Cigarette smoke retention and bronchodilation in patients with COPD. A controlled randomized trial

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KEYWORDS
COPD; Interaction; Smoke retention; Smoking

Introduction: Bronchodilators are the cornerstone for symptomatic treatment of chronic obstructive pulmonary disease (COPD). Many patients use these agents while persisting in their habit of cigarette smoking. We hypothesized that bronchodilators increase pulmonary retention of cigarette smoke and hence the risk of smoking-related (cardiovascular) disease. Our aim was to investigate if bronchodilation causes increased pulmonary retention of cigarette smoke in patients with COPD.

Methods: A double-blinded, placebo-controlled, randomized crossover trial, in which COPD patients smoked cigarettes during undilated conditions at one session and maximal bronchodilated conditions at the other session. Co-primary outcomes were pulmonary tar and nicotine retention. We performed a secondary analysis that excludes errors due to possible contamination. Secondary outcomes included the biomarkers C-reactive protein and fibrinogen, and smoke inhalation patterns.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a highly prevalent chronic condition, characterized by debilitating and progressive airflow obstruction. Unlike cardiovascular disease and other chronic conditions, prevalence and mortality of COPD still increase globally. In addition, patients with COPD often suffer from co-morbidity (cardiovascular disease; lung cancer) that contributes to mortality. Apart from an independent association between COPD and cardiovascular disease, both diseases share a common risk factor, i.e. cigarette smoking. Whereas approximately 90% of COPD is caused by smoking, about 50% of patients with diagnosed COPD continue to smoke, often despite intensive smoking cessation programs.

Bronchodilators are the cornerstone of the pharmacotherapeutic management of COPD patients, with a current trend towards the use of long-acting and ultra long-acting drugs. However, there is a controversy about the safety of bronchodilators, which in particular concerns their cardiovascular effects. Recent large randomized controlled trials observed a possible protective effect on (cardiovascular) mortality, but meta-analyses report either no or a hazardous effect.

We hypothesized an interaction between bronchodilators and smoking that may explain these discrepancies in safety profiles. Through a change in hyperinflation and/or breathing patterns, bronchodilators could affect COPD patients’ smoking behavior — including both increased and deeper smoke inhalation. Consequently, a more efficient smoke exposure and a subsequent increase in smoke retention could result in a modified risk profile to develop cigarette smoke-related diseases like cardiovascular disease. Potentially, this interaction might have implications in physicians’ decision making with regard to bronchodilator treatment in COPD patients who persist in their habit of smoking cigarettes.

Continuing our pilot study on interaction between smoking and bronchodilatation, the study reported in this paper hypothesized that maximal bronchodilation would increase the retention of cigarette smoke constituents, such as tar and nicotine, in COPD patients who smoke cigarettes, and consequently would increase biomarkers, such as C-reactive protein and fibrinogen.

Methods

We conducted a randomized, double-blind, double-dummy, placebo-controlled, crossover trial in patients with COPD, in which participants smoked cigarettes during both undilated and maximal bronchodilated conditions. Study methods have been ethnically approved by the CMO region Nijmegen-Arnhem (CMO 2009/037), registered at www.clinicaltrials.gov (NCT00981851), and published in detail.

Participants

COPD patients were recruited from respiratory clinics of the Radboud University Nijmegen Medical Centre (RUNMC), two neighboring general hospitals, and nine family practices. Selection criteria included: age 40–80 years; a diagnosis of COPD, GOLD stage 2 or 3; current smokers; absence of interfering pulmonary diseases, including asthma. Participants were advised on smoking cessation.

Interventions

Participants abstained from smoking and bronchodilators according to a pre-specified schedule. In a designated laboratory room of the RUNMC, participants smoked one Coresta Monitor No. 6 (CM6) cigarette before and one after inhalation of placebo aerosols in one session, and before and after maximal bronchodilation in another session, with one week between both sessions. During the measurements, participants were instructed to smoke as they normally did. Smoking conditions were standardized, including wearing a nose clip, electrical cigarette ignition, smoking up to 32 mm from the filter end, and exhaling through Cambridge filters. These filters trap 99.9% of particles larger than 100 nm, also referred to as tar (ISO 3308). CM6 cigarettes were conditioned at 22 °C and 60% relative humidity for at least 2 days (ISO 3308). Maximal bronchodilation was achieved by two aerosol inhalers: 5 μg tiotropium Respimat and 400 μg salbutamol via Volumatic spacer.

Outcomes and measurements

Changes of percentage of pulmonary retention of tar and nicotine were the co-primary outcomes. The proportional retention equals: (inhalation weight – exhalation weight)/ inhalation weight. Inhalation weights of tar and nicotine were calculated from a regression model based on cigarette filter nicotine weight analysis, including corrections from simultaneous blank filter measurements. As participants exhaled through Cambridge filters, tar exhalation weights were derived from filter weight increments after smoking, and nicotine exhalation weights from their substance analyses.
We also evaluated cardiovascular risk biomarkers from blood samples, smoke inhalation patterns from the Vivometrics Lifeshirt®, and pulmonary function by spirometry (Fig. 1). Consequently, our secondary outcomes included: plasma C-reactive protein and fibrinogen levels; smoke in- and exhalation volume and time; forced expiratory volume in one second (FEV₁) and forced volume capacity (FVC).

Sample size and randomization

Our statistician (RA) determined that in a crossover design with paired samples, a number of 34 patients is sufficient to demonstrate a pre and post mean difference of smoke retention between undilated and bronchodilated smoking conditions (assumptions: \( \alpha = 0.05, 1 - \beta = 0.80 \), two-tailed testing, \( c = 7.9 \)): \( n = c/\beta^2 + 2 \).¹⁹ We calculated a medium standardized effect size \( (\bar{d}) \) of 0.5, derived from a 10% standard deviation of smoke retention and a 5% increase of smoke retention (as due to a 20% change of FVC).¹⁹ The medication sequence was obtained by computer-generated block randomization with a block size of 2. An independent research nurse concealed allocation and secured double-blinding by preparing identical active medication and placebo canisters.

Statistical analyses

Individual statistical differences between undilated and bronchodilated smoking conditions were analyzed by linear mixed models, including adjustments for potential learning and carryover effects. As suspicions were raised of filter contamination in eight sessions (their unused blank filters showed positive measurements of nicotine), we performed a secondary analysis without these sessions to control for contamination. Furthermore, we analyzed the effect of bronchodilation on biomarkers, smoke inhalation patterns and pulmonary function. We evaluated associations between the level of smoke retention and the level of obstruction, by Pearson’s correlation analyses, to test for dose–effect relationships. All analyses were performed in SPSS 16.0. \( p \)-Values were set at 0.05 for statistical significance testing, confidence intervals (CI) at 95%.

Results

Study population

Patients were recruited from October 2009 to March 2011. Of 241 eligible candidates approached, we recruited 39 patients for participation, of whom 35 (90%) completed the study and were included in the final analyses (Fig. 2). Of 70 measurement sessions (each session includes one of the two measurement sessions of one patient), 14 sessions had missing values on nicotine exhalation due to values below the limit of quantification, and 5 sessions showed negative tar retentions (−3% to −11%). Eight sessions suffered from positive blanks, including 3 of the 5 sessions with negative tar retentions.

Table 1 shows the baseline characteristics at randomization: 18 males (51%); mean (± sd) age 59.5 years (±8.8); mean FEV₁, 1.74 l (±0.53) or 60% (±12) from predicted; mean cumulative smoking exposure 37.3 packyears (±25.7). FEV₁ and FVC both increased after bronchodilation (FEV₁, 277 ml (11% from predicted), \( p < 0.001 \); FVC 200 ml, \( p < 0.001 \) but not after placebo (FEV₁, −46 ml (−2% from predicted, \( p = 0.13 \)); FVC −50 ml, \( p = 0.20 \)). During 9 measurement sessions, patients were unable to properly smoke because smoking provoked coughing fits or dyspnoea. These coughing fits and dyspnoea resolved after the intervention at 4 of 5 sessions with active bronchodilation contrary to 1 of 4 sessions with placebos.

Effects on retention of smoke constituents

We observed mean (± sd) pulmonary retentions of nicotine and tar of 83% (±16) and 53% (±22), respectively, from smoking prior to inhalation of any medication. Mean inhalation weights of tar and nicotine were 18.0 mg and 1.5 mg, and did not statistically differ between the placebo or bronchodilators sessions (\( p > 0.90 \)). Linear mixed model analysis revealed that bronchodilation did not increase tar retention (−4.5%, 95% CI = −11.5% to 2.5%, \( p = 0.20 \)) or nicotine retention (−2.6%, −5.8% to 0.7%, \( p = 0.11 \)) (Tables 2 and 3, and Figs. 3 and 4). The secondary analysis that controlled for contamination revealed a potential decrease of tar and nicotine retentions: tar −3.8% (−8.7% to 1.2%, \( p = 0.13 \)), and nicotine −3.4% (−5.9 to −0.8%, \( p = 0.01 \)).

Effects on biomarkers and smoke inhalation patterns

Before smoking, mean (± sd) high sensitivity-CRP was 7.65 mg/l (±9.0) and mean (± sd) fibrinogen was 4129 mg/l (±826). Mean (± sd) inhalation and exhalation volumes and times were 831 ml (±652) and 1773 ml (±1025), and 1.9 s (±0.78) and 5.3 s (±1.7), respectively. Mean (± sd) smoke inhalation volume–FVC ratio was 28% (±18). Mean (± sd) smoking time and number of puffs were 7.1 min (±1.4) and 12.7 puffs (±2.9). We did not observe any statistical differences in changes of biomarkers or smoke inhalation patterns between the placebo aerosols and active bronchodilators sessions (Table 4).

Correlations on smoke retention and pulmonary function

No correlation was found between level of airflow obstruction and tar and nicotine retention. For both the change in FVC and change in FEV₁% from predicted we observed a possible weak inverse correlation with change of
nicotine retention: Pearson’s $r = -0.25$ ($p = 0.07$) and $-0.24$ ($p = 0.07$), respectively, and between change in FEV₁% from predicted and tar retention: $-0.22$ ($p = 0.08$). None of these reached statistical significance.

**Discussion**

Our study did not confirm our hypothesis that bronchodilation increases the pulmonary retention of cigarette smoke as measured by tar and nicotine retention, nor could we confirm that bronchodilation affects the smoke inhalation pattern or smoking-related biomarkers. When excluding the sessions with suspected contaminated filters, we observed a potential decrease of smoke retentions.

Several studies reported the effects of bronchodilators on mortality, with varying results. However, these trials usually suffered from methods not primarily designed to study (cardiovascular) mortality and did not adjust for the possible interaction between bronchodilators and smoking. To the best of our knowledge, our study is the first to address smoke retention in COPD patients and in particular in relation to bronchodilation. The only studies we found to compare our findings with, looked at cigarette smoke retention in healthy subjects: with normal smoke inhalation patterns, mean tar retention (50–80%) and mean nicotine retention (90–100%) seem similar to our results. These figures support our method to measure smoke retention. Furthermore, for comparative purposes smoking inhalation volumes are usually measured as

### Table 1 Baseline demographic and clinical characteristics of the 35 patients included in the study trial and analyzed, according to randomization.

<table>
<thead>
<tr>
<th></th>
<th>Total ($N = 35$)</th>
<th>Bronchodilation first visit ($N = 20$)</th>
<th>Placebo first visit ($N = 15$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>59.5 (8.8)</td>
<td>59.9 (8.2)</td>
<td>59.0 (9.8)</td>
</tr>
<tr>
<td><strong>Sex (male)</strong></td>
<td>18 (51%)</td>
<td>9 (45%)</td>
<td>9 (60%)</td>
</tr>
<tr>
<td><strong>Packyears</strong></td>
<td>37.3 (25.7)</td>
<td>34.6 (20.1)</td>
<td>41.0 (32.1)</td>
</tr>
<tr>
<td><strong>Pulmonary function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁% (post BD)</td>
<td>60 (2)</td>
<td>59 (13)</td>
<td>62 (12)</td>
</tr>
<tr>
<td>Reversibility (%)</td>
<td>4.2 (4.4)</td>
<td>3.9 (4.6)</td>
<td>4.7 (4.1)</td>
</tr>
<tr>
<td>FVC (post BD)</td>
<td>3.61 (1.10)</td>
<td>3.57 (1.26)</td>
<td>3.67 (0.88)</td>
</tr>
<tr>
<td>FEV₁/FVC (post BD)</td>
<td>0.50 (0.12)</td>
<td>0.47 (0.12)</td>
<td>0.52 (0.10)</td>
</tr>
<tr>
<td>TLC%</td>
<td>106 (16)</td>
<td>110 (18)</td>
<td>101 (11)</td>
</tr>
<tr>
<td>Diffusion (DLco)%</td>
<td>57 (15)</td>
<td>55 (16)</td>
<td>59 (13)</td>
</tr>
<tr>
<td><strong>Cardiovascular disease</strong></td>
<td>11 (31%)</td>
<td>6 (30%)</td>
<td>5 (33%)</td>
</tr>
</tbody>
</table>

Dichotomous variables are presented as total number (percentage), continuous variables as means (standard deviation).

Packyears, calculated by former and current smoking habit; FEV₁%, forced expiratory volume in one second as percentage from predicted; FVC, forced vital capacity (litre); TLC%, total lung capacity as percentage from predicted; Diffusion (DLco)%, carbon monoxide diffusing capacity as percentage from predicted; Cardiovascular disease includes cardiac, cerebral en peripheral vascular disease (not hypertension).

$^a$ Response on bronchodilator >10 min after administration.
a proportion of the vital capacity. In healthy subjects, previous studies revealed a smoking inhalation volume similar to our results of around 25% of the vital capacity, which indicates our method for measuring smoke inhalation patterns is valid as well.

**Strengths and limitations**

Apart from our original hypothesis, the crossover design, standardization, and baseline measurements of our outcomes are important strengths. These methods decreased variation and, consequently, reduced the number of patients needed for the study. Another strength is that patients finished participation within a month and hence our study would not have a significant impact on any intermediate smoking cessation attempts. Furthermore, we diminished cigarette variation by standardization and using conditioned CM6 cigarettes, known for their minimal variability. In particular, we countered the variation of between-patient differences in smoke inhalation and puff patterns by the crossover design. In addition, we minimized day-to-day variations by comparing individual smoke retentions of cigarettes smoked on the same morning; before and after medication. On the other hand, the standardized settings like exhaling through Cambridge filters may have interfered with the 'natural' smoking behavior as participants seemed to exhale relatively long, whereas the nose clips may have also modified inhalation through affecting the patients' sense of dyspnoea.

A possible limitation of our study was the fact that smoking provoked cough and/or dyspnoea in a number of patients. This may have interfered with our measurements, and consequently with the findings on smoke retention. Problems in the measurement of tar and nicotine may have influenced the findings: we calculated negative tar retentions (i.e., more tar exhaled than the amount initially inhaled) in 5 sessions. These negative tar retentions were accompanied by low nicotine retentions, which suggest a possible mathematical error in the lower retention ranges. In addition, we observed 14 sessions with nicotine exhalation values below the limit of quantification, reflecting a near 100% nicotine retention suggesting possible underestimation of the mean nicotine retention (83%) in our study. Another limitation is that our sample size calculation was designed to detect a 5% change of smoke retention, which makes it impossible to preclude effects below this level.

The positive nicotine measurements of unused filter blanks at several sessions need further explanation. If the nicotine was derived from cigarette smoke, the nicotine values of the positive blanks indicate an amount that would have been visible as a change in the filter color. As filters appeared blank on observation, the most likely explanation is a laboratory-based contamination of the filters or extract solutions with nicotine, for which no further clarification or source could be found. According to ISO-standards one should discard and recommence these measurements. However, filters could not be analyzed again since their contents were already fully extracted. We could not

### Table 2

<table>
<thead>
<tr>
<th>Effect</th>
<th>p-Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary analysis (35 patients, 70 sessions)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change of tar retention</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>2.8%</td>
<td>0.43</td>
</tr>
<tr>
<td>Bronchodilation</td>
<td>−4.5%</td>
<td>0.20</td>
</tr>
<tr>
<td>Visit</td>
<td>0.5%</td>
<td>0.88</td>
</tr>
<tr>
<td>Sequence</td>
<td>−1.2%</td>
<td>0.73</td>
</tr>
<tr>
<td>Change of nicotine retention</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>6.3%</td>
<td>0.03</td>
</tr>
<tr>
<td>Bronchodilation</td>
<td>−2.6%</td>
<td>0.11</td>
</tr>
<tr>
<td>Visit</td>
<td>0.0%</td>
<td>0.99</td>
</tr>
<tr>
<td>Sequence</td>
<td>−5.2%</td>
<td>0.15</td>
</tr>
<tr>
<td>Secondary analysis (35 patients, 62 sessions)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change of tar retention</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>−2.3%</td>
<td>0.41</td>
</tr>
<tr>
<td>Bronchodilation</td>
<td>−3.8%</td>
<td>0.13</td>
</tr>
<tr>
<td>Visit</td>
<td>−0.1%</td>
<td>0.98</td>
</tr>
<tr>
<td>Sequence</td>
<td>3.6%</td>
<td>0.26</td>
</tr>
<tr>
<td>Change of nicotine retention</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>2.0%</td>
<td>0.16</td>
</tr>
<tr>
<td>Bronchodilation</td>
<td>−3.4%</td>
<td>0.01</td>
</tr>
<tr>
<td>Visit</td>
<td>0.6%</td>
<td>0.62</td>
</tr>
<tr>
<td>Sequence</td>
<td>−0.7%</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Table 2: Linear mixed model analysis for changes of smoke retention due to medication, adjusted for learning (visit) and carryover (sequence) effects.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Visit 1 Before medication</th>
<th>After medication</th>
<th>Visit 2 Before medication</th>
<th>After medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tar retentions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sequence A</td>
<td>57.2%</td>
<td>54.7% (BD)</td>
<td>56.1%</td>
<td>57.6% (P)</td>
</tr>
<tr>
<td>Sequence B</td>
<td>48.3%</td>
<td>51.6% (P)</td>
<td>46.3%</td>
<td>44.5% (BD)</td>
</tr>
<tr>
<td>Nicotine retentions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sequence A</td>
<td>89.3%</td>
<td>88.9% (BD)</td>
<td>85.6%</td>
<td>86.7% (P)</td>
</tr>
<tr>
<td>Sequence B</td>
<td>75.5%</td>
<td>82.3% (P)</td>
<td>82.5%</td>
<td>83.4% (BD)</td>
</tr>
</tbody>
</table>

Table 3: Mean smoke retentions before and after medication, categorized by sequence and visit.

Sequence A is first visit bronchodilators and second visit placebo; Sequence B vice versa; BD is after bronchodilators; P is after placebo.
conduct these measurements again and hence corrected for these positive blanks with the assumption that the contamination was similar for either all Cambridge filters or all cigarette filters that were analyzed in the same batch. In addition, we performed a secondary analysis that excluded the sessions with possible systematic errors due to laboratory contamination. This secondary analysis did not confirm an increase of smoke retention due to bronchodilation either, but instead a possible decrease.

Finally, our study results cannot be directly translated to all COPD patients that persist in their smoking habit, although our findings seem relevant for a substantial part of the COPD population. Even more, our study aim was to demonstrate any existence of interaction, not generalization.

**Interpretation**

Our results do not confirm that bronchodilators in COPD patients increase cigarette smoke retention, smoking-related biomarkers or smoke inhalation patterns. Hence, it would be unlikely that bronchodilators increase the risk to develop cigarette smoke-related (cardiovascular) diseases through these mechanisms. However, final smoke exposure and translation into smoking-related cardiovascular risk profiles depends on various factors, including the number of cigarettes smoked, puff patterns, smoke inhalation patterns, and pulmonary smoke retention, penetration and transposition. Our study aimed to demonstrate that bronchodilators in COPD patients who continue to smoke might modify some of these factors, i.e., smoke retention and smoke inhalation patterns. The effect of bronchodilators on the other factors remains unclear and further studies on the interactive effect in the general COPD population and to what extent this interaction affects final cardiovascular disease are needed to address this. Further studies could add to a more deliberate prescription of bronchodilators to COPD patients who persist in their smoking habit, in particular those who already suffer from cardiovascular disease.

In addition, different COPD phenotypes may be affected differently by these factors of smoke exposure. Specifically, the possible effect of less smoke retention due to bronchodilation as observed in our secondary analysis, would be rather plausible in our cohort. Whilst the reversibility of obstruction, as measured by FEV1% and FVC, might be inversely correlated with smoke retention (near statistical significance), this reversibility appeared higher in patients that received bronchodilation, which may suggest that reduction of obstruction may cause an improved exhalation of hazardous smoke constituents and hence less retention. More specific, air turbulence in the airways of

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**Figure 3** Change of tar retention as modified by both bronchodilation and placebo. ——— are individual changes, whereas — line is the mean change.

**Figure 4** Boxplots for change of tar retention (%) from smoking a cigarette, after administration of both bronchodilator and placebo.
COPD patients may increase impaction and retention of smoke constituents. Although in the current study bronchodilators did not affect smoke inhalation patterns, the reduced obstruction could result in less turbulence and deposition, resulting in less retention.

Conclusions

Our results suggest that the use of bronchodilators in COPD patients that continue cigarette smoking is unlikely to increase smoke retention. Moreover, we observed a trend towards the opposite. As yet, there is a need to confirm our findings in an independent study sample and on other risk factors, and ultimately to study the effect of possible interaction on cigarette smoke-related (cardiovascular) diseases.

Author contributions

Van Dijk: study concept and design, performing of experiment, acquisition of data, interpretation of data, drafting of the manuscript; Heijdra: study concept concerning pulmonary measurements, interpretation of data and critical revision of the manuscript; Lenders: study concept concerning biomarkers, interpretation of data and critical revision of the manuscript; Klerx: study concept concerning smoke retention, filter analyses, interpretation of data and critical revision of the manuscript; Akkermans: study design concerning statistical advice, interpretation of data and critical revision of the manuscript; Van der Pouw: recruitment and critical revision of the manuscript; Scheepers: study concept concerning smoke retention, interpretation of data and critical revision of the manuscript; Schermer: study initiation, concept, and design, supervision, interpretation of data, and critical revision of the manuscript.

Conflicts of interest

There are no conflicts of interest of any kind related to this paper. Conflicts of interest, not related to this paper:

Walther Klerx, as government official from the Dutch Food and Consumer Product Safety Authority (VWA), is involved in law enforcement of tobacco products, though independent of the tobacco industry. In addition, the VWA (and Walther Klerx as technical expert) is a member of the task force tobacco laboratories of the WHO and EU and therefore obliged to comply article 5.3 of the FCTC (to prevent conflicts of interest with the tobacco industry of any kind). Chris van Weel received unrestricted fees or grants for his department for research, education, equipment, salaries, etc. from Bayer, NovoNordisk, Astra Zeneca, Boehringer Ingelheim, GlaxoSmithKline and Novartis. Tjard Schermer received unrestricted fees or grants for his department used for research, education, equipment, salaries, etc. from NutsOhra Fund, GlaxoSmithKline, Astra Zeneca and Boehringer Ingelheim. Wouter van Dijk, Yvonne Heijdra, Jacques Lenders, Reinier Akkermans, Anouschka van der Pouw and Paul Scheepers do not have any conflict.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.rmed.2012.09.019

References