Can an ozone system generator reduce indoor triggers in asthmatic patient?

Alessandro Zanasi¹, Paola De Nuntiis², Massimiliano Mazzolini³, Chiara Ciantelli², Matteo Alemanni⁴, Carla M.S. Ierna³, Marianna Mastroroberto⁵, Elena Nardi⁶, Antonio M. Morselli Labate⁶

INTRODUCTION

Asthma is a common, chronic respiratory disease affecting 1-18% of the general population in different countries. Manifestations of this disease are heterogeneous with different symptoms including: wheeze, shortness of breath, chest tightness and/or cough, and variable expiratory airflow limitation. These symptoms are often triggered by different factors: exercise, exposure to allergens or irritant substances, weather changes or viral respiratory infections [1].

During last decades, an increase in the prevalence of asthma and other allergic diseases has been recorded, together with modifications in the living environment and consequent changes in the quality of indoor air. Indoor environment is favorable to the proliferation of allergens such as: house dust mites, fungal spores and cockroaches. The primary action to be undertaken for an effective eradication of infectious agents constitutes in modifying the house environmental conditions, which make it favorable to infestations. Ozone can play a sanitize role, but at the same time it can cause inflammation, especially in the lung. The aim of this study was to verify the role and safety of ozone in the sanitation of the bedroom of a subject suffering from asthma.

RESULTS

Our analysis confirms that low ozone levels induced a marked reduction of indoor air microbiological pollution without adverse effects on lung functionality of the asthmatic patient we considered. Our observations warrant further investigation on the role that ozone-based sterilization might have in controlling asthmatic symptoms.

ABSTRACT

Objective: During the last decades, an increase in the prevalence of asthma and other allergic diseases has been recorded, together with modifications in the living environment and consequent changes in the quality of indoor air. Indoor environment is favorable to the proliferation of allergens such as: house dust mites, fungal spores and cockroaches.

The primary action to be undertaken for an effective eradication of infectious agents constitutes in modifying the house environmental conditions, which make it favorable to infestations. Ozone can play a sanitize role, but at the same time it can cause inflammation, especially in the lung. The aim of this study was to verify the role and safety of ozone in the sanitation of the bedroom of a subject suffering from asthma.

Methods: A daily ozone treatment was carried during a 14-day time period in the bedroom of an asthmatic patient. Aerobiological sampling in indoor air, microbiological sampling and detection of ATP bioluminescence on the surface were performed before and after treatment at the first day, as well as after treatment at the 7th and 14th day of the study. An aerobiological measurement was also performed outdoor of the patient’s bedroom only for the first day.

Results: Our analysis confirms that low ozone levels induced a marked reduction of indoor air microbiological pollution without adverse effects on lung functionality of the asthmatic patient we considered.

Conclusion: Our observations warrant further investigation on the role that ozone-based sterilization might have in controlling asthmatic symptoms.
According to the recent European Academy of Allergy and Clinical Immunology (EAACI) position paper [12], the effectiveness of interventions that reduce allergen exposure can be properly evaluated only when appropriate allergen measurements are performed, together with an accurate diagnosis of pathology and a comprehensive management of patients, in the context of routine surveillance of indoor environment (e.g., work environment surveillance), interventional studies, or population-based studies assessing exposure-response relationships [12]. Nevertheless, exposure reduction or cessation are considered a key step in prevention and management of occupational asthma and allergy [13-15].

The use of bedding covers as the sole measure to prevent allergy-triggered attacks showed small or no benefit in improving quality of life and reduce symptoms of patients affected by asthma and rhinitis [16-18]. To minimize allergens exposure, it is important to reduce the presence of in situ sources like carpets, upholstered furniture, as well as stuffed animals and to regularly vacuum-clean floors using devices with high-efficiency particular air filters [19]. Although the Environmental Protection Agency (EPA) recommends air filtration, a careful control of the sources of allergy-causing pollution and adequate indoor ventilation is considered to be more relevant. On the other hand, efforts should be made to eradicate house dust mites, fungi and bacteria infestations. The primary action for an effective eradication constitutes in modifying the house environment conditions that make it favorable to infestations. The use of modern dehumidifiers to maintain the relative humidity below 50% can reduce mite population and thus allergen exposure [20, 21]. In temperate climates, humidity regulation is an easy and effective measure, but in warm and humid regions, suitable humidity levels may be difficult to gain. Because of high temperature sensitivity of dust mites (> 50°C) [22], steam cleaning and hot air treatment (> 110°C) were reported as effective methods to kill them and to reduce environmental allergic concentration [23, 24]. Unfortunately, these maneuvers have a limit: if temperature does not reach the target level, the steam could contribute itself to mite and mold proliferation. Several chemical agents have also been tested to control the levels of mite allergens, but evidences of their effectiveness in standard conditions are still scarce [25, 26].

Ozone is a trivalent oxygen molecule, and a gas present in nature. It was discovered in 1840 and is characterized by high instability and a peculiar chlorine smell. Ozone is commonly found in stratosphere and its concentration is maintained by the ozone-oxygen cycle. The ozone molecule is formed because of the action of short-wave ultraviolet radiations (mostly UVC) which split an oxygen (O₂) molecule in two oxygen (O) atoms. Atomic oxygen reacts with molecular oxygen to form an ozone (O₃) molecule, according to following reactions:

\[
\begin{align*}
O_2 + UV &\rightarrow 2O \\
O + O_2 &\rightarrow O_3
\end{align*}
\]

UV rays with a wavelength between 240 and 310 nm are absorbed by ozone molecules, which are demolished to a molecule and an atom of oxygen:

\[
O_3 + (240 \text{ nm} < UV < 310 \text{ nm}) \rightarrow O_2 + O
\]

This mechanism is at the base of the so-called ‘ozone shield’, which, among the other effects, is essential in preventing oxidative damages of the DNA of living beings [27].

The ozone found at lower altitudes is formed instead through the interaction between sunlight and hydrocarbons or nitrogen oxides and it significantly contributes to air pollution [28]. In the ground atmosphere ozone is detectable at 0.02-0.04 ppm and even small concentrations of ozone can mask other smells, a characteristic that suggested its use as air purifier. Ozone usefulness for “air cleansing” was already a matter of debate in the early 20th century [29] and in recent times the disinfecting properties of ozone were better investigated. Ozone has to be produced immediately before its use because of its short half-life at atmospheric pressure. Most of the ozone generators use the corona discharge effect, which produces ozone plasma [30]. Ozone is already widely used for disinfection of wasted waters [31, 32] and it is approved by FDA for fruit cleaning. The microbicidal activity of ozone is influenced by several environmental factors such as relative humidity, temperature and pH. The gaseous ozone has been already tested on different bacterial strains [33]. Sharma and Hudson [34] demonstrated the in vitro efficacy of ozone generators in reducing Gram positive and Gram negative bacteria colonies; better results were observed with high levels of relative humidity (90%) and ozone concentrations of 25 ppm. In another study, higher concentrations (50-500 ppm) of ozone and hydrogen peroxide were tested in a large room (90 m³) on healthcare-associated pathogens. After the gaseous ozone exposure, a significant reduction of the tested pathogens and spores were observed [35]. Ozone is also used in food industry in USA since 1997 (and earlier in Europe) with several applications [36] and research is still active. A recent study demonstrated its effectiveness to control mite infestations on smoked ham (speck): after 15 days (8 h/day) of treatment with ozone at the concentration of 0.4 ppm, a complete elimination of food mites was observed [37]. Others beneficial effects of ozone have been reported in the treatment of several conditions such as vertebral disc herniation [38-40], atherosclerosis [41] and ischemic insults [42, 43], mostly by means of infiltrations. The therapeutic action of ozone is thought to be mediated by an up-regulation of antioxidant activity following its administration [44, 45]. The most important precaution to ozone exposure, particularly in patients with asthma, is to avoid excessive concentrations. Indeed, prolonged exposure to high concentrations of ozone may result in a strong inflammatory response of the airways [46, 47], vagal stimulation, change in pulmonary function including bronchoconstriction [48], although studies on lower ozone concentrations (0.02 ppm) have shown controversial results [49, 50]. The recommended limit of exposure suggested by the World Health Organization (WHO) is 100 µg/m³ (approx. equivalent to 50 ppb) for 8 h/day.
Zanasi et al: Ozone and the asthmatic patient

(75 ppb for the United States Environmental Protection Agency) [51]. Because of the risk of dangerous effects of ozone on health, laboratory concentrations used to kill mites are not reachable in home settings. However, it has been demonstrated that subjects exposed to ozone under controlled conditions below 72 ppb had a non-statistically different change in lung function when compared to subjects exposed to filtered air. Moreover, when exposed to concentrations greater than 72 ppb, the subjects showed no adverse effect on lung functionality because observed effects were transient, reversible, low in severity and did not establish a progressive respiratory dysfunction [52].

Ozone can play a sanitizing role but, at the same time, it can cause inflammation, especially in the lung. The aim of this study was to verify the role and safety of ozone in the sanitation of the bedroom of a subject suffering from asthma.

MATERIALS AND METHODS

Patient characteristics

A 15-year-old male subject presented an intermittent, non-productive, hacking cough since infancy. His medical history was positive for atopy, and diagnosis of asthma was made in 2005. Postnasal drip syndrome, gastroesophageal reflux and postinfectious cough were excluded. Review of systems was unremarkable and the results of physical examination were normal. He had got no pets in his apartment. Spirometry revealed normal pulmonary function with reversibility after bronchodilator challenge with a β2-agonist. Skin-prick test was positive for house dust mites. He used to record asthma symptoms by means of the Asthma Control Test (ACT) [53], and he had a good but not complete symptom control (ACT score equal to 23) during his therapy with inhaled corticosteroids, long acting β2-agonist and antileukotriene drugs. Dextromethorphan was used as cough suppressant on demand.

In the last month he reported ACT score 23, because he had cough at night. Therefore, we decided to test the efficacy of bedroom conditioning with ozone prior to sleep by making environmental measurements in order to verify if a reduction of the microbial load both in the air and on surfaces was present during the test and to test if the patient had no change in his asthmatic status.

Protocol to detect sanitization effects of ozone treatment

The following procedure was developed for this study by the Institute of Atmospheric Sciences and Climate (ISAC) (National Research Council - Consiglio Nazionale delle Ricerche; CNR, Bologna, Italy). A daily ozone treatment was carried during a 14-day time period in the bedroom of the patient (12 m²) using an ozone generator (ProWind MG100, MET Srl, San Lazzaro di Savena, Bologna, Italy) working for 60 min in order to keep air ozone at the fixed concentration of 0.06 ppm (60 ppb), followed by 120 min of post-exposure decanting process (depletion phase). The whole treatment was performed while maintaining door and windows closed and started at 7:00 pm. Only at the end of this 3 h period, the patient could sleep in the bedroom with door and windows closed. The ozone generator was located at 1.5 m above the ground. ProWind is a microprocessor-controlled device that gives the possibility of many treatment periods, on the basis of environmental contamination, without changing the levels of ozone concentration.

Experimental design

Sampling was performed weekly in the patient’s bedroom during three different days (1st day, November 28th, 2013: twice, both pre- and post-ozone treatment; December 5th, 2013: after 7 days of ozone treatment; and December 12th, 2013: after 14 days of ozone treatment). Each sampling was carried-out collecting the following measures (Table 1):

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Sample ID</th>
<th>Measures</th>
<th>Location and timing of sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>Ce</td>
<td>Airborne bioaerosol</td>
<td>Outside – air</td>
</tr>
<tr>
<td></td>
<td>C1_0</td>
<td>Airborne bioaerosol</td>
<td>Inside, O₂ pre-treatment – air</td>
</tr>
<tr>
<td></td>
<td>S1_0</td>
<td>Deposited bioaerosol</td>
<td>Inside, O₂ pre-treatment – surface</td>
</tr>
<tr>
<td></td>
<td>B1_0</td>
<td>ATP bioluminescence</td>
<td>Inside, O₂ pre-treatment – surface</td>
</tr>
<tr>
<td>7th day</td>
<td>C7_3</td>
<td>Airborne bioaerosol</td>
<td>Inside, 3 h O₂ post-treatment (60’) and depletion (120’) – air</td>
</tr>
<tr>
<td></td>
<td>S7_3</td>
<td>Deposited bioaerosol</td>
<td>Inside, 3 h O₂ post-treatment (60’) and depletion (120’) – surface</td>
</tr>
<tr>
<td></td>
<td>B7_3</td>
<td>ATP bioluminescence</td>
<td>Inside, 3 h O₂ post-treatment (60’) and depletion (120’) – surface</td>
</tr>
<tr>
<td>14th day</td>
<td>C14_3</td>
<td>Airborne bioaerosol</td>
<td>Inside, 3 h O₂ post-treatment (60’) and depletion (120’) – air</td>
</tr>
<tr>
<td></td>
<td>S14_3</td>
<td>Deposited bioaerosol</td>
<td>Inside, 3 h O₂ post-treatment (60’) and depletion (120’) – surface</td>
</tr>
<tr>
<td></td>
<td>B14_3</td>
<td>ATP bioluminescence</td>
<td>Inside, 3 h O₂ post-treatment (60’) and depletion (120’) – surface</td>
</tr>
</tbody>
</table>

Each sampling was coupled with temperature, humidity and ventilation measures
aerobiological sampling of airborne bioaerosol in indoor air, microbiological sampling (deposited bioaerosol) and detection of ATP bioluminescence on the surface; details of these measurements are given in the next sections. An aerobiological measurement was also performed outdoor of the patient’s bedroom only for the first day. Temperature and relative humidity were measured during the whole course of the study (outside and inside before treatment at the 1st day as well as inside after the 3 h ozone treatment at the 1st, 7th, and 14th days) with a portable digital thermo-hygrometer (Thermo-Higro, LPS Technologies, San Marcos, CA, USA). A portable hot-wire anemometer (TA 888, Dostmann Electronic GmbH, Wertheim, Germany) was used to measure indoor ventilation. The investigation of building structure (walls) of the bedroom was also performed in order to identify internal sources of biological contaminants (mold, fungi, etc). The study protocol was approved by the Italian Association for Cough Study institutional review board and conformed to the ethical guidelines of the Declaration of Helsinki. The study started after the informed consent was signed.

**Aerobiological sampling**

Active air sampling was performed by a portable surface air system (SAS super-100 sampler, PBI International, Milan, Italy) with a capacity of 100 l/min and a single-stage sampling head. Ninety millimeter Petri dishes, containing agar culture medium, were placed below the filter to trap viable particles. In detail, a plate count agar (PCA) (Oxoid Standard Plate Count Agar, Thermo Fisher Scientific Inc, Waltham, MA, USA) was used to grow viable bacteria, and a Sabouraud dextrose agar with chloramphenicol (SDA+CAF) (Oxoid Sabouraud Dextrose Agar, Thermo Fisher Scientific) was utilized for yeasts and molds. Before each sampling, the sampler was disinfected with denatured ethanol. Only for the first day, the aerobiological sampling was carried out in two different points: one at the center of the room for a volume of 100 liters of air (60 s of time), the other outside the room, for a volume of 33.3 l of air (20 s of time). In both cases, the sampler was placed at approximately 150 cm from the ground.

Subsequent aerobiological samplings were performed only inside the room, according to the scheme reported in Table 1. Collected Petri dishes, two for each culture medium, were stored at 25°C for 5-7 days (SDA+CAF) or at 22°C for 3 days (PCA). Following incubation, grown colonies were counted. Double-replicate plates were used. Positive-hole correction was applied to the raw value of colony forming units (CFU) as obtained from each plate, and then used along with sampling time and flow rate to calculate concentration, with the final value expressed as CFU per cubic meter of air (CFU/m³). The results were expressed in total microbial load (TML), as well as in bacterial and fungal loads.

**Surface sampling**

Surface monitoring was performed on a stiff horizontal shelf, about 1 m above ground level, as reported in Table 1.

The area investigated was continuous and without specific cleaning treatment. Sampling was carried out directly from the surface using a 55 mm contact plate with the same two culture media used for air sampling: PCA for viable bacteria, SDA+CAF for yeasts and moulds. All microbiological samplings were carried out twice on adjacent surfaces for each point and cultural medium type. A constant pressure of 500 g was applied on the plate for 10 s, as requested by ISO 18593 [54] procedure, to perform a standardized sampling. In order to evaluate microbial contamination, the number of colonies grown after incubation (with incubation conditions and counting procedure as detailed above for aerobiological sampling) was examined at the CNR-ISAC aerobiology laboratory. The results of surface monitoring were expressed as CFU/cm².

An assessment of biological contamination was also carried out directly on the bedroom surface through the detection of ATP bioluminescence (which is extensively used in the food industry as a tool for monitoring surface hygiene). We used a portable sampler (Hygenia System-Sure Il Plus, RG Strumenti Srl, Parma, Italy) with disposable swabs provided with the necessary pre-dosed reagents. After sampling on horizontal surface squares of 10 x 10 cm, the swab was inserted into a reader and a quick numeric reading was produced within 15 s. The results were expressed as relative light units (RLU). We decided to perform such measurements in order to indirectly detect the presence of viable organisms on sampled surfaces, and in particular the amount of mites, since this assay has been already used as a suitable method able to detect mites viability by Ebina and Ohto in 2007 [55].

**Asthma Control Test (ACT)**

The ACT was firstly described by Nathan et al in 2004 [53]: it is a simple test for asthmatic people older than 12 years that analyzes the trend of the disease symptoms and the consequent effectiveness of therapy in the last four weeks. The test is composed by five questions that evaluate the following 5-point scales: limitation of activity, frequency of shortness of breath, night symptoms’ severity (wheezing, coughing, shortness of breath, chest tightness or pain), necessity of rescue medication, and patient’s point of view about his/her asthma control. Asthma control is achieved when the patient shows satisfaction about asthma in the last month, he/she does not declare coughing or wheezing, no night-time interruptions and no emergency visits to the doctor or hospital. The final score is a number between 5 and 25, with a total score of 19 or less meaning that the disease is not controlled, a score between 20 and 24 meaning that asthma is controlled, although not completely, and a score of 25 suggesting full disease control.

ACT was submitted to the patient the first day before the treatment start and one day after the last ozone treatment.

**Data reporting**

Data are reported as mean and standard deviation (SD) of the two replicates. No inferential statistics were applied because of the preliminary nature of the study that investigated a single case.
The analyzed room had a natural ventilation, domestic heating and no air-conditioning. Indoor wind speed was always less than 0.3 m/s. Air temperature was between 17-19°C indoor and 10-12°C outdoor. Indoor and outdoor relative humidity was 40-45% and 70%, respectively. The bedroom was free from damage by building moisture or visible molds.

RESULTS

Aerobiological result

Figure 1 shows the colonies grown on Petri dishes after the incubation period and before morphological analysis. The morphological evaluation of the total microbial load, as well as the bacterial and fungal loads, in air resulting from the aerobiological analysis (airborne bioaerosol) is reported in Figure 2. The outdoor value indicates a microbiological pollution lower than the internal one (Ce 185 CFU/m³ vs C1_0 225 CFU/m³; Figure 2a) which is characterized by a naturally high environmental variability. The concentration value after treatment (C1_3, C7_3 and C14_3) showed a conspicuous breakdown all over the time: there was an 84% reduction for the total microbial load by comparing pre-and post-treatment concentration of the first sampling day (C1_0 vs C1_3) and the TML remained low on subsequent sampling (C7_3, C14_3).

Consistently with what observed with the TML, in both cases both loads abruptly decreased after treatment in the first day of the study (C1_3 vs C1_0: 87% and 70% reduction for bacteria and fungi were observed, respectively) and stayed low in the following two weeks (C7_3 and C14_3). Notably, fungal load broke down completely at the end of the study period. The measurement variability before and after treatment showed a good stability of indoor environment.
Surface result

The microbiological analysis of surfaces is shown in Figure 3. A rather low level of internal pollution was found even before treatment (the mean values of TML were always less than 100 CFU/cm²). As far as the behavior of the two agents is concerned, the comparison between pre-and post-treatment values of bacterial load evaluated in the first day showed no evidence of sanitization, and afterwards the post-treatment bacterial load showed a decrease after two weeks only. On the other hand, the post-treatment fungal load showed a progressive increase during the two weeks of treatment, even if the values remained at a very low level.

Bioluminescence data showed a decrease with the treatment of the first day (B1_0: 126 RLU; B1_3: 44 RLU; before and after 3 ozone treatment, respectively) with an irregular behavior of the post-treatment values measured after 7 (B7_3: 179 RLU) and 14 (B14_3: 53 RLU) days. There was no apparent correlation between ATP bioluminescence and microbial loads, as shown in Figure 4.

Clinical result

Before ozone treatment the asthma was not completely controlled (ACT score 23) because the patient reported cough and the patient subjective evaluation of asthma was “well controlled” (both scored 4 in the questionnaire), while limitation of activity, frequency of shortness of breath, and necessity of rescue medication scored 5. At the end of the two-week time period of the study, although the patient reported a value of ACT score increased to 24 the asthma was still not completely controlled because, even if he reported not at all waking for coughing (score 5) during the 14-day chamber exposure study, he still defined his disease as “well controlled” (instead of “completely controlled”).

DISCUSSION

In the present report, we assessed the effect of an ozone-based sterilizing technique on the indoor allergen load in the bedroom of an asthmatic patient. The first noteworthy observation we made was that indoor microbiological air pollution (assessed as TML) was higher than that observed outdoor. This confirms the effect of bioaerosol accumulation in absence of natural dispersion driven by wind, as well as, it suggests that lifestyles and sanitation strategies adopted by inhabitants may greatly affect indoor levels of allergens. As a consequence, indoor measurements seem to give more accurate indications on the exposure of people to pollutants, especially in industrial countries, where about 90% of the time is spent indoor.

Moreover, we evidenced that TML in air decreased after ozone treatment. This seems to confirm the efficacy of ozone in sanitizing indoor air, regardless of environmental re-contamination due to daily room opening. The same
applies to fungal and bacterial loads. The ozone sterilizing technique we employed was able to significantly reduce both bacterial and fungal indoor pollution since reference values of “very low” microbiological air pollution levels in domestic environments are less than 100 CFU/m³ for bacterial load and less than 50 CFU/m³ for fungal load [56]. Interestingly, while after 14 days of treatment bacterial load was clearly below the 100 CFU/m³ threshold, fungal air pollution was completely abolished. On the contrary, no clear effect was observed for surface pollution. The preliminary nature of our observations makes risky to draw a general conclusion and our results should be confirmed by further analyses; however, many different explanations may account for the ambiguous effects observed for surface pollution. For instance, it is possible that the ozone surface treatment might require a longer period to be effective, or that longer exposures to ozone at each session are needed to achieve an effective surface sanitization. Additionally, considering the very low values initially observed, an ozone-related sanitization effect might have been hidden by background variability.

ATP bioluminescence data are in good agreement with sanitization. The relative light unit (RLU) decreased from 126 to 44 with the first treatment, and maintained a value of 53 after the last one, but surprisingly the intermediate treatment showed a value even higher than that detected with no treatment (179 RLU). It is hard to explain this result, but we have to mention that we could not control the cleaning activities of people living inside the apartment, although the inhabitants were properly trained in maintaining a constant and reproducible cleaning during the two-week time of this study.

The comparison between ATP bioluminescence and microbiological data showed no clear correlation. A larger sampling is indeed needed, but we may suggest that ATP bioluminescence might not be accurate enough as a measure of viable organisms: for example, fungal spores have a complex wall and, being resistant forms allowing fungi to survive under unfavorable conditions, they are not metabolically active. Our first intention was to use this method for indirectly detecting mite contamination since this measure has been applied to detect mite viability [55]. The results of this study do not support our hypothesis for the same above mentioned reasons and more analyses are needed in order to evaluate a possible role of ATP bioluminescence in detecting mite contamination.

Although, ozone seems to be useful in reducing indoor microbiological pollution, at least in the air, it should be kept in mind that some cautions are required when ozone is used. The most important one is to avoid excessive concentrations when ozone is used in sanitization for patients with asthma. Exposition to high concentrations of ozone may result in an increased inflammatory response of airways and in a reduction of pulmonary function [46, 47, 49, 50]. The used ozone concentrations were in line with the recommendations of the World Health Organization [51], and despite this ‘limited exposure’, an effective reduction of the total microbial load in the air was reached (Figure 2a), whereas this tendency was not confirmed by the analysis of surface pollution (Figure 3a). The killing effect of ozone on house microorganisms and dust mites is probably mediated by the production of oxidizing agents arising from the peroxidation of polyunsaturated fatty acids and from oxidation of lipids and thiols, which could damage cell walls, membranes, cytoplasm components and DNA. Prolonged expositions to gaseous ozone could lead to a progressive damage of core constituent of microorganisms without significant adverse effects on patient’s health [57], as well as the internal space was preserved since the surfaces were not physically damaged during the treatment with ozone.

It is difficult to anticipate whether the reduction of air TML we observed may have a clinical relevance in controlling asthma symptoms, since the patient we considered already had a good level of disease control, making hard the detecting of a further improvement in his symptomatology. Nevertheless, the moderate improvement of ACT score that was found at the end of the treatment period (due to the improvement of the night cough) confirms the safety of this maneuver, since an excessively high level of ozone would have likely led to symptoms deterioration and thus to ACT score reduction. Therefore, our observations on air TML, coupled with the absence of negative effects observed in the asthmatic patient, pose the basis for a further investigation on this technique involving a larger number of asthmatic patients, particularly by selecting those with a poorer control of symptoms.

Like the Roman God Janus, sterilization using ozone has two faces: it is effective in reducing the indoor microbial load but, if it is obtained through high ozone levels, it might pose a health risk, especially in asthmatic patients. Nevertheless, our analysis confirmed the effectiveness of low ozone levels to induce a marked reduction of air microbiological pollution, although the same effect was not apparent for surface pollution. At the same time, the low levels of ozone employed were safe for the asthmatic patient we considered, since we observed no deterioration in his symptomatology. Taken together, our observations warrant further investigation on the role ozone-based sterilization might have in controlling asthmatic symptoms.

ACKNOWLEDGEMENT

We thank to Dr. Elisabetta Maiolini for her support to environmental monitoring.