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Pidotimod for the prevention of acute respiratory infections in healthy children entering into daycare: A double blind randomized placebo-controlled study

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ABSTRACT

Acute respiratory tract infections (ARTIs) are very common in pediatric age and reach a peak in the first 4 years of life, especially in children attending daycare. Pidotimod, a synthetic immunostimulant, may reduce the incidence of ARTIs in children with predisposing risk factors. Nevertheless studies on healthy children are presently lacking. We performed a double-blinded randomized placebo-controlled trial study to assess the efficacy of Pidotimod in a population of 3-year-old healthy children who just entered kindergarten. The main outcome was the incidence of respiratory infections in this population and the secondary outcome was the prescription of antibiotics.

The study group consisted of healthy 3-year-old children who had not yet attended day-care centers. Patients were enrolled by a convenience sample of 17 family pediatricians (FP). Children were randomized to receive either Pidotimod 400 mg *per os* or placebo twice daily for the last 10 days of each month from October 2013 to April 2014. Any time a child presented to his/her FP with fever and ARTI was diagnosed, clinical and therapeutic data were collected.

A total of 800 children were pre-screened, 733 did not meet the inclusion criteria and 10 refused to participate. Of the 67 eligible subjects, 57 were successfully enrolled within the study recruitment period and randomized to receive Pidotimod ($n = 29$) or placebo ($n = 28$). Eight children were lost to follow-up. In the final analysis were thus included 24 children who received Pidotimod and 25 who received placebo. The incidence rate ratio for respiratory infections was 0.78 (95%CI 0.53 to 1.15, $p = 0.211$) for Pidotimod vs. placebo. The corresponding risk ratio for antibiotic usage was 0.56 (95%CI 0.27 to 1.16, $p = 0.120$).

In our trial, Pidotimod did not prove to be statistically superior to placebo for the prevention of ARTI in a population of healthy children who entered kindergarten. However, Pidotimod showed some potential as a means for reducing antibiotic usage in these children.

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Abbreviations: ARTIs, acute respiratory tract infections; CI, confidence interval; IRR, incidence rate ratio; NK, natural killer; PI, principal investigator; SD, standard deviation.

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1. Introduction

Worldwide acute respiratory tract infections (ARTIs) are the most common forms of infections in children. In temperate countries, ARTIs have higher incidence in colder seasons such as autumn and winter [1]. During childhood, ARTIs incidence peaks in the first 4 years of life, especially in children attending daycare [1].

Given the increasing proportion of working mothers during the past decades, day care centers constitute an important common childhood environment, where children are exposed to conditions that may influence their respiratory health. Compared to their home-attended pairs, children attending day care centers are exposed to a larger variety and amount of pathogens, given their closer contact with other children every day. Upon entry to day care, the youngster are the most vulnerable and more susceptible to develop respiratory infections [2]. A tendency toward hyporesponsive immune responses in early life, characterized by both reduced innate and adaptive immune responses, plays an important role in ARTIs recurrence in the first 6 years of life [3]. Moreover exposure to dampness, molds, parental smoke and pollution are known environmental risk factors for ARTIs [1,4–7].

ARTIs contribute to healthcare expenses up to 20% of medical consultations, emergency room admittance, hospitalization and prescription of symptomatic treatments and antibiotics [7,8]. ARTIs are responsible of 75% of antibiotics prescription in pediatric age and may contribute substantially to the rise in antibiotic resistance [8,9]. ARTIs also lead to indirect costs such as parental absence from work and loss of productivity: up to 30% of parental work-days are lost because of their child's respiratory infection [9,10].

Current epidemiological and socio-economical data, call for alternative approaches to the most well-studied and known therapies. Non-specific enhancement of the antimicrobial immune responses or the innate immunity mechanisms are functional approaches to prevent and treat ARTIs considering the immaturity and naivety of childhood immune system [3,9].

A variety of biologically active substances of natural and synthetic origins – known as immunostimulants have been introduced in some countries for the prevention of ARTIs in children [11]. Pidotimod (3-L-Pyroglutamyl-L-thiazolidine-4-carboxylic acid) is a synthetic immunostimulant authorized for use in subjects 3 years of age or older [11]. Pidotimod has been shown to improve the maturation of dendritic cells, the functioning of natural killer (NK) lymphocytes phagocytosis, and the differentiation of T-cells toward the Th1 phenotype [11–15]. A recent study in children with Down syndrome showed that Pidotimod can up-regulate genes involved in the innate immunity activation [16]. However, the efficacy of immunostimulants in preventing ARTIs is still debated [9]. Some studies reported a reduction of ARTIs following the administration of immunostimulants [9,16–18]. Of note, these studies were all performed in small and heterogeneous populations, and potential confounding factors such as age, concomitant disorders, daycare attendance, parental smoking and seasonality of infections were often not taken into consideration [9]. On the other hand, a more recent study by Licari et al. showed that Pidotimod may prevent respiratory infections in children with an history of recurrent respiratory infections [19].

To date, the preventive effect of Pidotimod on ARTIs incidence in healthy children entering daycare is unknown.

We aimed to assess the efficacy of Pidotimod in preventing ARTIs in a cohort healthy children entering into daycare.

2. Materials and methods

2.1. Study protocol

This double-blinded randomized placebo-controlled trial study was first designed to assess the incidence of ARTIs in the study population. The secondary outcome was to assess the prescription of antibiotics. The study was performed on a convenience sample of 17 family pediatricians (FPs) working for the Italian National Health System in two cities, Milan and Vicenza. FPs were enrolled by word of mouth by the Center for Research and Education of Family Pediatricians, the Pediatric Society of Primary Health Care and the principal investigator (PI). Recruitment took place from October 1 to October 19, 2013. Eligible for the study were healthy Caucasian 3-years old children entering into daycare in fall 2013. Children were excluded if had history of: (1) exposure to passive smoking, (2) one or more siblings attending a day-care center, (3) congenital abnormalities of the respiratory tract, (4) congenital or acquired immunodeficiency including cysytic fibrosis, (5) cardiovascular disease, (6) neurological disease, (7) hematological disease, (8) renal diseases, (9) Down syndrome and (10) previous use of immunostimulants. Each FP provided the PI with a anonymous list of potentially eligible children, in which the pediatrician code, an arbitrary patient number and the gender and birthdate of each child. Children were randomized using permuted-block randomization (4:1) to receive Pidotimod (Axil® – Valeas, Milan) 400 mg *per os* or placebo twice daily for the last 10 days of each month from October 2013 to April 2014. An alpha numeric string was generated by a statistician for the randomization; numbers were printed on a random assignment card and placed in an opaque envelope.

Pidotimod and placebo pills were prepared in order that they were of the same number, size, appearance and taste by Valeas S.p.A and they were administered to the participant children by their FP. Blindness of patients and pediatricians was ensured by using packages that reported only the progressive number of the subject. Subjects were followed-up from October 20, 2013 to May 31, 2014. Any time a child presented to his/her FP with fever (defined as axillary temperature >38 °C), a complete clinical examination was performed. Were considered ARTIs: common cold, influenza, tonsillitis, pharyngitis, bronchitis and otitis media. If the diagnosis of ARTI was made, a case report form including the specific diagnosis and the prescription of any drug was completed. Upon enrollment, FPs were instructed to perform phone calls follow up every 15 days, to remind the study procedures to the parents and monitor participants' adherence to the protocol, documenting the number of missed doses if any. The study was performed in accordance with the declaration of Helsinki and was approved by the Ethical Committee Luigi Sacco Hospital of Milan. The parents of each child gave their written consent to participate.

2.2. Statistical analysis

We considered 0.30 to be the minimally clinical relevant difference in the rate of respiratory infections between the Pidotimod and placebo groups. Considering that an average of 5 respiratory infections per year are reported for 3-year-old children and that they are expected to occur mostly during the autumn–winter season of interest, here [20], we calculated that a number of 40 children per group gives a power of 93% to detect an incidence rate ratio (IRR) of 0.70 at an alpha level of 0.05 [21]. The main outcome (IRR of respiratory infections) was evaluated using a Poisson regression model having the number of infections developed during the study as response variable and the treatment group (0 = placebo; 1 = Pidotimod) as predictor [22]. The secondary outcome (risk ratio [RR] of use of antibiotics) was evaluated using a binomial regression model having the use of antibiotics at any time during the study (0 = no; 1 = yes) as response variable and the treatment group (0 = placebo; 1 = Pidotimod) as predictor [21]. Statistical analysis was performed using Stata 13.1 (Stata Corporation, College Station, TX, USA).

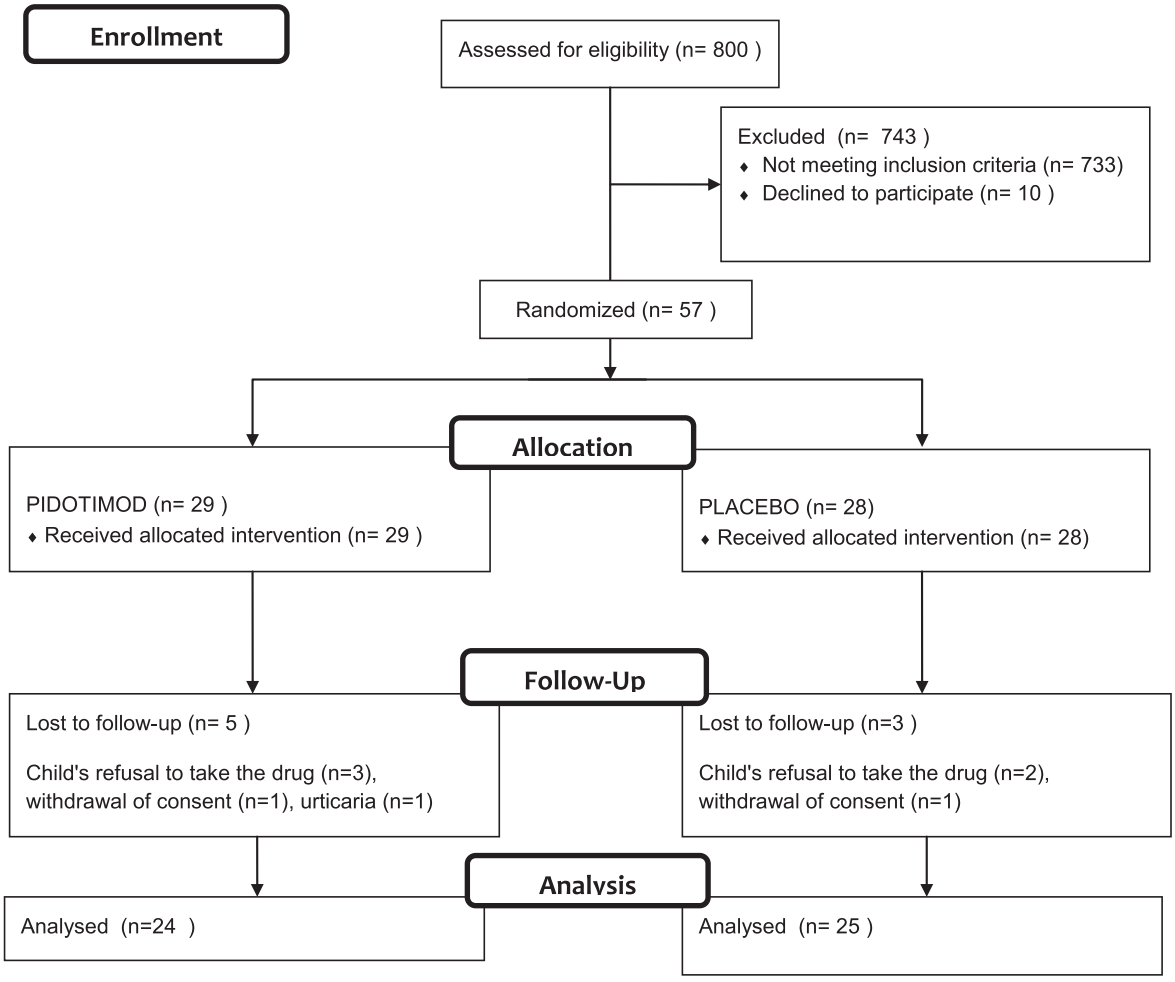

Fig. 1. Flow of patients during the study.

3. Results

3.1. Flow of patients during the study

Fig. 1 reports the flow of children during the study.

A total of 800 children were evaluated for potential enrollment by 8 FP in Vicenza and by 9 FP in Milan. Seven hundred thirty three subjects did not meet the inclusion criteria and 10 refused to participate. Fifty seven children were successfully enrolled: 29 were randomized to receive Pidotimod and 28 to placebo. Eight children were lost to follow-up, 5 from the Pidotimod and 3 from the placebo group (Fig. 1). Twenty four subjects were thus available for analysis in the Pidotimod group and twenty five in the placebo group.

Except for the number of children lost at follow-up for drug refusal, no differences in treatment adherence were found between the two groups.

3.2. Baseline measurements

Table 1 gives the baseline measurements of the Pidotimod and placebo groups. The mean (standard deviation, SD) age was 3.3 (0.3) years in both groups. Moreover both groups were comparable for the other features of interest.

Table 1
Characteristics of the subjects.

	Placebo		Pidotimod	
	N	%	*N*	%
Gestational age				
<37 weeks	1	4.0	4	16.7
>37 weeks	23	92.0	20	83.3
Not available	1	4.0	0	0.0
Total	25	100.0	24	100.0
Immunization with hesavalent vaccine				
Yes	25	100.0	24	100.0
Total	25	100.0	24	100.0
Immunization with pneumococcal 13-valent vaccine				
No	3	12.0	4	16.7
Yes	22	88.0	20	83.3
Total	25	100.0	24	100.0
Previous hospitalization for a respiratory infection				
No	24	96.0	21	87.5
Yes	1	4.0	3	12.5
Total	25	100.0	24	100.0

3.3. Main outcome

The incidence rate ratio (IRR) for ARTIs was 0.78 (95%CI 0.53 to 1.15, $p = 0.211$) for Pidotimod vs. placebo, corresponding to infection rates of 1.9 (95%CI 1.3 to 2.4) and 2.4 (95%CI 1.8 to 3.0) respectively (Fig. 2).

3.4. Secondary outcome

The RR for antibiotic prescription was 0.56 (95%CI 0.27 to 1.16, $p = 0.120$) for Pidotimod vs. placebo, corresponding to prescription

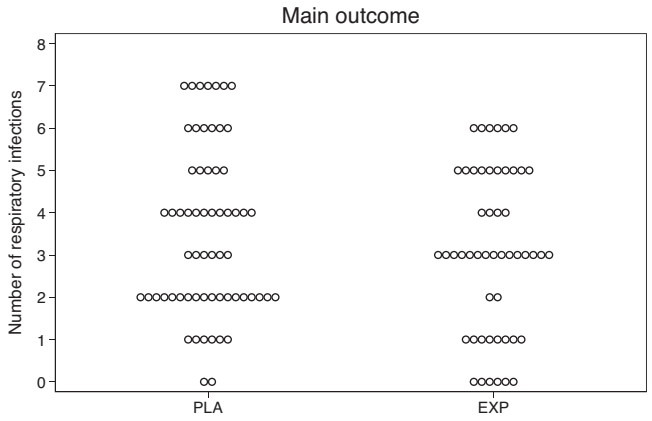

Fig. 2. Number of respiratory infections in Pidotimod and placebo-treated group. EXP: Pidotimod; PLA: placebo.

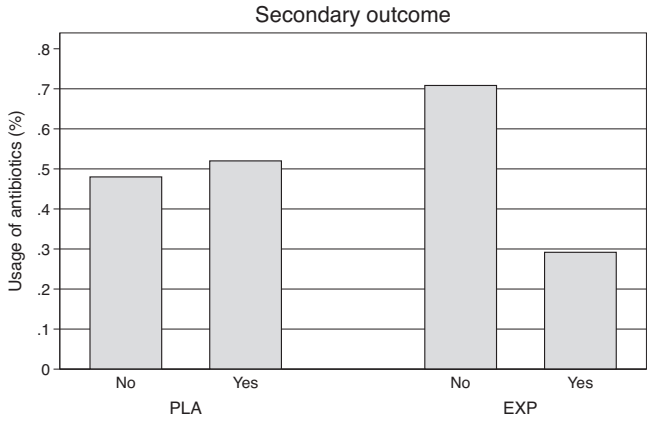

Fig. 3. Antibiotic use in Pidotimod and placebo-treated group. EXP: Pidotimod; PLA: placebo.

rates of 0.29 (95%CI 0.11 to 0.47) and 0.52 (95%CI 0.32 to 0.72) (Fig. 3).

3.5. Adverse events

The only reported adverse event was for a child treated with Pidotimod. Such child had urticaria, which resolved within 1 week after Pidotimod discontinuation.

4. Discussion

Acute respiratory infections represent a common problem in childhood, especially when children enter into day care, due to the naivety of the immune system in the first years of age [1–3]. Achieving the goal of primary prevention of ARTIs is still ambitious and could involve mechanism-oriented studies alongside clinical intervention trials to test biologically plausible prevention ideas. In this scenario Pidotimod, a synthetic immunostimulant that shown to be active on both the adaptive and the innate immune responses, could be an innovative strategy. At the light of this consideration, we firstly designed this double-blinded randomized placebo-controlled study aimed to assess the efficacy of Pidotimod for the prevention of ARTIs in healthy children within a very narrow age range entering into daycare.

When we designed the study, we considered 0.30 to be the minimally clinical relevant relative difference in the incidence rate of respiratory infections between the Pidotimod and placebo groups. We were however not able to enroll the 80 subjects (40 per group) needed to evaluate this difference within the time frame specified in the study protocol. Data collected from the 49 enrolled subjects documented a 22% reduction of the rate of ARTIs in the children who received Pidotimod compared to those who received placebo. However, the precision of this estimate is low (from a 47% reduction to a 15% increase). Our inability to reach the postulated sample size was due to two main reasons. First of all, the children were selected according to very strict criteria in order to overcome a common bias detected in most clinical trials of Pidotimod [10]. Such trials were performed in children of different ages and with different predisposing risk factors for ARTIs. This choice was however a double-edged sword as only 0.7% of the 800 children evaluated for inclusion revealed to be eligible for recruitment. Secondly, the 2013–2014 winter season was characterized by an unpredictably lower circulation of respiratory pathogens [23]. The European Center for Disease Prevention and Control reported that in 2013 influenza started 4–6 weeks later and that its burden on the primary healthcare systems was lower than in previous winter seasons [23].

Despite with these limitations, the 22% reduction of the rate of ARTIs in the children who received Pidotimod compared to those who received placebo merit attention and pave the way to further studies that may assess the clinical impact of this immunomodulatory preventive strategy.

We found a 44% decrease in the relative prescription of antibiotics in the Pidotimod vs. the placebo group. While this difference may be clinically relevant, its 95%CI was remarkably wide (−73% <95%CI < +16%). The reduction in antibiotic use in Pidotimod-treated children seemed to be due to the occurrence of less severe respiratory infections compared to those experienced in placebo-treated group. Therefore, according to our data, Pidotimod seems to have immunomodulant properties reducing the severity of ARTIs. This finding however needs to be confirmed by larger studies.

Antibiotic overuse is common in Italy and the antibiotic prescription rate is significantly higher compared with other European countries [24]. Of note, in the outpatient setting, is that almost half of the antibiotics prescribed (mainly penicillin) are for the treatment of ARTIs. Accordingly, in 2013, Italy was among the European countries with the highest levels of antibiotic resistance for a wide variety of bacteria with increasing trend for penicillin [25]. Therefore the administration of Pidotimod in healthy children may help to reduce antibiotic prescription for ARTIs. Given the association between unneeded antibiotics and the development of antibiotic resistance, Pidotimod could help to reduce both in the long term.

5. Conclusion

In this Pidotimod was not statistically superior to placebo for the prevention of ARTIs in healthy children entering into daycare. Pidotimod shows some potential as a means for reducing antibiotic usage.

Competing interests

The authors declare that they have no competing interests.

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Appendix A. AX-working group

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