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The bacterial lysate Lantigen B reduces the number of acute episodes in patients with recurrent infections of the respiratory tract: The results of a double blind, placebo controlled, multicenter clinical trial

Fulvio Braido a, Giovanni Melioli a,*, Piero Candoli b, Andrea Cavalot c, Mario Di Gioacchino d, Vittorio Ferrero e, Cristoforo Incorvaia f, Carlo Merue g, Erminia Ridolo h, Giovanni Rolla i, Oliviero Rossi j, Eleonora Savì k, Libero Tubino l, Giorgio Reggiardo m, Ilaria Biaiardini a, Eddi di Marco n, Gilberto Rinaldi o, Giorgio Walter Canonica a

Lantigen Study Group,

Carlo Accorsi c, Claudia Bossilino i, Laura Bonzano h, Michela DiLizia d, Barbara Fedrighini c, Valentina Garelli a, Vincenzo Gerace l, Sara Maniscalco g, Ilaria Massaro j, Alessandro Messi b, Manlio Milanese g, Silvia Peveri k, Arminio Penno c, Stefano Pizzimenti i, Tiziana Pozzo e, Alberto Raie i, Sergio Regina e, Francesca Scilò a

a Allergy & Respiratory Diseases Department, University of Genoa, IRCSS A.O.U. San Martino – IST, Irg. Benzi, 10, 16132 Genova, Italy
b Divisione Pneumologia, Ospedale Lugo di Romagna Viale Dante, 10, 48022 Lugo, RA, Italy
c Ospedale S. Croce Moncalieri (TO), P.zza A. Ferdinando, 3, 10024 Moncalieri, TO, Italy
d Dipartimento di Medicina e Scienze dell’Invecchiamento, Immunologia e medicina del lavoro, Università G. D’Annunzio, Chieti Via dei Vestini, 31, 66100 Chieti, Italy
e Ospedale Gradение Turino, C.so Regina Margherita, 8, 10153 Turino, Italy
f Istituti Clinici di perfezionamento, Milano Via Bignami, 1, 20126 Milano, Italy
g Centro Asma, Struttura Complessa Pneumologia, Ospedale Santa Corona, Via XXV Aprile, 38, Pietra Ligure, SV, Italy
h Ambulatorio di Allergologia Pedigotne Barbieri 2 piano, Dipartimento di Scienze Cliniche, Università degli Studi di Parma, Via Gramsci, 14, 43125 Parma, Italy
i Allergologia e Immunologia Clinica, Dipartimento di Scienze Mediche dell’Università di Torino & AO Ordine Mauriziano, Largo Turati, 62, 10128 Torino, Italy
j Azienda Ospedaliero Universitaria Careggi, D.A.I. Biomedicina – S.O.D. Immunologaerdia PAD, 13, Largo Brambilla 3, Firenze, Italy
k U.O. di Allergologia, AUSL di Piacenza, Via Taverna, 49, 29100 Piacenza, Italy
l Ospedale di Chivasso (TO), C.so Gallies Ferrari, 3, 10034 Chivasso, Italy
m Biostream unit, Mediservice srl, Milano, Italy
n Laboratorio di Analisi, Istituto G. Gaslini, Via G. Gaslini 5, 16147 Genova, Italy
do Direzione Medica, Braschetti srl, Via Isonzo 5, 16147 Genova, Italy

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A B S T R A C T

Studies in the 1970s and 1980s reported that bacterial lysates (BL) had a prophylactic effect on recurrent respiratory tract infections (RRTI). However, controlled clinical study procedures have evolved substantially since then. We performed a trial using updated methods to evaluate the efficacy of Lantigen B®, a chemical BL. This double blind, placebo controlled, multi-center clinical trial had the primary objective of assessing the capacity of Lantigen B to significantly reduce the total number of infectious episodes in patients with RRTI. Secondary aims were the RRTI duration, the frequency and the severity of the acute episodes, the use of drugs and the number of missed workdays. In the subgroup of allergic patients with RRTI, the number of allergic episodes (AE) and the use of anti-allergic drugs were also evaluated. One hundred and sixty patients, 79 allocated to the treated group (TG) and 81 to the placebo group (PG), were enrolled; 30 were lost during the study and 120 (79 females and 38 males) were evaluated. The PG had 1.43 episodes in the 8-months of follow-up while the TG had 0.86 episodes (p = 0.036). A similar result was observed in the allergic patients (1.80 and 0.86 episodes for the PG and the TG, respectively, p = 0.047).

* Corresponding author. Tel.: +39 346 5168826.
E-mail address: giovannimelioli@gmail.com (G. Melioli).

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The use of antibiotics was reduced (mean 1.24 and 2.83 days of treatment for the TG and the PG). Logistic regression analysis indicated that the estimated risk of needing antibiotics and NSAIDs was reduced by 52.1 and 30.6%, respectively. With regard to the number of AE, no significant difference was observed between the two groups, but bronchodilators, antihistamines and local corticosteroids were reduced by 25.7%, 56.2% and 41.6%, respectively, in the TG. Lantigen B significantly reduced the number of infectious episodes in patients with RRTI. This finding suggests a first line use of this drug for the prophylaxis of infectious episodes in these patients.

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1. Introduction

Recurrent respiratory tract infections (RRTI) are less frequent in adults than in the children, but the frequency of patients affected remains significant [1]. The therapy is based on anti-inflammatory drugs and antibiotics, and only rarely is infection prophylaxis provided. Bacterial lysates (BL) are a family of “biological” drugs that were introduced in therapy many decades ago, but only in the last 10 years has their mechanism of action been elucidated. Since the first in vivo studies, it was hypothesized that the clinical effects observed were related to the capacity of BL to elicit a specific immune-response against bacterial antigens. Along this line, since the mid 1970s, specific IgA directed against bacterial antigens have been observed in the salivary fluid of treated patients [1–4] following the oral administration of bacterial lysates. During the 1990s, the description of the toll-like receptor (TLR) family and its functions [5] within the larger context of the pattern recognition receptor (PRR) system explained how the innate immune system was alerted by the presence of different stimuli, mainly represented by bacterial derived structures. In that same period, the central role of dendritic cells (DC) in the generation of an efficient immune-response was described [6]. Starting from these lines of evidence, different bacterial lysates were shown to be able to induce significant activation and maturation of DC in vitro [7,8]. More recently, using a specific member of the bacterial lyase family (the Polyvalent Mechanical Bacterial Lyase – PMBL), other relevant effects were observed on B cells, T cells and NK cells [9,10] and these effects were also correlated with the clinical results [11,12]. Bacterial lysates are obtained by physical or chemical lysis. The former are in general composed of fragments of bacterial bodies. The latter may be constituted by either only low molecular weight proteins or a mixture of bacterial bodies and soluble proteins. Lantigen B belongs to the latter family, being a suspension of bacterial antigens obtained from Strepococcus pneumoniae type 3, Streptococcus pyogenes Group A, Branhamella catarrhalis, Staphylococcus aureus, Hemophilus influenzae type b and Klebsiella pneumoniae. The in vivo effects of Lantigen B were first described in the mid 1970s [11–13]. Since that time, few studies have been performed on this specific drug. Although the first studies were considered obsolete from a procedural point of view [14–16], the drug remained in the pharmacological armamentarium of both general practitioners and specialists. In this context, other studies even if not totally in line with modern clinical trial practice have been published [17–19], further demonstrating the clinical efficacy of Lantigen B. In particular, the main effect observed was the reduction of infections during the observation period. Indeed, by analyzing all the abovementioned studies, it can be calculated that a mean of 1.73 episodes/year were observed in treated patients vs. 2.63/year in the placebo group, corresponding to a reduction of 34% of the number of infectious episodes. In addition, in the same studies, the reduction of cough, of lost days and antibiotics use was also observed. Of note, only one non-positive study on the common cold [20] was published, while all the others demonstrated the efficacy of the treatment.

In this study, using modern and effective clinical practice tools, we re-evaluated the efficacy of Lantigen B in a double blind randomized placebo-controlled clinical trial in inducing a significant reduction in the number of infectious episodes in an unselected (real life-like) population of patients with RRTI. This type of study is needed not only to verify previous results using an up-dated methodology but also for the availability of drugs (such as third generation anti-histamine, local corticosteroids and modern bronchodilators) that have recently demonstrated a deep impact on the management of allergic patients who represent a significant fraction of patients with RRTI.

2. Material and methods

2.1. Definition of acute infectious episodes

Acute episodes of the upper respiratory airways were identified by the continuous presence of rhinorrhea (both sero-mucous and purulent), pharyngitis, and cough, lasting at least 48 h, with or without fever. Acute episodes of the lower respiratory airways were defined as the continuous presence of stridor, wheeze, crackles and rhonchi, an indrawing respiratory frequency > 50 cycles/min, or cyanosis lasting at least 48 h, with or without fever. Acute episodes of otitis were defined as the continuous presence of pain, erythema and a reduction in or loss of tympanic membrane mobility. Finally, an acute infectious episode was defined as new if at least 72 h had passed with a complete absence of symptoms from the resolution of the previous episode. The identification of an infectious event was based on patient’s report (fever or cough or dyspnea or use of drugs) as well as on doctor’s report (visits and therapeutic indications). The protocol provided that when an infectious episode occurs, the patient might contact to the primary care physician or the study center that enrolled her/him. In both cases, the diagnosis and the therapy were reported in the patient’s diary and in the Case Report Form.

2.2. Study design

This was a national multi-center longitudinal, prospective randomized double blind versus placebo controlled clinical phase IV study, conducted on two parallel groups for an observation period of 8 months. The protocol EudraCT code was 2011-00-3229-76. The primary goal was the evaluation of the efficacy of the Lantigen B treatment on the number of infectious episodes (IE) in the group of treated patients. Secondary goals were (a) the total number of days with IE (in a 6 month follow-up); (b) the frequency and severity of episodes of allergy (AE), in particular asthma and rhino-conjunctivitis; (c) the disease free period evaluated as the time of occurrence of an IE after the end of one of the treatment cycles; (d) any other drug consumption in the 8 month period, including anti-infectious, anti-inflammatory and anti-allergic drugs; (e) the enumeration of days of absence from the community (school, work); (f) the safety and tolerability of the treatment.
2.3. Study approval

The study was conducted in accordance with good clinical practice was also approved by the Ethical Committee (EC) of the University of Genova and the AOU San Martino, reference of the Institution of the Principal Investigator. The other 10 ECs of the remaining 11 centers also approved the protocol.

2.4. Study timing

The study recruitment started in September 2012 and finished at the end of December 2012. The follow-up finished and the study was closed in September 2013. The clinical and statistical data were available from December 2013.

2.5. Patient selection

The study was carried out at 12 different centers, representative of the different Italian climates. This study enrolled men and woman between the ages of 18 and 65 years. Inclusion criteria were: (a) patients complaining two or more infections of the upper respiratory in the year preceding this study, possibly associated with respiratory allergy, (b) the absence of any type of malformation or other significant diseases (i.e., cancer, immunological disorders), and (c) the capacity to understand the study and collaborate with the investigators. Exclusion criteria were: (a) recurrent respiratory infections in the last 7 days before enrollment; (b) acute diseases (either infectious or not infectious) requiring hospitalization; (c) gastro-esophageal reflux; (d) cystic fibrosis, α1-antitrypsin defects or ciliar dyskinesia; (e) any chronic systemic disease; (f) body mass <3th percentile for age; (g) autoimmune diseases; (h) any drugs such as immunoglobulins, immunostimultants, antineoplastic drugs, cytokines, interferons, interleukins, immunosuppressors, or systemic corticosteroids; (i) known allergy or intolerance to the study product or its excipients; (k) patients who could not be contacted by telephone during the study; (l) patients recruited into any other clinical study in the last month before the enrollment or during the study.

2.6. Randomization protocol

The randomized list was based on the “RANUNI" random number generator of the SAS software (SAS Institute, Cary, NC, USA). The type was a 1:1 randomization of two groups.

2.7. Treatment

The treated group received Lantigen B (Bruschettini Srl.) in oral drops. Lantigen B is a suspension of bacterial antigens obtained from S. pneumoniae type 3, S. pyogenes group A, B. catarrhalis, S. aureus, H. influenzae type b and K. pneumoniae in distilled water, pH 7.30, containing undetectable doses of chlorhexidine diacetate (0.02 mg/mL). The placebo control had the same characteristics (namely, aspect, pH, and chlorhexidine), but the bacterial antigens were not present in the bottles. A 4-week cycle of treatment represented by a total of 30 drops in two different daily administrations (15 + 15), morning and evening, fasting, was scheduled, followed by an interval of 2 weeks and another 4 weeks of treatment. After a 6-week observation period, two additional 4-week treatments were scheduled, with an interval of 2 weeks. At the end of the second treatment cycle, the patients were followed-up for another 6 weeks. Table 1 shows the treatment schedule. During the period of the study, immune-stimulant drugs, cytokines, interferons, immune-suppressors and anti-neoplastic drugs were not allowed. Systemic steroids, if used continuously for more than 2 weeks, were also not allowed. The use of forbidden drugs caused the exclusion of patients from the study. Any other drug was allowed and recorded in the patient’s personal diary.

2.8. Visits

Three visits were scheduled during the treatment: a first visit, which included the enrollment, a second visit before the beginning of the second 4 + 4 week cycle and a third visit at the end of the second follow-up period. During the first visit, the patients were evaluated to define whether the inclusion and the exclusion criteria were met. In positive cases, the patients were asked to be enrolled in the clinical trial. If enrolled, in the same visit, the patients underwent a physical examination for the evaluation of vital signs and they were further evaluated for their life style, clinical history and smoking habits. The enrolled patients signed the informed consent and were included in the randomization procedure by receiving the study drug. All the patients were then trained in the use of the drop dispenser and were instructed by the investigator regarding when and how to take the daily treatment, according to the provided schedule. Finally, the patients were exhaustively instructed to compile a daily diary and were asked to record any relevant events that occurred. Thus, in the presence of a decline in a patient’s conditions, the symptoms were recorded and the intensity was evaluated using a 4-point scale from 0 (absence) to 3 (presence of severe symptoms). The administered drugs, medical visits and possible hospitalization were also recorded in the diary. The patients were also asked to carefully record the use of the study drug according to the provided schedule. They were also required to return the bottles and the containing boxes to the investigator at their next visit. During the second visit, the investigators recorded any adverse reactions, hospitalization periods, modifications of life style, work or school habits and drug use (in particular antibiotics, anti-inflammatory drugs etc.). At the same visit, the first phase diary was retired and both infectious and allergic episodes were recorded in the Case Report Form (CRF). The remaining drug was also retired and the drug for the second cycle was given together with the second part of the diary. The third and final visit (at the end of the second period of observation) was used to record the same param-

| Table 1 | Study plan. |
|---------|-------------|
|         | First cycle |
| Month   | 0 | 1 | 2 | 3 | 4 |
| Week    | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Treatment Visit | X |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Month   | 5 | 6 | 7 | 8 | End |
| Week    | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 |
| Treatment Visit |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | X |
eters as the second visit and to retire the remaining drug and the diary.

2.9. Patient consent withdrawal

Patients were allowed to withdraw from the treatment for any reason. When possible, the adverse reactions and the use of drugs were recorded for the retired patients and the personal diary was retired. Any relevant information was recorded in the CRF.

2.10. Patient compliance control

The compliance to the treatment was evaluated by comparing the registered doses and the amount of remaining drug in the bottles.

2.11. Safety assessments

The patients and the investigators were asked to monitor the possible occurrence of adverse reactions (ARs). These were classified as certain, likely, possible, unlikely, unrelated and not evaluable, according to the definition of the protocol. ARs were also classified as slight (if not interfering with daily activities), intermediate (if the AR interfered with the day activities) and severe (if the AR inhibited daily activities).

2.12. Exclusion of patients from the study

Patients were excluded from the study in the presence of a severe AR incompatible with the continuation of participation in the study.

2.13. Viro-epidemiological survey of the study period

The frequency of viral infections in the general population during the period of the study and the previous year was evaluated. The results of molecular biology-based research on influenza viruses, para-influenza viruses, bocaviruses, coronavirus, rhinoviruses, enteroviruses, adenoviruses and Respiratory Syncytial virus were recorded. The frequency of viral infections was based on the percentage of positive samples as described [10]. Thus, an increase in the percentages of positive samples was considered representative of an increased circulation of pathogens in a given period.

2.14. Statistical analysis

For the sample size calculation, a total of 190 patients (85 patients per group) were required to be recruited, by considering an expected drop out of 15% of the patients enrolled with the intention to treat (ITT). Thus, a total of 160 patients were expected to be evaluable. The primary efficacy analysis was based on the ITT. The efficacy of the treatment on the two groups was evaluated by an analysis of covariance (ANCOVA). For this analysis, the total number of infective episodes was the dependent variable, the treatment group was the factor and the number of previous year infective episodes was the covariate. In the ANCOVA model, the adjustment for baseline covariates was performed. The subgroup analyses were performed in compliance with the rules suggested in the European Medicine Agency Guideline on the investigation of subgroups in confirmatory clinical trials. Binary variables measuring the effects of treatment on the secondary objectives were analyzed by a \( \chi^2 \) test. Finally, a logistic regression (multivariate) analysis was used to evaluate the probability of using drugs: antibiotics and NSAID, for the whole population and local corticosteroids, antihistamine drugs and bronchodilators for the subset of allergic patients. All these statistics were carried out by using SPSS on data extracted from the CRFs.

3. Results

3.1. Study population

A total of 160 patients (79 in the treated group and 81 in the placebo group) were enrolled. The age of the placebo group was 42.4 ± 15.14 (mean ± standard deviation) and the age of the treated group was 42.4 ± 13.94 years. One hundred and seventeen patients (58 in the treated and 59 in the placebo group) completed the study (Fig. 1). The drop out was observed both in the Lantigen B group (17 patients) and in the placebo group (23 patients). Some patients were lost for inadequate compliance or violation of the protocol (seven patients), three for adverse reactions, five were lost in the follow-up and 23 (corresponding to the 57.5%) for consent withdrawal. Finally, two were lost for other reasons. In patients who completed the study, the genders were equilibrated between the placebo (20 males and 38 females) and in the treated group (18 and 41, respectively) (\( \chi^2 = 0.21, p = 0.65 \)). Table 2, in the lanes related to the reference period (2011–2012) shows the clinical characteristics of these patients, in line with the eligibility criteria.

3.2. Post-hoc power

The number of patients recruited and the number of drop-outs could have impacted the power of the study results. However, because the differences between the placebo and treatment were significant and the efficacy of the treatment was higher than could have been expected on the basis of the published literature, the post-hoc power of the study was confirmed. Indeed, a sample size of 58 in each group had an 84% power to detect a probability of 0.34 that an observation in the Lantigen B group was less than an observation in the placebo group (Wilcoxon Rank-sum test with a 0.05 two sided significance level). Of note, a power of 80% is considered acceptable by the scientific community for clinical studies. Thus, the original design of this study had a power suitable to achieve complete statistical significance even in the presence of a conspicuous drop-out of the enrolled patients.

3.3. The primary objective

The primary objective of the study was the reduction of the number of IE during an 8 months follow-up period. In the whole group of enrolled patients, the mean frequency of IE in the year preceding the study was 3.66/year (C.I. 3.38–3.93). The frequency was 3.34/year for males and slightly higher, 3.81/year for females, corresponding to a mean 2.56 IE in 8 months (2.5 and 2.9 in 8 months, respectively). The distribution of IE between the placebo

| Table 2 | Clinical characteristics of the patients, number of patients with a given diseases and number of episodes of disease in the period of the study (2012–2013). |
|---------|----------------------------------------------------------------------------------------------------------------------------------|
|         | Patients with disease | Number of disease episodes                                    |
|         | Placebo | Lantigen B | Placebo | Lantigen B |
| Otitis  | 2       | 1          | 2       | 1          |
| Tonsillitis | 5     | 2          | 7       | 2          |
| Sinusitis | 9      | 3          | 18      | 6          |
| Pharyngotonsillitis | 17   | 11         | 23      | 15         |
| Rhinopharyngitis | 20   | 18         | 32      | 21         |
| Bronchitis | 15     | 8          | 23      | 12         |
| Pneumonia | 0       | 1          | 0       | 1          |
| Total   | 68      | 44         | 105     | 58         |
The assessed population, the group

...the period of the study, the placebo group (58 patients) experienced a mean of 1.43 (C.I. 1.01–1.86) episodes in the 8 month study period, while 0.86 (C.I. 0.54–1.19) episodes were recorded in the 59 patients of the active group (t-test = 2.129, p = 0.036). To further support this difference, a non-parametric analysis of the number of infectious episodes showed a median value of 0 in the treated group and 1 in the placebo group. To better define this result, the difference in episodes in the single patients was also evaluated. For this, the frequency of the patients with 0, 1, 2 etc. IE was analyzed. Fig. 2 shows the frequency of IE in the pre-study population, in the Lantigen B treated group and in the placebo group. The placebo group had more IE/patients (χ² = 14.7, p = 0.023) when compared with the active group. With regard to the diseases suffered by the patients, the frequency was 16% for pharyngotonsillitis (PT), 36% for rhinopharyngitis (RP) and 22% for bronchitis (B); the remaining 26% were otitis, tonsillitis, sinusitis and, rarely, pneumonia. After the treatment with Lantigen B, the total number of infections was significantly reduced, as stated before, but the relative frequency of different clinical pictures remained virtually unmodified (25% for PT, 41% for RP, 18% for B), while others were slightly reduced to 16% (Table 2). A subgroup analysis of those patients suffering from respiratory allergy (22 in the placebo and 22 in the treated group, according to the evidence collected during the eligibility visit) was also performed. Of these patients, only 24 had allergic episodes during the study period: 9 had ocularrhinitis, 11 had asthma and 4 had both. Of note, the frequency of IE in those patients with allergy was slightly higher (3.80 IE/8 months) that in the non-allergic patients (3.58 IE/8 months) even if not significant (t = −0.81, p = 0.42). Like the original population, non-significantly different effects were observed when the gender (12 males and 32 females) of the allergic patients was evaluated. Similarly, no significant effects on the treatment results were observed when the smoking habits and the different clinical centers were evaluated (ANCOVA, F = 1.473, p = 0.227 and F = 1.817, p = 0.087, respectively).
3.4. Secondary objectives

The duration of the IE in the two groups was different (10.14 days in the placebo and 5.83 in the Lantigen B group), but a statistical significance was not achieved ($F = 2.724, p = 0.102$). The intervals between IE were evaluated at the end of the first and second treatment cycle. In the first interval, the differences in the number of events (eight in the placebo and five in the treated group) as well as the mean intervals (in days) were not significant. However, the cumulative hazard of suffering from an IE was 15% for the placebo and 9% for the Lantigen B group. Similar results, even if not statistically significant, were observed for the second interval (six EI for the placebo and two for the Lantigen B group). The relevant cumulative hazards of developing an IE in the second interval were 0.11 and 0.035 for placebo and Lantigen B groups. The concomitant drug use in the treated and in the control groups was also evaluated by a logistic regression analysis. For this, three families of drugs the use of antibiotics and NSAIDs was evaluated in the whole population, while anti-allergic drugs, which included bronchodilators, local corticosteroids and anti-histamine were evaluated in the subset of allergic patients. No significant differences were observed between the treated and control groups, even if a clear trend was evident: indeed, the treated patients were always treated less. Despite the logistic regression analysis demonstrated that the differences were not significant ($p = 0.421, p = 0.134, p = 0.283, p = 0.519$ and $p = 0.667$ for antibiotics, NSAID, anti-histamine, local corticosteroids and bronchodilators, respectively), the same statistics showed that probability (Exp(B), also called the odds-ratio) of using antibiotics was reduced by 52.1% (being Exp(B) = 0.479) in the treated patients, of NSAID by 30.2% (Exp(B) = 0.698), of anti-allergic drugs by 56.2% (Exp(B) = 0.438), of corticosteroids by 41.2% (Exp(B) = 0.588) and of bronchodilators by 25.7% (Exp(B) = 0.734) in the treated groups. Thus, not only the logistic regression showed that for all the drugs the trend was toward a reduction, but further confirmed the significance of the reduction of the number of IE ($p = 0.021$ and $p = 0.044$, for the whole group and the subset of allergic patients). Finally, in both groups, the mean days of absence from work were very low (0.79 for placebo and 0.74 days for Lantigen B, $p = 0.925$).

3.5. Adverse reactions (ARs)

With regard to the AR, 21 were recorded for Lantigen B and 23 for placebo. The ARs are described in detail in Tables 3a and 3b. Briefly, the AR of the Lantigen B group occurred in 10 different patients, while to 17 different patients in the placebo group. Some AR could be related to the administration of Lantigen B and the placebo, such as a dry and burning mouth (two episodes in one patient of the group), stomatitis, odinophagia, and oropharynx burning (five episodes in the placebo group). The last three AR required the suspension of the therapy. Other AR included hypothyroidism, distortion of the left ankle, arthralgia, lung cancer, headache, urinary tract infection, vaginal candidiasis, viral gastroenteritis, oral herpes, hypertension, nausea and gastro-enteric reflux and were not related to the treatment. Only lung cancer required the suspension of the drug. The ARs were also classified according to the severity and the need for therapy interruption (TI). In the placebo group, the 23 ARs were divided into 11 slight (1 requiring TI), 10 intermediate (requiring TI) and 2 severe, both interrupted. In the Lantigen B group were 12, 7 and 1 (requiring TI), respectively.

3.6. Epidemiological surveys of the study period

During the study period (namely, September 2012–June 2013), the circulation of respiratory viruses (both in qualitative and quantitative terms) was largely superimposable on that of the previous year, used as the reference interval for the calculation of the number of IE for patient recruitment. Fig. 3 shows the results of this survey; the RSV and influenza viruses were clearly present in the December–April interval of the two different periods, while other viruses, such as adenoviruses and rhinoviruses were detected in the remaining period.

4. Discussion

In this report, the efficacy of a chemical bacterial lysate, Lantigen B, in the reduction of the number of IE in patients with RRTI has been clearly demonstrated using a rigorous scientific and clinical approach. Indeed, this phase IV study was conducted at 12 different centers, using a double blind, placebo controlled study protocol. The authors are aware that few studies on the effects of bacterial lysates have been conducted in the past, using controlled clinical trials in Good Clinical Practice (GCP) trained hospitals. For this study, the foreseen number of patients to be recruited was 190 and 30 were expected to be lost during the study. However, the clinical centers, even though strongly committed, were able to recruit 160 patients. Of these, more than 25% were lost and only 117 patients could be evaluated at the end of the study. The loss of one-fourth of
Table 3b
Adverse reaction in the placebo group.

| Patient | Adverse reaction          | Seriousness | Intensity | Action                          | Relationships with the drug |
|---------|---------------------------|-------------|-----------|--------------------------------|------------------------------|
| 4       | Dry and burning mouth     | Not serious | Light     | None                           | None                         |
| 5       | Dry and burning mouth     | Not serious | Light     | None                           | None                         |
| 25      | Surgery (lipoma removal)  | Not serious | Intermediate | None                  | None                         |
| 26      | Pre-menopausal syndrome   | Not serious | Light     | None                           | None                         |
| 42      | Dental abscess            | Not serious | Intermediate | None                  | None                         |
| 43      | Constipation              | Not serious | Light     | Discontinuation of therapy     | Unlikely                     |
| 65      | Backache                  | Not serious | Light     | None                           | None                         |
| 65      | Odinophagia               | Not serious | Severe    | Discontinuation of therapy     | Possible                     |
| 81      | Dyspepsia                 | Not serious | Intermediate | None                  | None                         |
| 81      | Viral gastro-enteritis    | Not serious | Intermediate | None                  | None                         |
| 89      | Hypertension              | Not serious | Intermediate | None                  | None                         |
| 97      | Hip arthroplasty          | Serious     | Severe    | Discontinuation of therapy     | None                         |
| 100     | Oropharynx burning        | Not serious | Intermediate | Discontinuation of therapy     | Possible                     |
| 122     | Stomatitis                | Not serious | Intermediate | Discontinuation of therapy     | Possible                     |
| 133     | Oral herpes               | Not serious | Light     | None                           | None                         |
| 161     | Thoracoalalgia            | Not serious | Light     | None                           | None                         |
| 165     | Migraine                  | Not serious | Light     | None                           | None                         |
| 165     | Migraine                  | Not serious | Light     | None                           | None                         |
| 168     | Conjunctivitis            | Not serious | Light     | None                           | None                         |

Fig. 3. The cumulative frequencies of virus isolation in the reference period (2011–2012) and in the study period (2012–2013) are represented as histograms. It is evident the increased frequency of virus infection in the late winter–early spring periods.

the patient cohort is a relevant phenomenon. Despite this reduction, the post-hoc analysis showed that the number of evaluable patients was still sufficient to maintain the statistical relevance of the study and thus the validity of the observed results. Moreover, the adverse reactions recorded as well as the causes of dropout were extremely heterogeneous and all virtually unrelated to the treatment. A significant reduction in the number of IE was observed in the treated group: an average of 0.86 episodes in the 8 month study period was observed in the Lantigen B treated patients, while in the placebo group the mean was 1.43 episodes. In this context, it should be noted that, for the population recruited, the average number of previous IE was 5.34/year (corresponding to 3.56/8 months). As stated before, in the period of the study, the mean number of IE in the placebo group was 1.43/8 months (females 1.24 IE, males 1.80 IE). This difference may have different explanations. Different weather and epidemic conditions in the two subsequent years, differences in winter and spring climates such as a warmer temperature or a less aggressive flu virus epidemic may reduce the number of respiratory tract infections, allowing the placebo to mimic a pharmacologically positive effect. Moreover, a certain susceptibility to the effects of a placebo (more evident in females than in males) and a certain tendency to increase the number of IE in both groups when describing the severity of their conditions may have also influenced the study results. With regard to the different period epidemiology, it should be noted that the survey of virus infections in the study season, when compared with that of the previous “cold” season, showed virtually identical results, with the main viruses (influenza, rhinoviruses, adenoviruses and RSV) having the same prevalence in the two period. Therefore, this finding did not support the hypothesis of different “infectious” environments causing a different number of respiratory tract infections in the study population. The reduction in the number of IE in the placebo group may have depended on a well-known bias, the Hawthorne effect. This phenomenon, originally defined in an industrial setting [23] has been extended to the realm of medical research [24,25] and suggests that the subjects’ behavior or the study results may be altered by the subjects’ awareness that they are being studied or if they received additional attention.
It should also be noted that recruitment was based on the number of respiratory tract infections in the previous year. Even if not generalized, a certain tendency of patients to (involuntary) increase their reported number of infectious episodes might have occurred, and this fact should at least in part explain the differences observed. However, to overcome these problems, double blind placebo controlled studies (like the present one) are mandatory to measure the differences between the treatment and placebo in the same period of the year and in the same locations. In this context, the finding that neither the doctors nor the patients really recognized the superior activity of the treatment is also intriguing. Of course, the positive effects of the placebo, as discussed above, had a certain role. But, more importantly, the differences observed were homogeneously distributed between the placebo and the treated groups. This clearly means that the positive effects of the administration of bacterial lysates could be observed at a population level while they were less evident at individual level. In addition, this can also explain why the medical community is sometimes skeptical about the use of these drugs. Indeed, just using personal daily experience and feelings, it is difficult to establish whether this family of drugs is active.

Some interesting, if inconclusive data can be derived from the analysis of the use of the drugs in the two groups. No statistically significant differences were observed between the placebo and the treated groups for antibiotics, NSAIDs, bronchodilators, anti-histamine and local steroids. However, a clear trend was observed in the treated groups; the use of drugs was always reduced in this group. With regard to the small group of allergic patients, the first observation was that in the population of RRTI patients, patients with allergy symptoms represented 40% of the total. It was evident that the treatment with Lantigen B did not significantly alter the number of allergic episodes or the number of days with allergy. This finding was not expected even if the strict correlation between the allergy symptoms and respiratory infectious diseases could suggest a potential positive effect [20]. A certain effect was observed, as a trend, in the use of anti-allergy drugs: indeed, in allergic patients, logistic regression showed that the probability of using drugs specific for allergy was reduced. Some criticism can be raised on this study: (a) the number of patients recruited, even if statistically correct, is not very large; (b) during patient’s recruitment and follow up, functional and laboratory tests were performed only if some IE or adverse reaction occurred; (c) concomitant diseases, potentially mimicking symptoms of the respiratory tract (such as unknown primary immune deficiency – undiagnosed defect however rare in a study cohort aged between 18 and 65 years) were not specifically considered; (d) the non-perfect synchronism of the recruitment (started in September and closed in December 2012) associated to the 8-month follow-up, that could at least in part dilute the effects of typical infections of the winter–spring period. However, strengths of the study are several. In particular, (a) the rigorous randomization homogeneously distributed the abovementioned biases in the two cohorts; (b) the statistical analyses (ANOVA and logistic regression) not only documented the effects of Lantigen B on the number of IE, but also identified a clear trend toward reduction of the use of concomitant therapies in Lantigen B treated patients; (c) the real life-like enrollment of patients (as stated by the heterogeneity of adverse reactions not related to the administration of the drug) is representative of the population that has daily access to the offices of general practitioners and specialists: thus, the observed results are reliable.

In conclusion, the administration of Lantigen B, evaluated in a double blind controlled clinical study, resulted in a significant reduction in the number of IE in the treated group. In particular, not only was the frequency of IE reduced at the population level but also at the level of the individual, the number of episodes was lower in the treated than in the placebo group. The control of respiratory tract infections, at least in the familial or work environment, results in a reduced spread of the pathogen and in a reduced use of drugs such as antibiotics, with evident advantages for the patient and the community. In conclusion, the described results, together with the significant reduction in concomitant therapies and the virtual absence of any adverse reactions, indicates that this drug may be used as an effective first line drug for the prophylaxis of infectious episodes in patients with RRTI. The capacity of controlling respiratory tract infections is a finding particularly relevant in this period, when not only a global increase of respiratory infections is recorded [24], but also new antibiotics will not be available on the market for the next years [25].

Conflicts of interest

G. Rinaldi, MD declares that he is full-time Scientific Director of Bruschettini S.r.l.; G. Melioli, MD is an advisor of Bruschettini S.r.l. for Research and Development. The authors report no other conflicts of interest in this work.

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