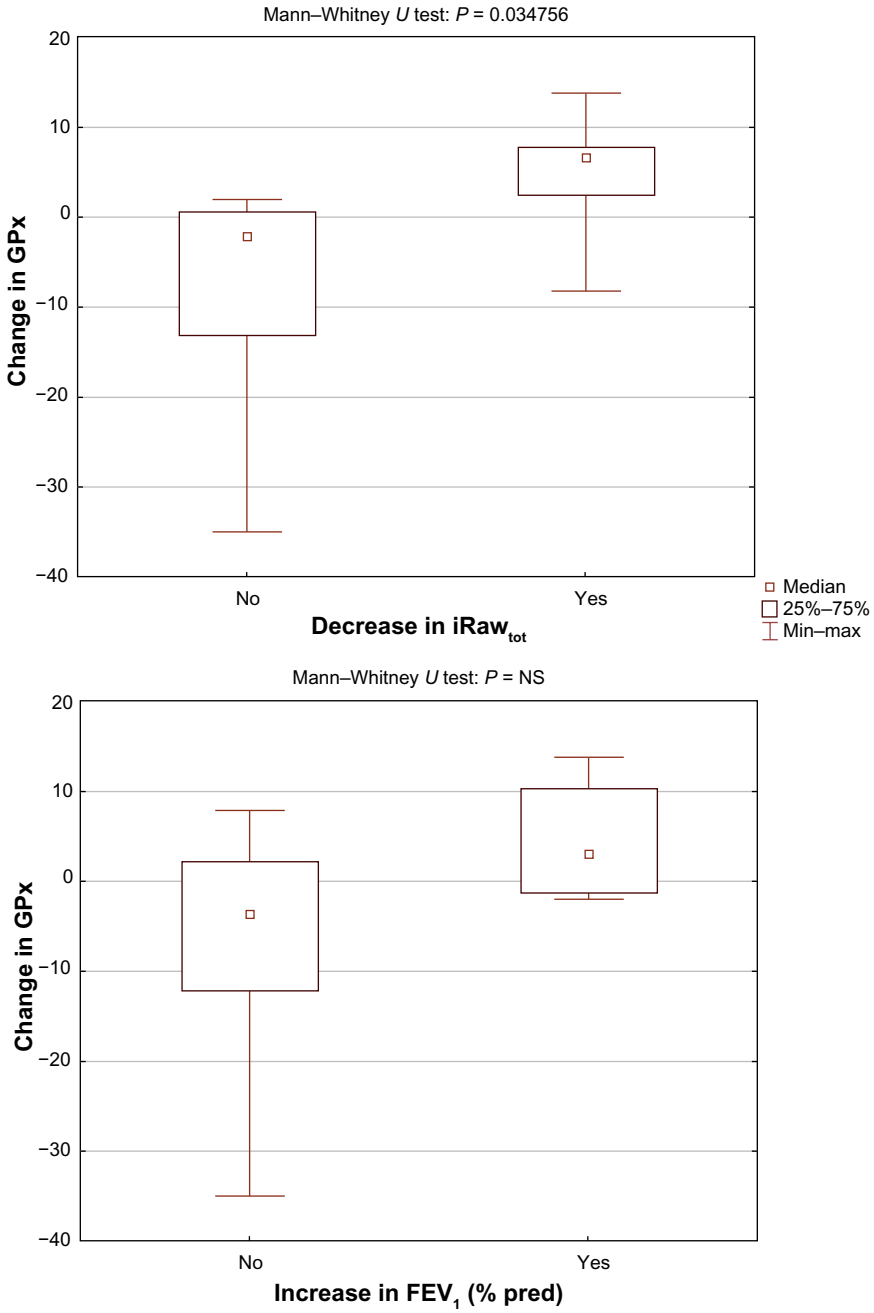

Figure 7 Significant difference in change in GPx between responders and nonresponders in terms of $iRaw_{tot}$ (top) and no significant difference in change in GPx between responders and nonresponders in terms of FEV_1 (bottom).
Abbreviations: iRaw, image-based resistance; GPx, glutathione peroxidase; FEV_1, forced expiratory volume in one second.

inhalation medication consistently to limit the decline in lung function.

The role of glutathione peroxidase appears to be interesting. The current study clearly shows a correlation between the baseline and placebo values for glutathione peroxidase and the change in resistance after treatment. It is known that the biochemical function of glutathione peroxidase is to reduce free H_2O_2 to water, thereby protecting the organism from oxidative stress. We could speculate that patients with low baseline glutathione peroxidase have additional reserves that could be activated by high doses of NAC, eventually resulting in a reduction in airway resistance. This hypothesis needs to be investigated further in a larger clinical trial in which the role of cytokines, not studied in the current trial, could also be explored further. If the hypothesis is confirmed, the level of glutathione peroxidase could be the basis for selecting patients who would benefit from treatment with NAC in addition to their current therapy.

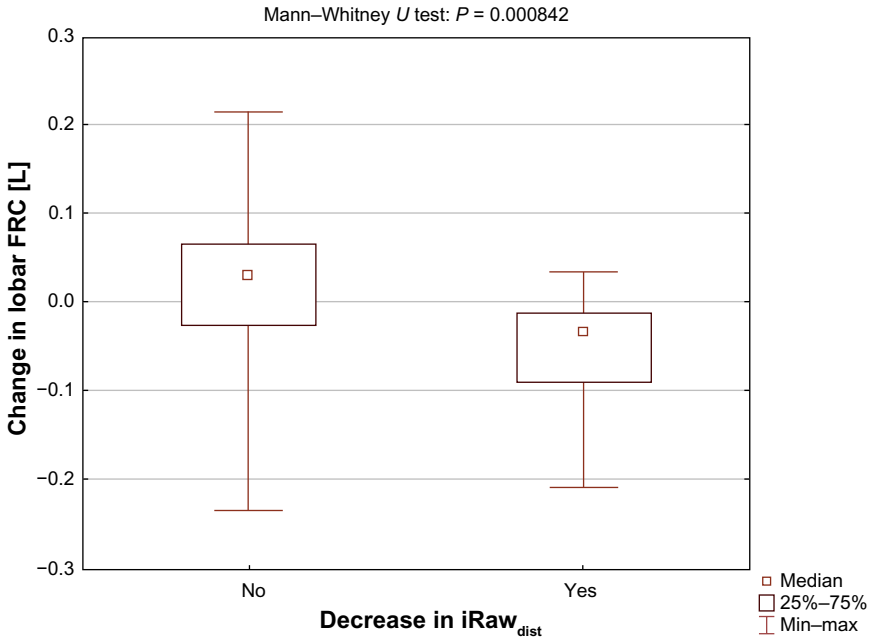

Figure 8 Significant difference in reduction of hyperinflation (lobar FRC volumes) between responders and nonresponders in terms of $iRaw_{dist}$.
Abbreviations: iRaw, image-based resistance; FRC, forced residual capacity.

Further, the current pilot study demonstrated that FEV_1 was not a good predictor for changes in glutathione peroxidase induced by NAC therapy. The $iRaw_{dist}$ on the other hand was able to group patients according to the difference in glutathione peroxidase with statistical significance. The latter can be explained by the enhanced signal-to-noise ratio of imaging. By focusing on a specific clinically relevant region, ie, the central and distal airways, it is possible to eliminate "noise" from the upper airway and the patient's effort. However, the spirometer's inherent black box approach does not allow this. In line with previous studies, it was demonstrated that NAC could have a beneficial effect on hyperinflation in a subset of patients. The functional residual capacity level of the lobes was significantly reduced in patients showing a decrease in distal airway resistance. Inspiratory capacity at a lobar level was improved in the best $iRaw_{dist}$ responders. Even though this was only in two patients, the improvement in SGRQ symptom score potentially indicates a beneficial effect of reduction in hyperinflation on the patient's quality of life. Recent large-scale studies also point in the same direction, whereby hyperinflation appears to be an important determinant of the patient's clinical condition.[24,25]

Table 3 Lobar inspiratory capacity decreased significantly after placebo and increased after treatment with NAC in patients with a reduction in $iRaw_{dist} < 0.002$ kPa/L

	Baseline	After placebo	After NAC
Inspiratory capacity (L) on lobe level (TLC-FRC)	0.63 ± 0.27	0.59 ± 0.26	0.65 ± 0.28
Mann–Whitney *U* test with baseline, *P*-value	–	0.011	0.028
Mann–Whitney *U* test with placebo, *P*-value	0.011	–	0.011

Abbreviations: NAC, N-acetylcysteine; TLC, total lung capacity; FRC, functional residual capacity; iRaw, image-based resistance.

The current pilot study demonstrates how new imaging tools can assist research into the pathophysiology of COPD and the subsequent effect of inhalation therapies. However, the sample size of the current study was very small and the results need to be confirmed in larger clinical trials. Nonetheless, there is increasing evidence that these novel imaging methods could complement existing pulmonary function tests and patient-reported outcome parameters to provide better phenotyping of COPD patients in order to enhance and expedite future development of respiratory drugs.

Author contributions

JDB, WV, SV, CVH, RC, PMP and WDB were responsible for the conception and design of the study, along with its analysis and interpretation. JDB, WV, SV, CVH, RC, PMP, and WDB drafted and revised the manuscript for important intellectual content.

Disclosure

JDB is a founder/shareholder of FluidDA NV, Kontich, Belgium. WV, SV, and CVH are employed by FluidDA NV, and

WDB is the director of FluidDA NV. PMP and RC have no conflicts of interest to report. The study was supported by Zambon SpA, Bresso, Italy.

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