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Original Article

# Strategies for optimizing compliance of paediatric patients for seasonal antibacterial vaccination with sublingually administered Polyvalent Mechanical Bacterial Lysates (PMBL)

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**Abstract.** The objective of this pilot study was to evaluate efficacy, tolerance and compliance of paediatric patients vis-à-vis a cycle of PMBL treatment (a sublingual tablet taken for ten consecutive days over three consecutive months). The study enrolled 89 children (65 randomised to the treated group and 24 to the control group). The study protocol included an enrolment check-up (T0) and follow-ups at two months (T1), three months (T2) and nine months (T3) following the end of treatment, during which episodes of RRI were recorded; the main blood chemistry, immunology and phlogosis parameters were measured, together with hepatic, renal and bone marrow toxicity indexes. The administration of PMBL led to a significant decrease in RRI in the treated group, not only among the same children in relation to the previous winter, but also in comparison with untreated children during the same winter (mean number of infective episodes per patient 7.84 vs. 4.78, $p<0.05$, in the first case; 6.78 vs. 4.78, $p<0.05$, in the second case). White blood cell count showed a drop in the treated group as opposed to an increase in the untreated group, but there were no statistically significant differences in the intergroup analysis or in the intragroup one. Phlogosis indexes (PCR and plasma mucoprotein) in the treated group fell following treatment with PMBL, and this is statistically significant not only in the intragroup analysis but also the intergroup one. Mean values of B-lymphocytes in the treated group seemed to increase significantly following treatment, which was not the case in the untreated group. The variations in all the blood chemistry indexes for toxicity were far from significant and they remained within the norm, without significant clinical manifestations of side-effects of drug intolerance. As to evaluation of patient compliance, use of the device we describe enabled acceptable compliance with treatment even in the youngest children, similar to the compliance observed among appropriately motivated older children.

**Parole chiave:** Recurrent respiratory infections, polyvalent mechanical bacterial lysates, sublingual, paediatric compliance

## Introduction

Acute respiratory infections (ARI) and recurrent ones (RRI) of the upper and middle respiratory tracts, and of the middle ear, are among the leading clinical problems faced in general paediatrics, particularly during the winter (1-5).

This type of pathology, which generally has a viral etiopathogenesis at onset but is frequently then overlapped by a bacterial component, has increased notably over the past few decades, not only on a clinical level, but also in terms of its burden on the health and social system (6-8). Numerous causes have been theorised for this phenomenon. First of all, we can cite the environmental conditions, which have shown a constant decline in air quality during the industrial era, particularly in major urban centres and in the highly industrialised areas of Central Europe (dubbed "the blue banana", a term used to define the enormous area extending from southern England to the middle of continental Europe across to northern Italy; in nocturnal satellite images it is shaped more or less like a banana). Steps to remedy the situation have been taken only recently. It is widely known that the respiratory tract is subject to a cumulative effect of air pollution, cold weather and constant exposure to the potential sources of reinfection that we must face in our daily lives. Particularly among younger patients, this lifestyle involves a high degree of social "promiscuity". Simply by way of example, we can cite day-care centres, kindergartens and elementary schools: it has become normal for children to spend many hours in closed rooms, elbow to elbow with their schoolmates who, in turn, often represent a full-fledged incubation medium for germs as well as viral or bacterial respiratory infections.

In a perverse mechanism, this is compounded by the relative immaturity typical of the immune system of young children. These subjects often reach school age after spending the first few years of their lives in completely protected conditions and in wholly comfortable environments, having minimal contact not only with the rigours of the outside environment but also with their peers. Thus, there arises a vicious cycle of infection, irritation of the respiratory tract and reinfection, which can trigger a seasonal series of infective episodes – better known as RRI – that can frequently arrive at six-seven episodes a year (9-13). If we consider the fact that these episodes tend to be concentrated during the cold season, e.g. from September to March-April (Fig. 1) (14), this means that, in the worst cases, these children can spend more time at home, sick or convalescing, than at school.

In these cases, intervention strategies are well known, not only in the case of clear infection, but also during the preventive phase. In terms of prevention, there is now wide consensus among experts on the benefits that can be achieved through several simple and timely actions.

The role of antiviral vaccination campaigns (particularly flu vaccines) has garnered enormous attention, and evaluation on the merit of whether or not to recommend these vaccinations to our patients and/or to their closest relatives and persons in their homes must be handled on a case-by-case basis. Instead, there is no doubt that children at risk of RRI must categorically be protected against passive smoke and futile contact with outside sources of environmental pollution. Along these lines, there are systems – some of which more effective than others – for filtering air in closed environments, and they can help to some extent. Likewise, it is possible to provide simple but often very helpful information to parents on the need for attentive household hygiene, the hours when it is best to air out the home, and when to take young children outside for a walk (15, 16).

Lastly, there is consensus regarding an active preventive approach, based on full-fledged antibacterial

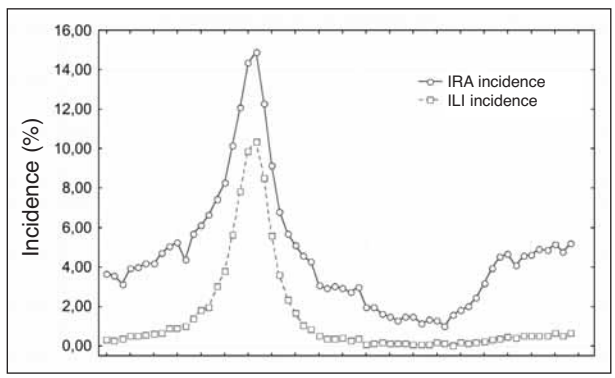


Figure 1. IRA and ILI (Influenza-Like-Illness) annuation (from week 42 in 2002 to week 49 in 2003)

vaccination. This can be done through the seasonal use of products pertaining to the class of polyvalent mechanical bacterial lysates (PMBL), which can be administered sublingually; the leading one is Ismigen® (6, 15, 17-19).

According to extensive and well-established literature, PMBL treatment seems to yield various beneficial effects, including a significant increase in antibody titre even after just one treatment cycle (Fig. 2), in terms of IgM, IgG and IgA (Fig. 3). This has a positive therapeutic effect on the amplitude of the spectrum of immunological production and on the production of opsonising antigens (6, 17). In particular, sublingual administration guarantees effective protection of the respiratory mucosae, which represent the first barrier to infection (Fig. 4), making it possible to bypass the gastroenteric tract. This avoids denaturing the antigens and puts them directly in contact with

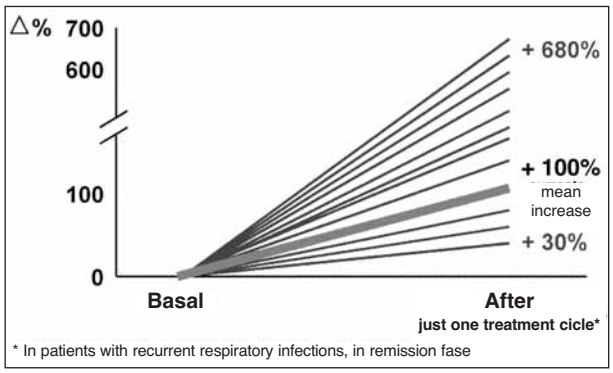


Figure 2. Significant variation in antibody titre using a polyvalent mechanical bacterial lysates (PMBL)

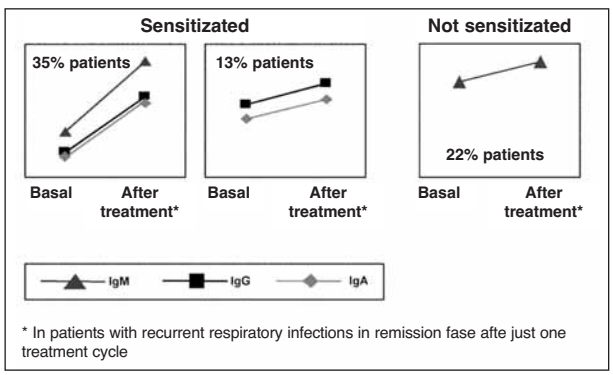


Figure 3. Significant increase in terms of specific antibodies (IgM, IgG, IgA) using a polyvalent mechanical bacterial lysates (PMBL)

| Antigen features | Immunological consequences | Therapeutical results |
|---|---|---|
| Mixture of bacterial wale fragments | Intact antigen | Production of opsonising antibodies |
| Different bacteria | Antigenic heterogenicity | Wide spectrum of immunological action |
| Sublingually | Immunization in leading site | Protection of the respiratory mucose |

Figure 4. Objectives for an effective antibacterial vaccine-prophylaxis

the cells that best carry out the task of antigen-presenting cells, namely the Langherans cells. The outcome is the development of optimum immune response, demonstrable in the saliva via antibody assay after even just one treatment cycle (15, 20-23).

We must emphasise that the recommended PMBL treatment envisages three ten-day cycles over the course of three consecutive months. In clinical terms, this treatment can guarantee a significant reduction in the number and gravity of ARI episodes during the winter in the groups of treated patients.

PMBL treatment thus seems to be an elegant preventive approach that is relatively advantageous from an economic standpoint, effective and well tolerated by geriatric and, in this specific case, paediatric patients alike.

Nevertheless, there is a problem that few have addressed so far: i.e. patient compliance. Particularly with very young children, this is an aspect that should not be underestimated.

In fact, for optimal sublingual administration of PMBL, the preparation must be kept under the tongue for several minutes (at least 5-7). This is done to permit prolonged contact with the sublingual mucosae, which are very rich in cells pertaining to the immune system. In our experience, this procedure can be conducted only with children who are old enough and sufficiently motivated to understand the need for respecting this “tedious” procedure. In effect, this almost always occurs in children over the age of five years, to a variable extent in those between four and five years of age, and almost never in children under four.

These considerations convinced us to conduct a pilot study in order to evaluate the adherence of paediatric patients to sublingual treatments such as the

ones cited above, based on different practical approach strategies: the use of devices in younger children and simple educational protocols in older ones.

## Materials and methods

This study was conducted on a group of children, selected randomly from subjects referred consecutively in the autumn and winter of three consecutive years (2001-2002-2003) for repeated episodes of RRI. Inclusion criteria for the study were age ranging from 10 months to 10 years (Table 1) at the time of enrolment, and diagnosis of RRI based on case history with reference to the winter prior to enrolment.

Specifically, to be considered eligible for enrolment the subjects had to have a history of at least three episodes/year for children under the age of 12 months, at least five for those aged 13 to 24 months, at least seven for those aged 25 to36 months, at least eight for those aged 37 to 48 months, at least nine for those aged 5 years, and at least four episodes in the past year for subjects aged six years and older.

Exclusion criteria were the presence of congenital metabolic disorders (diabetes), cancer, chronic alterations of immune and cardiovascular functions, and the assumption of drug treatments that could interfere with the immune system during the three months prior to enrolment. Children manifesting acute lower respiratory tract infections during the study would automatically be excluded from the protocol.

Table 1. Enrolled children classified by age at time of enrolment

| Age | Treated group | Control group | |
|---|---|---|---|
| 10-12 months | 2 | - | |
| 13-24 months | 3 | 1 | |
| 25-36 months | 5 | 2 | |
| 37-48 months | 4 | 1 | |
| 49-60 monthsmesi | 25 | 10 | |
| six-ten years | 26 | 8 | |
| Total enrolled | 65 | 24 | |
| Mean age (months) | 63.6±38.3 | 57.8±41.2 | ns |
| Total patients at end of study | 62 | 23 | |
| Mean age (months) | 61.9±36.8 | 58.5±40.3 | ns |

In all eligible cases, prior to enrolment the parents were provided with exhaustive information and were required to give written consent to include their children in the study. Based on these criteria, 89 children were eligible to be enrolled in the study; 65 were put in the treated group and 24 in the untreated group. Sixty-two children in the first group and 23 in the second group completed the study. Three patients dropped out of the study due to inadequate compliance with treatment, which involved one tablet of Ismigen® in the morning for ten days, repeated for three consecutive months during autumn. One of the untreated subjects did not complete the follow-up programme because the child's family moved to another city. None of the enrolled subjects contracted lower respiratory tract infections.

For all patients, the study protocol envisaged a check-up at enrolment (T0) and follow-ups at two (T1), three (T2) and nine (T3) months after the end of treatment.

The primary objective of the study was the clinical evaluation of treatment efficacy in terms of reducing infective episodes and assessment of variations in immune and phlogosis indexes both within the group (winter 2003 vs. winter 2002) and between groups (treated subjects vs. untreated subjects in winter 2003).

During all check-ups, the young patients underwent in-depth general clinical evaluation and examination of medical history, recording all ARI episodes of both the upper and middle respiratory tract. With regard to the evaluation of immune and inflammation indexes obtained through numerous lab tests scheduled at times T0, T1 and T2, the measured parameters are listed in Table 2.

Lastly, tolerance to treatment was evaluated based on clinical data and medical history, and through careful evaluation of the relevant indexes obtained from blood chemistry tests.

Statistical evaluation was performed through parametric tests (Student's T test for paired data, and analysis of variance).

The secondary endpoint of the study was to evaluate compliance with treatment among very young patients. Consequently, when our patients were enrolled in the study, we divided them into two groups, set-

Table 2. Laboratory tests envisioned by the study protocol

| |
|---|
| *Indexes of phlogosis and immune function* |
| White blood cells |
| Lymphocytes |
| Neutrophils |
| Monocytes |
| Basophils |
| PCR |
| Plasma mucoproteins |
| B-lymphocytes |
| T- lymphocytes |
| NK |
| Phagocytic activity |
| Chemotaxis |
| Spontaneous lymphocyte proliferation (without stimulation) |
| *Bone marrow toxicity indexes* |
| Erythrocytes |
| Leukocytes |
| Platelets |
| *Renal toxicity indexes* |
| Serum creatinine |
| Phosphorus |
| Plasma electrolytes (Cl, Na, K) |
| *Hepatic toxicity indexes* |
| SGOT |
| SGPT |
| Gamma-GT |

ting the age of 48-60 months as the cut-off based on the behavioural differences noted above.

Patients younger than 48 months and those aged 48 to 60 months who were still immature were taught to use a simple handmade device we invented. In essence, we put the crushed tablet into a dummy. The tablet was inserted into the bulb of the dummy through the back opening on the dummy, which was then blocked by pressing piece of candy onto it (fruit gelatine). A hole was made in the tip of the dummy using a red-hot needle. As the child sucked on the dummy, the tablet dissolved and passed through the hole into the sublingual cavity. The child was checked at two and at five minutes to verify that the entire amount had gone into the oral cavity.

Older or more motivated subjects were given conventional commercially available tablets and these children were patiently instructed on the importance of taking the tablet correctly. At times, "reward" strategies were used (e.g. "if you keep this little candy under your tongue for the time it takes me to read you this fairytale, I'll give you…".).

Table 3. Classification of treated subjects according to PMBL intake procedure

| Age | Device gruppo | Motivated group |
|---|---|---|
| <48 months | 13 | 1 |
| 49-60 months | 18 | 7 |
| >61 months | - | 26 |

The subgroup of non-compliant subjects (device group) included all children under the age of 48 months (with the exception of a 46-month-old subject who adamantly refused to take the tablet unless he could take it the way his older brother did; however, this child scrupulously followed our instructions) and 18 five-year-old children. The other seven in the latter age group proved sufficiently motivated to follow the procedure used for subjects over the age of six (motivated group) (Table 3).

The objective of this phase of the study was simply to evaluate – based on medical history and with parent collaboration – if use of the device made it possible to achieve compliance in the younger group comparable to the optimal compliance of the older children.

For evaluation of merit, compliance was considered optimal if the child took at least 28 of the 30 tablets envisaged by the administration protocol, acceptable if at least 26 out of 30 were taken, and unsatisfactory if less than 26 tablets were taken.

## Results

In terms of clinical efficacy of the treatment, Tables 4, 5 and 6 show that the administration of PMBL led to a significant decrease in the number of ARI episodes in the treated group, not only with regard to the number observed in the same children the previous winter, but also in comparison to the untreated children in the same winter (mean number of infective episodes per patient 7.84 vs 4.78, $p<0.05$, in the first case; 6.78 vs 4.78, $p<0.05$, in the second case). Inversely, the comparison performed in the untreated group did not reveal statistically significant differences in the rate for the two winters examined.

Table 4. Rate of infective episodes of the respiratory tract in the group treated with PMBL: comparison between winter 2001-2002 (no treatment) and winter 2002-2003 (treatment)

| | Winter 2001-2002 Without PMBL | Winter 2002-2003 With PMBL | |
|---|---|---|---|
| Number of patients | 65 | 62 | |
| Number of infective episodes | 496 | 297 | |
| Mean per patient | 7.84 | 4.78 | p<0.05 |

Table 5. Rate of infective episodes of the respiratory tract in the untreated group: comparison between winter 2001-2002 and winter 2002-2003

| | Winter 2001-2002 Without PMBL | Winter 2002-2003 With PMBL | |
|---|---|---|---|
| Number of patients | 24 | 23 | |
| Number of infective episodes | 173 | 156 | |
| Media per paziente | 7.20 | 6.78 | ns |

In terms of blood chemistry, it must be noted that the leukocyte count showed a drop from mean values of 10,848 before treatment to 9723 after treatment in the treated group vs an increase from 11,231 to 11,983 in the untreated group. However, there were no statistically significant differences in the intergroup and intragroup comparisons.

Table 6. Rate of infective episodes of the respiratory tract: comparison between the untreated group and the treated group in winter 2002-2003

| | Winter 2002-2003 Without PMBL | Winter 2002-2003 With PMBL | |
|---|---|---|---|
| Number of patients | 23 | 62 | |
| Number of infective episodes | 156 | 297 | |
| Mean per patient | 6.78 | 4.78 | p<0.05 |

Variations in the phlogosis indexes (PCR and plasma mucoprotein) were likewise significant. In the treated group these indexes dropped following treatment with PMBL, and this is statistically significant not only in the intragroup analysis but also the intergroup one.

Differences in the mean values of B-lymphocytes were also significant. In the treated group they seemed to increase significantly following treatment, which was not the case in the untreated group.

Variations in other immune indexes are currently being assessed and these data will be published at a later date.

Lastly, the variations in all blood chemistry indexes of toxicity proved to be entirely marginal and far from significant. These indexes remained strictly within normal range, confirming the excellent tolerability of PMBL.

It is also important to emphasise that from a clinical standpoint, there were no significant manifestations of side-effects or of intolerance to the drug.

Table 7. Variations in blood chemistry indexes of efficacy: comparison between the untreated group and the treated group in winter 2002-2003

| | Winter 2002-2003 Without PMBL | | | | Winter 2002-2003 Wiht PMBL | | | |
|---|---|---|---|---|---|---|---|---|
| | T0 | T1 | T2 | T3 | T0 | T1 | T2 | T3 |
| White blood cells (n.v. 6200-10100) | 11231 | 10984 | 12546 | 11983 | 10848 | 10634 | 10962 | 9723* |
| Lymphocytes (%) | 31 | 30 | 31 | 30 | 30 | 31 | 31 | 32 |
| Neutrophils (%) | 60 | 61 | 59 | 60 | 58 | 60 | 61 | 60 |
| Monocytes (%) | 5 | 5 | 4 | 5 | 5 | 4 | 5 | 5 |
| Basophils (%) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Eosinophils (%) | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 |
| PCR (n.v. <5 ng/100 ml) | 16.98 | 15.78 | 16.53 | 16.32 | 17.54 | 13.65 | 10.76 | 6.84* |
| Plasma mucopr. (v.n. 2-5 mg/100 ml) | 9.02 | 8.54 | 8.95 | 8.32 | 8.75 | 6.44 | 4.84 | 3.88* |
| B-lymphocytes (n.v. 8-15%) | 7.1 | 6.6 | 7.4 | 5.9 | 6.7 | 6.9 | 8.1 | 9.9* |

* p<0.05

Table 8. Compliance with treatment based on age and administration strategy in subjects taking PMBL

| | Device group | Behavioural group |
|---|---|---|
| Total subjects | 31 | 34 |
| Regula intake | 24 | 29 |
| Irregular but acceptable intake | 5 | 4 |
| Unsatisfactory intake | 2 | 1 |

As to evaluation of patient compliance, which was a secondary endpoint of the study, Table 8 shows that use of the device we describe here makes it possible to achieve compliance with treatment even in the youngest children, comparable to the compliance observed among appropriately motivated older children.

Lastly, on an anecdotal level, we must note that parental satisfaction with this type of therapeutic approach seemed very good, substantially limiting any doubts, particularly among the parents of the youngest children.

## Conclusions

Based on the results of this experience, we can state that in terms of the primary objective of the study, i.e. evaluating the efficacy and tolerability of PMBL treatment in a heterogeneous group of children like the one enrolled in our study, hypothesis H0 seems to be confirmed on a clinical level and on the basis of laboratory indexes. If taken correctly, PMBL can significantly lower the rate of RRI in otherwise healthy children, not only in terms of intragroup analysis but also in comparison with an untreated control group.

From both a clinical and laboratory standpoint, tolerability also appeared to be excellent. Moreover, any doubts about the effective possibility of administering preparations absorbable sublingually to very young children can be overcome by using an appropriate educational strategy and with the aid of adequate but extremely simple devices, such as a slightly modified conventional dummy.

To conclude, PMBL is confirmed as a well-tolerated and effective treatment (15, 19, 24-26). This is reflected by the decrease in mean values – and in the end values above all – of white blood cells, PCR and plasma mucoproteins following treatment. The decrease of the maximum end values is particularly interesting, as this documents a reduction in the stimulus determined by microbial flora. It is likely that the contrasting action exerted by bolstering the immune system against microbial, viral and, above all, bacterial aggression reduces its pathogenic potential, with an ensuing decrease in the extent of inflammatory reactions, documented by the decrease in the relevant indexes.

Among immune parameters, a significant increase in B-lymphocytes was noted following treatment. In all other cases, conclusive assessments have not been possible yet. Lastly, tests conducted to monitor product tolerability demonstrated that there are no side-effects either clinically or in terms of bone marrow, renal and hepatic function indexes.

Therefore, the adopted strategy can be indicated as a rational seasonal approach for a large range of paediatric patients at risk for respiratory problems triggered by colds.

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