



Contents lists available at ScienceDirect

Clinical and Translational Radiation Oncology

journal homepage: www.elsevier.com/locate/ctro

Original Research Article

Fractional exhaled nitric oxide as a potential biomarker for radiation pneumonitis in patients with non-small cell lung cancer: A pilot study



W.M. Szejniuk^{a,b,h,*}, M.S. Nielsen^c, D. Brønnum^d, Z. Takács-Szabó^e, U.M. Weinreich^{f,h}, L. Pilegaard Thomsen^g, M. Bøgsted^{b,h}, I. Jensen^c, T. McCulloch^{a,b,h}, U.G. Falkmer^{a,b,h}, J. Carlⁱ, O.D. Røe^{a,b,h,j}

^a Dept. of Oncology, Aalborg University Hospital, Denmark^b Clinical Cancer Research Center, Aalborg University Hospital, Denmark^c Dept. of Medical Physics, Aalborg University Hospital, Denmark^d Centre for Clinical Research, North Denmark Regional Hospital, Hjørring, Denmark^e Dept. of Radiology, Aalborg University Hospital, Denmark^f Dept. of Respiratory Diseases, Aalborg University Hospital, Denmark^g Dept. of Health Science and Technology, Aalborg University, Denmark^h Dept. of Clinical Medicine, Faculty of Medicine, Aalborg University, Denmarkⁱ Dept. of Oncology, Naestved Hospital, Zealand University Hospital, Denmark^j Dept. of Clinical Research and Molecular Medicine, NTNU, Trondheim, Norway

ARTICLE INFO

Article history:

Received 13 September 2019

Accepted 20 September 2019

Available online 26 September 2019

Keywords:

Non-small cell lung cancer

Radiation pneumonitis

Fractional exhaled nitric oxide

Thoracic radiation therapy

ABSTRACT

Introduction: The aim of the study was to investigate repetitive fractional exhaled nitric oxide (FeNO) measurements during high-dose radiation therapy (HDRT) and to evaluate the use of FeNO to predict symptomatic radiation pneumonitis (RP) in patients being treated for non-small cell lung cancer (NSCLC). **Materials and methods:** A total of 50 patients with NSCLC referred for HDRT were enrolled. FeNO was measured at baseline, weekly during HDRT, one month- and every third month after HDRT for a one-year follow-up period. The mean FeNO_(visit 0-6) was calculated using the arithmetic mean of the baseline and weekly measurements during HDRT. Patients with grade ≥ 2 of RP according to the Common Terminology Criteria for Adverse Events (CTCAE) were considered symptomatic.

Results: A total of 42 patients completed HDRT and weekly FeNO measurements. Grade ≥ 2 of RP was diagnosed in 24 (57%) patients. The mean FeNO_(visit 0-6) \pm standard deviation in patients with and without RP was 15.0 ± 7.1 ppb (95%CI: 12.0–18.0) and 10.3 ± 3.4 ppb (95%CI: 8.6–11.9) respectively with significant differences between the groups ($p = 0.0169$, 95%CI: 2.3–2.6). The leave-one-out cross-validated cut-off value of the mean FeNO_(visit 0-6) ≥ 14.8 ppb was predictive of grade ≥ 2 RP with a specificity of 71% and a positive predictive value of 78%.

Conclusions: The mean FeNO_(visit 0-6) in patients with symptomatic RP after HDRT for NSCLC was significantly higher than in patients without RP and may serve as a potential biomarker for RP.

© 2019 The Author(s). Published by Elsevier B.V. on behalf of European Society for Radiotherapy and Oncology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

High-dose radiation therapy (HDRT) is the main treatment for patients with localized non-small cell lung cancer (NSCLC) who are ineligible for surgery [1,2]. One of the most serious side effects of HDRT is radiation pneumonitis (RP), which is characterized by inflammatory damage to the irradiated lung parenchyma and can

lead to severe respiratory distress or even death [3]. The incidence of RP ranges from 8 to 50% depending on the diagnostic criteria [3–5]. One of the most frequently used diagnostic grading scale is the Common Terminology Criteria for Adverse Events (CTCAE).

Typical symptoms of RP include cough, dyspnea, fever and fatigue, which usually occur between 4 and 12 weeks after the end of radiotherapy [6]. In few cases RP develops during HDRT or shortly afterwards. The earlier RP is observed, the more severe the grade [7]. Treatment guidelines are based on nonrandomized clinical trials [8] and recommend prednisolone 1 mg/kg per day for several weeks [9].

* Corresponding author at: Dept. of Oncology, Aalborg University Hospital, Hobrovej 18-22, 9000 Aalborg, Denmark.

E-mail address: wms@rn.dk (W.M. Szejniuk).

Candidate predictive biomarkers lack satisfactory sensitivity and specificity [10–12]. Dose-volume histogram (DVH)-based HDRT allows minimization of side effects for organs at risk [5]. However, DVH estimation cannot assess the individual risk of RP [13]. Therefore, predictive markers are necessary.

One of the proposed markers of RP is fractional exhaled nitric oxide (FeNO), which may be increased in patients with lung cancer [14]. Some studies showed a decrease in FeNO after HDRT, with the exception of patients with RP [15–18]. Measurement of FeNO is noninvasive and reproducible [19–21], currently used to monitor inflammation in asthma [22].

This pilot study explores the value of repetitive FeNO measurements during HDRT to predict symptomatic RP in patients being treated for NSCLC. The study was performed at the Department of Oncology, Aalborg University Hospital, Denmark.

2. Materials and methods

2.1. Patients

Patients with NSCLC verified by histopathology or cytology and referred for HDRT were considered eligible. Staging was determined using the 8th edition of the tumor, node and metastasis (TNM) classification system for NSCLC [23]. Chemotherapy was administered according to the Danish National guidelines [1]. The exclusion criteria were previous thoracic radiotherapy, cystic fibrosis or sarcoidosis. Each patient signed an informed consent form before the enrolment. The follow-up period was censored at 12 months from HDRT or at the time of diagnosis of progressive disease by computed tomography (CT) scan. The study was performed in accordance with the principles of the Helsinki declaration. The project was approved by the National Committee of Health Ethics of North Denmark (reg. no N-20120029) and reported to the Data Protection Agency in Denmark (2008-58-0028).

2.2. Imaging assessment

Baseline CT or positron emission tomography-CT (PET-CT) of the chest was performed on average within 8 weeks before the HDRT. Follow-up CT scans were scheduled 4–6 weeks after the last HDRT fraction was administered and every three months until 12 months after HDRT. All imaging was re-evaluated by one senior consultant radiologist (ZTS) who was blinded to the clinical findings. Each CT was analyzed according to the Response Evaluation in Solid Tumors (RECIST) criteria version 1.1 [24].

2.3. Clinical assessment

Performance status (PS), weight, smoking status and administration of medicine were reported at each visit. Clinical signs of RP were investigated and assessed using the CTCAE version 4. Grade 1 was defined as asymptomatic RP with no medical intervention; grade 2 as symptomatic RP requiring steroid administration; grade 3 as severe symptomatic RP requiring oxygen treatment; grade 4 as life-threatening RP with respiratory compromise; and grade 5 as death due to RP [25].

2.4. High-dose radiation therapy

Target and normal tissue delineation and dose calculations were performed on PET-CT scans. The delineation of gross tumor volume (GTV), clinical target volume (CTV) and planning target volume (PTV) was performed according to the International Commission on Radiation Units & Measurements (ICRU 62) [26] and

the Danish National guidelines [1]. The radiotherapy dose plans were calculated using the Eclipse™ Treatment Planning System (TPS) from Aria® Oncology Information System, Varian Medical System (California, USA). Plans were optimized using either three dimensional (3D) conformal or intensity modulated radiotherapy (IMRT). Due to changes in the TPS algorithm during the inclusion period of the study, plans were recalculated using the anisotropic analytic algorithm (AAA) 13.7 with fixed beam data. The prescribed mean dose for the CTV was 60–66 Gray (Gy)/30–33 fractions/5 fractions per week with a target dose homogeneity within 95–107%. Constraints to the organs at risk were as follows: mean lung dose (MLD) < 20 Gy, V_5 (percentage of lung volume exceeding 5 Gy) ≤ 60%, V_{20} (percentage of lung volume exceeding 20 Gy) ≤ 40% for both lungs excluding GTV, V_{50} (percentage of heart volume exceeding 50 Gy) ≤ 20% for the heart, global hotspot < 115%, total dose of ≤ 66 Gy for the esophagus and ≤ 45 Gy for the spinal cord.

2.5. Chemotherapy

Carboplatin (AUC 5) was administered intravenously (i.v.) on day 1 and 60 mg/m² of vinorelbine per os (p.o.) was administered on day 1 and 8 every 3 weeks, and 3 cycles in total were given. Patients treated concomitantly received HDRT simultaneously with the second chemotherapy cycle. In the case of induction chemotherapy, HDRT was delivered after 3–4 cycles. Chemotherapy according to the Navelbine and Radiotherapy in Locally Advanced Lung cancer [27] protocol was also allowed (induction carboplatin 5 AUC i.v. on day 1 and vinorelbine 60 mg/m² p.o. on days 1 and 8 during the first cycle and 80 mg/m² p.o. on days 1 and 8 during the second cycle followed by concomitant vinorelbine (50 mg on three days of a week) during HDRT. If the patient was considered not eligible for concomitant treatment, HDRT alone was delivered.

2.6. Fractional exhaled nitric oxide measurements

FeNO measurements were performed using chemiluminescence analyzers (NIOX MINO® and VERO®, Aerocrine, Solna, Sweden). The results were reported in parts per billion (ppb). A single FeNO measurement was performed at baseline, followed by weekly measurements during the six weeks of HDRT, 4–6 weeks after HDRT and every third month for a one-year follow-up period. Patients were instructed to inhale to near-total lung capacity and to exhale afterwards with a constant flow rate of 50 ml/s for 10 s according to the international guidelines [28]. The FeNO measurements were performed before pulmonary function tests (PFTs) if those were planned on the same day. The arithmetic mean FeNO of baseline and weekly measurements during the HDRT were calculated for every patient and named the mean FeNO_(visit 0–6). Steroid treatment and smoking status during radiation were registered.

2.7. Pulmonary function tests

Spirometry and automatic lung parameter estimator (ALPE) measurements were performed at baseline, one month after HDRT and at the 12 month visit with additional ALPE measurements made during the fourth week of HDRT. Baseline spirometry was performed with a SPIDA® spirometer, while during the follow-up period, an Easy One® spirometer was used. The forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC) and Tiffeneau-Pinelli index (FEV1/FVC ratio) were registered. The ALPE measurements were performed with an Essential® (Mermaid Care, Denmark) estimator [29].

2.8. Statistical analysis

Longitudinal data were collected from 11 visits, including the baseline visit. Patients with RP CTCAE grade ≥ 2 were defined as the RP group while patients with RP CTCAE grade < 2 were allocated to the non-RP group. Patient characteristics between the groups were compared using the *t*-test, Mann-Whitney's *U* test or chi-square tests depending on whether the variables were continuous or categorical. The mean FeNO_(visit 0-6) for each patient in the RP and NRP groups was compared using the *t*-test after logarithmic transformation. Univariate logistic regression was used to test associations between clinical variables and RP. Statistically significant variables from the univariate analyses were used in the multivariate logistic regression analysis. The optimal cut-off value of FeNO_(visit 0-6) as a predictor of symptomatic RP was obtained as the value that maximized the sum of sensitivity and specificity (i.e., Youden's index) and visualized by the area under the receiver operating curve (AUC). Cross-validation of the sensitivity, specificity and positive and negative predictive values with leave-one-out was performed to test the generalizability of Youden's index as a predictor for symptomatic RP. Tests with a two-sided $p < 0.05$ were deemed statistically significant. All computations were performed using the statistical program Stata, version 14.2.

3. Results

Between October 2012 and December 2016, 50 patients were included in the study (Fig. 1). The results are based on data from 42 patients who completed the treatment (Table 1). In 24 patients, CTCAE grade ≥ 2 RP was diagnosed (23 grade 2, 1 grade 3). The average time to RP diagnosis was 81 days (range, 3–166; median, 69 days) after administration of the last HDRT fraction. Oral steroid treatment unrelated to RP (due to nausea / fatigue) during HDRT was administered in four and one patient in the RP and non-RP groups, respectively. One patient in the RP group was treated for asthma. Nineteen patients completed the follow-up period of 12 months without recurrence or progressive disease (PD) (Fig. 1).

The mean FeNO_(visit 0-6) \pm standard deviation for RP patients was 15.0 ± 7.1 ppb (95%CI: 12.0–18.0) compared to that for non-RP patients of 10.3 ± 3.4 ppb (95%CI: 8.6–11.9). The difference between the groups was statistically significant after log-transformation ($p = 0.017$, 95%CI: 2.3–2.6) (Fig. 2). The difference in the mean FeNO_(visit 0-6) remained statistically significant after adjustment for non-RP-related steroid treatment during HDRT

(OR 6.3, 95%CI: 1.2–33.6, $p = 0.03$) and dose of radiation therapy (OR 6.6, 95%CI: 1.0–43.1, $p = 0.05$) in the multivariate analysis. However, after adjustment for smoking status, the difference in the mean FeNO_(visit 0-6) showed a statistical trend (OR 4.87, 95%CI: 0.85–27.9, $p = 0.075$). Ten patients who smoked during HDRT had mean FeNO_(visit 0-6) of 9.3 ppb compared to non-smokers with FeNO_(visit 0-6) of 14.1 ppb. The predictive value of the mean FeNO_(visit 0-6) for RP was denoted as AUC = 0.73 (Fig. 3). The optimal Youden's index was 14.8 ppb, by yielding a sensitivity of 50% and a specificity of 94%. After cross-validation of the cut-off value, the mean FeNO_(visit 0-6) of 14.8 ppb showed a sensitivity and specificity for predicting symptomatic RP of 0.50 (0.31, 0.69) and 0.71 (0.42, 0.92), respectively. The positive predictive value was 0.78 (0.52, 0.94), and the negative predictive value was 0.42 (0.22, 0.63).

The mean of FeNO among patients who developed RP was higher compared to the non-RP group and increased after HDRT (Fig. 4A), becoming most distinct 4.5 months after HDRT (Table 2). The differences in the mean FeNO between the groups were statistically significant despite high-dose steroid treatment for RP (Fig. 4B). Only patients who developed RP showed a statistically significant increase in FeNO compared to their baseline measurements 4.5 months after HDRT ($p = 0.02$).

The majority of patients were treated with 66 Gy in 33 fractions. Neither the radiation dose, tumor localization (upper versus middle or lower lobe), nor the affected lung (left versus right) had statistically significant impact on development of RP. There were 5 cases of post-operative HDRT in the RP group compared to only one case of post-operative HDRT in the non-RP group (Table 1). The differences in dosimetric parameters, the mean MLD and V_5 to V_{60} were not statistically significant between the RP and non-RP groups (Table 3).

The difference between the baseline FEV1 and FEV1/FVC was not statistically significant in the RP and non-RP groups ($p = 0.2$ in both). The change in FEV1 after HDRT was not significant in either of the groups ($p = 0.18$). The ALPE measurements were performed in 35 patients at baseline. Seven patients had low baseline oxygen normalization pressure, three of them developed RP, and two of those had ventilation/perfusion mismatch.

The majority of patients were either active or previous smokers. There was no statistically significant difference in smoking history between the RP and non-RP groups (Table 1). The average number of pack-years was comparable between the groups, with 43.5 pack years in the RP group versus 52.5 in the non-RP group ($p = 0.3$).

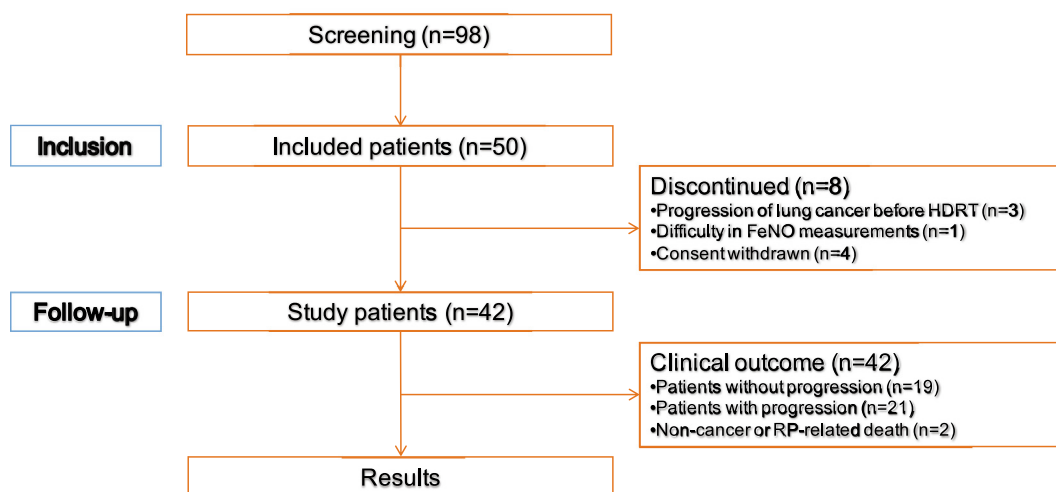


Fig. 1. Patient inclusion flow-chart.

Table 1
Patient characteristics related to radiation pneumonitis (RP).

	CTCAE grade ≥ 2 RP (n = 24)	CTCAE grade < 2 RP (n = 18)	p-Value
Age (y)			
Median	66	64	p = 0.93
Range	40–78	45–74	
Gender			
Female	10	8	p = 1.00
Male	14	10	
Performance status			
0	13	12	p = 0.53
1	11	6	
Smoking status			
Never	2	0	p = 0.07
Previous	19	11	
Current (during HDRT)	3	7	
Histopathology			
Adenocarcinoma	11	7	p = 0.92
Squamous cell carcinoma	8	7	
NSCLC, other type	5	4	
8th TNM stage			
IIA	1	0	p = 0.62
IIB	1	0	
IIIA	5	6	
IIIB	7	8	
IIIC	5	2	
IVA	0	1	
No tumor (postop. HDRT)	5	1	
Steroid treatment during HDRT			
Oral	4	1	p = 0.51
Inhalation	2	2	
Chemotherapy			
Concomitant	16	15	p = 0.45
Induction	6	3	
None	2	0	
Radiation therapy dose			
60 Gy/30 fx	6	7	p = 0.50
66 Gy/33 fx	18	11	
Radiation therapy type			
3D conformal	23	17	p = 0.68
IMRT	1	1	
HDRT side			
Left	7	6	p = 1.00
Right	17	12	
HDRT lobe localization			
Upper	12	11	p = 0.54
Middle/lower	12	7	

CTCAE – Common Terminology Criteria for Adverse Events; RP – radiation pneumonitis; HDRT – high-dose radiation therapy; NSCLC – non-small cell lung cancer; TNM – tumor, node, metastasis; IMRT – intensity-modulated radiation therapy.

4. Discussion

Increased FeNO in exhaled air is normally observed in patients with asthma, eosinophilic airway inflammation or high level of allergen exposure [28]. A physiologically elevated FeNO has been postulated to be a result of activation of a nitric oxide synthase, which activity remains increased in alveolar macrophages for three months after irradiation [30]. In our study, the levels of FeNO after HDRT were increased in both groups of patients, independent of RP. Interestingly, the levels of FeNO were already higher in patients with RP at baseline and throughout the HDRT, with the most significant increase in FeNO occurring approximately 4 months after HDRT. The FeNO_(visit 0-6) remained statistically significant even if accounting for radiation dose. Asthma was diagnosed in one patient from the RP group. However, this patient had a FeNO_(visit 0-6) < 10 ppb, which did not bias the value of the mean FeNO for the RP group. The standard deviations of the weekly FeNO measurements for every patient were higher than those reported in the literature, suggesting that weekly measurements can vary more than repetitive measurements and are of lower precision than

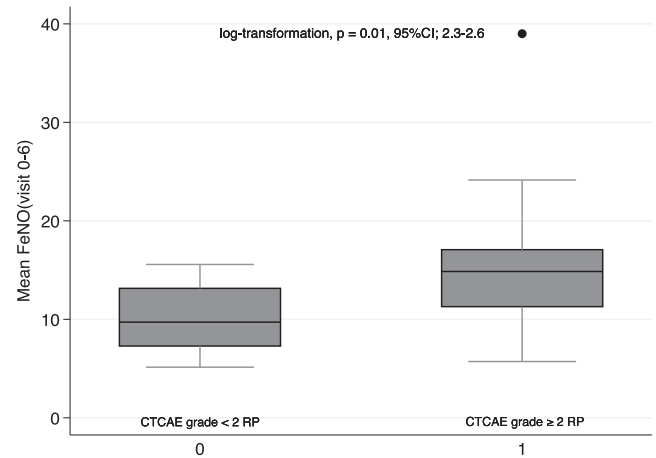


Fig. 2. The mean FeNO(visit 0–6) for the non-radiation pneumonitis (0 – non-RP) and radiation pneumonitis (1 – RP) groups.

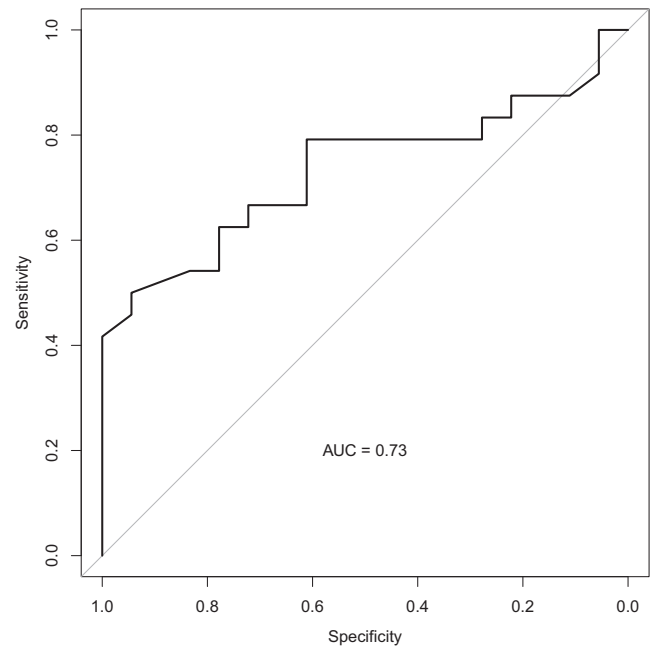


Fig. 3. Area under the receiver operating characteristic (ROC) curve (AUC) for the cut-off value of 14.8 ppb for the mean FeNO(visit 0–6).

3 ppb [19]. The FeNO measurements one month or later after the end of HDRT were not useful for predicting RP and therefore can be avoided in practice.

Dynamic changes in FeNO were described by Yamazaki et al. [31], who showed a decrease in FeNO immediately after HDRT in both groups of patients with and without RP. The authors calculated a threshold of the FeNO ratio (FeNO/minimum value of FeNO during HDRT) of 1.4 for RP symptoms, which had 100% sensitivity and 52% specificity. These findings confirmed results from previous studies of the predictive ability of an FeNO ratio > 1.4 to distinguish asymptomatic from symptomatic RP patients [16,17]. Our study cannot be used to reproduce the result from the mentioned studies as the FeNO was not measured immediately after the end of HDRT. However, the FeNO_(visit 0-6) in our study showed a higher specificity with AUC 0.73 compared previous studies.

Interestingly, FeNO levels were higher at baseline in patients with RP and persisted throughout the HDRT and follow-up period. The differences in FeNO levels between the groups could suggest a constitutive susceptibility trait to radiation-induced

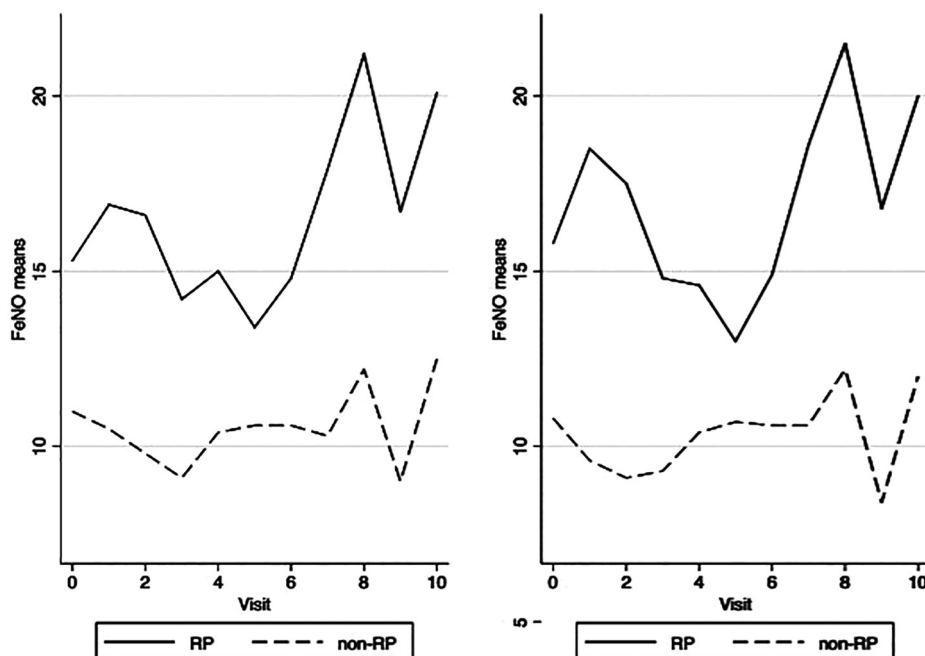


Fig. 4. Mean FeNO for the radiation pneumonitis (RP) and non-radiation pneumonitis (non-RP) groups including all patients (A) and excluding patients treated with steroids during HDRT (B).

Table 2

FeNO measurements at each visit in relation to the grade of radiation pneumonitis.

Mean FeNO \pm SD (ppb)	CTCAE grade \geq 2 RP n = 24	CTCAE grade < 2 RP n = 18	p-value
Visit 0 (baseline)	15.3 \pm 8.6 (95% CI: 11.4–19.2)	11.0 \pm 5.2 (95% CI: 8.4–13.6)	p = 0.08
Visit 1 (1. week of HDRT)	16.9 \pm 11.5 (95% CI: 11.9–21.9)	10.5 \pm 5.1 (95% CI: 7.8–13.2)	p = 0.03
Visit 2 (2. week of HDRT)	16.6 \pm 9.0 (95% CI: 12.7–20.5)	9.8 \pm 5.0 (95% CI: 7.4–12.3)	p = 0.02
Visit 3 (3. week of HDRT)	14.2 \pm 9.6 (95% CI: 10.0–18.4)	9.1 \pm 3.7 (95% CI: 7.3–10.9)	p = 0.02
Visit 4 (4. week of HDRT)	15.0 \pm 7.3 (95% CI: 11.2–18.1)	10.4 \pm 4.8 (95% CI: 8.0–12.8)	p = 0.03
Visit 5 (5. week of HDRT)	13.4 \pm 5.6 (95% CI: 11.0–15.7)	10.6 \pm 6.6 (95% CI: 7.3–13.9)	p = 0.07
Visit 6 (6. week of HDRT)	14.8 \pm 7.5 (95% CI: 11.6–17.9)	10.6 \pm 6.7 (95% CI: 7.1–14.0)	p = 0.03
Visit 7 (4–6 weeks after visit 6)	17.9 \pm 12.2 (95% CI: 12.6–23.2)	10.3 \pm 5.4 (95% CI: 7.6–13.0)	p = 0.02
Visit 8 (6 months after visit 1)	21.2 \pm 17.1 (95% CI: 13.4–28.9)	12.2 \pm 7.4 (95% CI: 7.9–16.5)	p = 0.01
Visit 9 (9 months after visit 1)	16.7 \pm 10.3 (95% CI: 11.6–21.8)	9.0 \pm 3.3 (95% CI: 7.0–10.9)	p = 0.01
Visit 10 (12 months after visit 1)	20.1 \pm 11.1 (95% CI: 13.4–26.9)	12.5 \pm 5.5 (95% CI: 8.3–16.8)	p = 0.1

FeNO – fractional exhaled nitric oxide; SD – standard deviation; ppb – parts per billion; CTCAE – Common Terminology Criteria for Adverse Events, RP – radiation pneumonitis; CI – confidence interval; HDRT – high-dose radiation therapy.

Table 3

Dosimetric parameters in relation to radiation pneumonitis.

Parameter	CTCAE grade \geq 2 RP n = 24	CTCAE grade < 2 RP n = 18	T-test (p-value)
MLD	14.2 (95%CI: 13.0–15.5)	14.1 (95%CI: 12.2–15.9)	T-test (p = 0.86)
V5 (%)	44.0 (95%CI: 39.5–48.6)	43.7 (95%CI: 38.7–48.8)	T-test (p = 0.92)
V10 (%)	34.0 (95%CI: 30.5–37.5)	34.3 (95%CI: 30.5–38.2)	T-test (p = 0.89)
V20 (%)	28.7 (95%CI: 25.9–31.5)	28.0 (95%CI: 24.6–31.4)	T-test (p = 0.75)
V40 (%)	25.2 (95%CI: 22.3–27.4)	24.6 (95%CI: 21.0–28.2)	T-test (p = 0.76)
V50 (%)	20.2 (95%CI: 17.9–22.6)	20.0 (95%CI: 16.5–23.5)	T-test (p = 0.88)
V60 (%)	14.3 (95%CI: 12.0–16.6)	14.6 (95%CI: 11.4–17.9)	T-test (p = 0.88)

CTCAE – Common Terminology Criteria for Adverse Events, RP – radiation pneumonitis; CI – confidence interval; MLD – mean lung dose; V5, 10, 20, 40, 50, 60 (%) – percentage of lung volume exceeding 5, 10, 20, 40, 50, 60 Gy, respectively.

lung injury and RP, expressed by elevated FeNO. Susceptibility to RP development have been described in patients with the rs189037 variant of the ataxia telangiectasia mutated (ATM) gene [32]. Similarly, different bronchial airway epithelial gene expression is correlated with FeNO in patients with various asthma phenotypes [33].

This study reported a rather high incidence of symptomatic RP compared to the literature. Different scoring methods and the lack of consensus on the definition of RP have been repeatedly discussed as a challenge in comparing both the severity and incidence of RP [34,35]. Several studies used either former version of CTCAE [7], different scoring systems, such as the Southwest

Oncology Group scale [4,36], late [37] or modified Radiation Therapy Oncology Group [3] system or unspecified scales [18,38,39]. Our study strictly followed the CTCAE version 4 grading considering only the clinical manifestations of radiation-induced lung injury with promptly steroid treatment at first appearance of symptoms of RP. The close and frequent monitoring of symptoms may be a possible explanation of the higher incidence of RP in our study compared to other retrospective studies or reports from clinical practice. Another plausible reason for the high RP incidence in our cohort was the relatively high radiation dose (60–66 Gy) compared to the average dose or radiation modalities used in other studies [4,16,40,41].

Smoking status seemed to be negatively correlated with RP, indicating a protective factor for RP development, similar to other reports [34,42]. Cigarette smoking leads to hypoxic saturation in normal and tumor parenchyma, which diminishes the effect of radiation. Therefore, the risk of side effects such as RP is lower. We observed that active smoking during HDRT influenced measurements of FeNO resulting in false negative and lower values of the mean FeNO_(visit 0-6), limiting its usefulness in prediction of RP.

Our study confirms that PFTs cannot predict the development of RP. Deterioration in PFTs, abnormal ventilation/perfusion mismatch and shunt were not correlated with RP. As shown in other studies [34], repetitive PFTs lack the ability to predict the development of RP.

This study reports a higher rate of successful measurements than other trials [19]. Only one out of fifty patients could not perform FeNO measurement, showing that longitudinal FeNO measurements are practically feasible. The measurement method is robust with high reproducibility, even when performed by different operators with stationary or portable devices [19,21,43].

The limitation of the study is a small sample size with possible impact of few cases on results and conclusions. A possible drawback of the method is the precision of <3 ppb for values <30 ppb [19]. Therefore, the margin of +/-3 ppb should be added to the cut-off value of 14.8 ppb for the mean FeNO_(visit 0-6). A mean FeNO_(visit 0-6) less than 14.8 ppb should be interpreted with awareness for the measurement uncertainty.

Weekly measurements of FeNO are feasible during HDRT. A cut-off value of 14.8 ppb for the mean FeNO_(visit 0-6) can be considered as a potential predictive marker of RP risk. Neither dosimetric parameters nor PFTs were predictive of RP. Regular clinical follow-up of at least 6 months is necessary for patients treated with HDRT in order to detect RP. The results should be further explored in a randomized trial with steroid treatment introduced after the end of HDRT in the group of patients with a high mean FeNO_(visit 0-6). Finally, the data indicate a possible genetic susceptibility for RP expressed in differential baseline FeNO levels.

Declaration of Competing Interest

The authors declare no competing financial interest.

Acknowledgment

This work was supported by the Ebba og Aksel Schøllins Fond (grant number 63801 HC).

References

- [1] Strålebehandling ved lungekræft. Dansk Onkologisk Lungecancer Gruppe. <http://dolg.dk/index.php?side=rekommandationer>. Published 2010. Accessed Mrc 03, 2019.
- [2] Krzakowski M, Provencio M, Utracka-Hutka B, et al. Oral vinorelbine and cisplatin as induction chemotherapy and concomitant chemo-radiotherapy in stage III. *J Thorac Oncol* 2008;3(9):994–1002. <https://doi.org/10.1097/ITO.0b013e31818396cb>.
- [3] Wang JY, Chen KY, Wang JT, et al. Outcome and prognostic factors for patients with non-small-cell lung cancer and severe radiation pneumonitis. *Int J Radiat Oncol Biol Phys* 2002;54(3):735–41. [https://doi.org/10.1016/S0360-3016\(02\)02994-2](https://doi.org/10.1016/S0360-3016(02)02994-2).
- [4] Jenkins P, Watts J. An improved model for predicting radiation pneumonitis incorporating clinical and dosimetric variables. *Int J Radiat Oncol Biol Phys* 2011;80(4):1023–9. <https://doi.org/10.1016/j.ijrobp.2010.03.058>.
- [5] Rodrigues G, Lock M, D'Souza D, Yu E, Van Dyk J. Prediction of radiation pneumonitis by dose-volume histogram parameters in lung cancer—a systematic review. *Radiother Oncol* 2004;71(2):127–38. <https://doi.org/10.1016/j.radonc.2004.02.015>.
- [6] Choi YW, Munden RF, Erasmus JJ, et al. Effects of radiation therapy on the lung: radiologic appearances and differential diagnosis. *Radiographics* 2004;24(4):985–7. <https://doi.org/10.1148/rg.244035160>. discussion 998.
- [7] Takeda A, Ohashi T, Kunieda E, et al. Early graphical appearance of radiation pneumonitis correlates with the severity of radiation pneumonitis after stereotactic body radiotherapy (SBRT) in patients with lung tumors. *Int J Radiat Oncol Biol Phys* 2010;77(3):685–90. <https://doi.org/10.1016/j.ijrobp.2009.06.001>.
- [8] Fujino M, Shirato H, Onishi H, et al. Characteristics of patients who developed radiation pneumonitis requiring steroid therapy after stereotactic irradiation for lung tumors. *Cancer J* 2006;12(1).
- [9] McDonald S, Rubin P, Phillips TL, Marks LB. Injury to the lung from cancer therapy: Clinical syndromes, measurable endpoints, and potential scoring systems. *Int J Radiat Oncol Biol Phys* 1995;31(5):1187–203. [https://doi.org/10.1016/0360-3016\(94\)00429-0](https://doi.org/10.1016/0360-3016(94)00429-0).
- [10] Hara R. Serum levels of KL-6 for predicting the occurrence of radiation pneumonitis after stereotactic radiotherapy for lung tumors. *Chest* 2004;125(1):340–4. <https://doi.org/10.1378/chest.125.1.340>.
- [11] Chen Y, Hyrien O, Williams J, Okunieff P, Smudzyn T, Rubin P. Interleukin (IL)-1A and IL-6: Applications to the predictive diagnostic testing of radiation pneumonitis. *Int J Radiat Oncol Biol Phys* 2005;62(1):260–6. <https://doi.org/10.1016/j.ijrobp.2005.01.041>.
- [12] Anscher MS, Kong F-M, Andrews K, et al. Plasma transforming growth factor β 1 as a predictor of radiation pneumonitis. *Int J Radiat Oncol Biol Phys* 1999;41(5):1029–35. [https://doi.org/10.1016/S0360-3016\(98\)00154-0](https://doi.org/10.1016/S0360-3016(98)00154-0).
- [13] Lind PA, Marks LB, Hollis D, et al. Receiver operating characteristic curves to assess predictors of radiation-induced symptomatic lung injury. *Int J Radiat Oncol Biol Phys* 2002;54(2):340–7. [https://doi.org/10.1016/S0360-3016\(02\)02932-2](https://doi.org/10.1016/S0360-3016(02)02932-2).
- [14] Liu CY, Wang CH, Chen TC, Lin HC, Yu CT, Kuo HP. Increased level of exhaled nitric oxide and up-regulation of inducible nitric oxide synthase in patients with primary lung cancer. *Br J Cancer* 1998;78(4):534–41. <https://www.ncbi.nlm.nih.gov/pubmed/9716040>.
- [15] Koizumi M, Yamazaki H, Toyokawa K, et al. Influence of thoracic radiotherapy on exhaled nitric oxide levels in patients with lung cancer. *Jpn J Clin Oncol* 2001;31(4):142–6. <http://www.ncbi.nlm.nih.gov/pubmed/11386459>.
- [16] Guerrero T, Martinez J, McCurdy MR, Wolksi M, McAleer MF. Elevation in exhaled nitric oxide predicts for radiation pneumonitis. *Int J Radiat Oncol Biol Phys* 2012;82(2):981–8. <https://doi.org/10.1016/j.ijrobp.2010.08.041>.
- [17] McCurdy MR, Wazni MW, Martinez J, McAleer MF, Guerrero T. Exhaled nitric oxide predicts radiation pneumonitis in esophageal and lung cancer patients receiving thoracic radiation. *Radiother Oncol* 2011;101(3):443–8. <https://doi.org/10.1016/j.radonc.2011.08.035>.
- [18] Enache I, Noel G, Jeung MY, et al. Can exhaled NO fraction predict radiotherapy-induced lung toxicity in lung cancer patients? *Radiat Oncol* 2012;7(1):117. <https://doi.org/10.1186/1748-717X-7-117>.
- [19] Gill M, Graff GR, Adler AJ, Dweik RA. Validation study of fractional exhaled nitric oxide measurements using a handheld monitoring device. *J Asthma* 2006;43(10):731–4. <https://doi.org/10.1080/02770900601031045>.
- [20] Takalo R, Piirilä P, Sovijärvi ARA. Repeatability of successive measurements with a portable nitric oxide analyser in patients with suggested or diagnosed asthma. *Scand J Clin Lab Invest* 2008;68(8):830–2. <https://doi.org/10.1080/00365510802621158>.
- [21] Khalili B, Boggs PB, Bahna SL. Reliability of a new hand-held device for the measurement of exhaled nitric oxide. *Allergy* 2007;62(10):1171–4. <https://doi.org/10.1111/j.1398-9995.2007.01475.x>.
- [22] Barnes PJ, Dweik RA, Gelb AF, et al. Exhaled nitric oxide in pulmonary diseases a comprehensive review. *Chest* 2010;138(3):682–92. <https://doi.org/10.1378/chest.09-2090>.
- [23] Goldstraw P, Chansky K, Crowley J, et al. The IASLC lung cancer staging project: Proposals for revision of the TNM stage groupings in the forthcoming (eighth) edition of the TNM Classification for lung cancer. *J Thorac Oncol* 2016;11(1):39–51. <https://doi.org/10.1016/j.jtho.2015.09.009>.
- [24] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancy J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer* 2009;45(2):228–47. <https://doi.org/10.1016/j.ejca.2008.10.026>.
- [25] National Institutes of Health. National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) version 4. https://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf. Published 2010. Accessed March 03, 2019.
- [26] I.C.R.U. report 62: Prescribing, recording and reporting photon beam therapy (supplement to ICRU report 50) Bethesda MD Int Com Radiat Units Meas. 1999.

- [27] Hansen O, Schytte T, Brink C, et al. NARLAL – Navelbine and radiotherapy in locally advanced. *Lung Cancer* 2009.
- [28] ATS/ERS. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. *Am J Respir Crit Care Med* 2005;171(8):912–30. <https://doi.org/10.1164/rccm.200406-710ST>.
- [29] Rees SE, Kjærgaard S, Thorgaard P, Malczynski J, Toft E, Andreassen S. The automatic lung parameter estimator (ALPE) system: non-invasive estimation of pulmonary gas exchange parameters in 10–15 minutes. *J Clin Monit Comput* 2002;17(1):43–52. <https://doi.org/10.1023/A:1015456818195>.
- [30] Giaid A, Lehnert SM, Chehayeb B, Chehayeb D, Kaplan I, Shenouda G. Inducible nitric oxide synthase and nitrotyrosine in mice with radiation-induced lung damage. *Am J Clin Oncol* 2003;26(4). <https://doi.org/10.1097/O1.COC.0000077940.05196.86>.
- [31] Yamazaki H, Aibe N, Nakamura S, et al. Measurement of exhaled nitric oxide and serum surfactant protein D levels for monitoring radiation pneumonitis following thoracic radiotherapy. *Oncol Lett* 2017;14(4):4190–6. <https://doi.org/10.3892/ol.2017.6691>.
- [32] Yan Z, Tong X, Ma Y, et al. Association between ATM gene polymorphisms, lung cancer susceptibility and radiation-induced pneumonitis: A meta-analysis. *BMC Pulm Med* 2017;17(1):1–8. <https://doi.org/10.1186/s12890-017-0555-7>.
- [33] Modena BD, Tedrow JR, Milosevic J, et al. Gene expression in relation to exhaled nitric oxide identifies novel asthma phenotypes with unique biomolecular pathways. *Am J Respir Crit Care Med* 2014;190(12):1363–72. <https://doi.org/10.1164/rccm.201406-1099OC>.
- [34] Vogelius IS, Bentzen SM. A literature-based meta-analysis of clinical risk factors for development of radiation induced pneumonitis. *Acta Oncol* 2012;51(8):1–7. <https://doi.org/10.1038/jid.2014.371>.
- [35] Faria SL, Aslani M, Tafazoli FS, Souhami L, Freeman CR. The challenge of scoring radiation-induced lung toxicity. *Clin Oncol (R Coll Radiol)* 2009;21(5):371–5. <https://doi.org/10.1016/j.clon.2009.01.017>.
- [36] Jenkins P, Welsh A. Computed tomography appearance of early radiation injury to the lung: Correlation with clinical and dosimetric factors. *Int J Radiat Oncol Biol Phys* 2011;81(1):97–103. <https://doi.org/10.1016/j.ijrobp.2010.05.017>.
- [37] Graves PR, Siddiqui F, Anscher MS, Movsas B. Radiation pulmonary toxicity: From mechanisms to management. *Semin Radiat Oncol* 2010;20(3):201–7. <https://doi.org/10.1016/j.semradonc.2010.01.010>.
- [38] Palma DA, Senan S, Haasbeek CJA, Verbakel WFAR, Vincent A, Lagerwaard F. Radiological and clinical pneumonitis after stereotactic lung radiotherapy: A matched analysis of three-dimensional conformal and volumetric-modulated arc therapy techniques. *Int J Radiat Oncol Biol Phys* 2011;80(2):506–13. <https://doi.org/10.1016/j.ijrobp.2010.02.032>.
- [39] Marks LB, Fan M, Clough R, et al. Radiation-induced pulmonary injury: symptomatic versus subclinical endpoints. *Int J Radiat Biol* 2000;76(4):469–75. <http://www.ncbi.nlm.nih.gov/pubmed/10815626>.
- [40] Palma DA, vanSörnsen de Koste JR, Verbakel WFAR, et al. A new approach to quantifying lung damage after stereotactic body radiation therapy. *Acta Oncol* 2011;50(4):509–17. <https://doi.org/10.3109/0284186X.2010.541934>.
- [41] Guckenberger M, Heilman K, Wulf J, Mueller G, Beckmann G, Flentje M. Pulmonary injury and tumor response after stereotactic body radiotherapy (SBRT): results of a serial follow-up CT study. *Radiother Oncol* 2007;85(3):435–42. <https://doi.org/10.1016/j.radonc.2007.10.044>.
- [42] Zhang X-J, Sun J-G, Sun J, et al. Prediction of radiation pneumonitis in lung cancer patients: a systematic review. *J Cancer Res Clin Oncol* 2012;138(12):2103–16. <https://doi.org/10.1007/s00432-012-1284-1>.
- [43] Alving K, Janson C, Nordvall L. Performance of a new hand-held device for exhaled nitric oxide measurement in adults and children. *Respir Res* 2006;7(1):67. <https://doi.org/10.1186/1465-9921-7-67>.