A randomized clinical trial indicates that levamisole increases the time to relapse in children with steroid-sensitive idiopathic nephrotic syndrome

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Mariken P. Gruppen[1], Antonia H. Bouts[1], Marijke C. Jansen-van der Weide[2], Maruschka P. Merkus[3], Aleksandra Zurowska[4], Michal Maternik[4], Laura Massella[5], Francesco Emma[5], Patrick Niaudet[6], Elisabeth A.M. Cornelissen[7], Thierry Schurmans[8], Ann Raes[9], Johan van de Walle[9], Mieke van Dyck[10], Ashima Gulati[11], Arvind Bagga[11] and Jean-Claude Davin[1,8]; all members of the Levamisole Study Group

[1]Department of Pediatric Nephrology, Emma Children's Hospital/Academic Medical Center, Amsterdam, The Netherlands; [2]Pediatric Clinical Research Office, Children's Hospital/Academic Medical Center, Amsterdam, The Netherlands; [3]Clinical Research Unit, Academic Medical Center, Amsterdam, The Netherlands; [4]Department of Pediatric and Adolescent Nephrology and Hypertension, Medical University of Gdansk, Gdansk, Poland; [5]Nephrology and Dialysis Unit, Pediatric Subspecialties Department, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [6]Service de Néphrologie Pédiatrique, Hôpital Necker-Enfants Malades, Paris, France; [7]Department of Pediatric Nephrology, Radboud University Medical Centre, Amalia Children's Hospital, Nijmegen, The Netherlands; [8]Department of Pediatric Nephrology, Hôpital Universitaire des Enfants Reine Fabiola, Brussels, Belgium; [9]Department Pediatric Nephrology, Ghent University Hospital, Ghent, Belgium; [10]Department of Pediatrics, University Hospital, Leuven, Belgium; and [11]Division of Nephrology, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, India

Levamisole has been considered the least toxic and least expensive steroid-sparing drug for preventing relapses of steroid-sensitive idiopathic nephrotic syndrome (SSINS). However, evidence for this is limited as previous randomized clinical trials were found to have methodological limitations. Therefore, we conducted an international multicenter, placebo-controlled, double-blind, randomized clinical trial to reassess its usefulness in prevention of relapses in children with SSINS. The efficacy and safety of one year of levamisole treatment in children with SSINS and frequent relapses were evaluated. The primary analysis cohort consisted of 99 patients from 6 countries. Between 100 days and 12 months after the start of study medication, the time to relapse (primary endpoint) was significantly increased in the levamisole compared to the placebo group (hazard ratio 0.22 [95% confidence interval 0.11–0.43]). Significantly, after 12 months of treatment, six percent of placebo patients versus 26 percent of levamisole patients were still in remission. During this period, the most frequent serious adverse event (four of 50 patients) possibly related to levamisole was asymptomatic moderate neutropenia, which was reversible spontaneously or after treatment discontinuation. Thus, in children with SSINS and frequent relapses, levamisole prolonged the time to relapse and also prevented recurrence during one year of treatment compared to prednisone alone. However, regular blood controls are necessary for safety issues.

Correspondence: *Mariken P. Gruppen, Emma Children's Hospital, Pediatric Nephrology, Academic Medical Center, Meibergdreef 15, Amsterdam, North Holland 1100 DD, The Netherlands. E-mail: m.p.gruppen@amc.nl*

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Idiopathic nephrotic syndrome (INS) is a rare disease with an incidence that varies between 2 and 7 cases per 100,000 children per year.[1,2] It is hypothesized that INS results from a defect in lymphocyte function.[3] Most children with INS are steroid sensitive. Seventy percent of the latter also experience ≥1 relapses.[1,2] Half of these children relapse frequently after cessation of corticosteroids (frequently relapsing nephrotic syndrome [FRNS]) or become steroid dependent.[4] Children with FRNS are then exposed to the side effects of steroids.[1,2] To reduce the relapse rate, several drugs have been used.[1,2] Among these, levamisole was considered the least toxic and least expensive and the only one not classified as an immunosuppressive agent until the pharmaceutical industry withdrew it from the market in 2004 for human use due to lack of clear indications.[5] Evidence of the efficacy of levamisole in FRNS was restricted to retrospective studies and a few clinical trials, all of which had some methodological limitations.[6–8] Since then, it has only remained available as a low-cost drug for veterinary use, given of its anthelminthic properties. We hypothesized that the addition of levamisole following complete remission with steroid therapy in children with FRNS or steroid dependency

would either prevent relapses or prolong the time to relapse during a 1-year treatment period.

For this reason, we conducted an international, multicenter, double-blind, placebo-controlled, randomized clinical trial (RCT) to assess the efficacy and safety of 1-year levamisole treatment in children with FRNS. Study medication (levamisole or placebo) started during prednisone treatment for a relapse. To study longer term efficacy and safety of levamisole, patients still in remission and on levamisole treatment at trial completion were evaluated in an additional longer term follow-up.

RESULTS

Patients

Between October 2007 and March 2012, 103 patients, recruited from 13 sites in 6 countries (The Netherlands, Belgium, France, Italy, Poland, and India), were randomized. Ninety-nine patients were included in the modified intention-to-treat population. Three randomized patients could not be included because they did not start the study medication; for 2 of them, the study medication (placebo) did not arrive on time, and proteinuria developed in the third patient (levamisole) before the start of study medication. One patient (placebo) was excluded due to missing primary outcome information. Eight patients prematurely discontinued study medication (4 levamisole and 4 placebo) and were censored. No patients were lost to follow-up (Figure 1). Nonadherence with study treatment was not detected in any patient.

Demographic and baseline characteristics were similar across both study treatment groups (Table 1). Analysis of age and steroid dependency (SD) by region showed a similar age distribution for both regions, but a difference in the distribution of SD between Europe and India (Table 2). The range and distribution of the lowest prednisone doses necessary to prevent a relapse before the start of study are depicted in the Supplementary Material Table S1.

Efficacy endpoints

Time to relapse (primary endpoint). Kaplan-Meier analysis in the primary analysis (modified intention-to-treat) population showed similar cumulative relapse-free survival probabilities for both treatment groups during the first 100 days of study medication (Figure 2). However, afterward, a difference in the time to relapse became apparent in both treatment groups in favor of the levamisole group (log-rank analysis, total period, $P = 0.015$). By the end of the 1-year study period, 6% of placebo (3/49) versus 26% (13/50) of levamisole patients were still in remission and on study medication (Figure 2). Since Kaplan-Meier curves showed that proportional hazards could not be assumed throughout the 1-year study period, a time-dependent Cox proportional hazards regression analysis was performed incorporating the stratification factors. During the first 100 days of study medication similar hazards for a relapse needing prednisone were observed in both treatment groups (hazard ratio [HR]: 1.14, 95% confidence interval [CI] 0.56–2.34, $P = 0.72$). Afterward, a significantly lower hazard was observed in the levamisole group compared with the placebo group (HR: 0.22, 95% CI 0.11–0.43, $P = 0.001$) (Table 3). The HR was not confounded by the prespecified covariates of sex, age, and ethnicity. Exploratory subgroup analyses suggested insufficient evidence of treatment effect modification of the time to relapse by region (India vs. Europe), SD (yes vs. no), sex, and age (2–5 years vs. ≥6 years) (formal tests for treatment by subgroup interaction; P values of 0.79, 0,64, 0.35, and 0.51, respectively); see Kaplan-Meier curves for region and SD/FR in the Supplementary Material Figures S1 and S2.

Occurrence of prednisone-needing relapse (secondary endpoint). At 1-year follow-up, a lower cumulative proportion of patients with a relapse requiring prednisone was seen in the levamisole group (33/50, 66%) compared with the placebo group (42/49, 86%) (relative risk estimate/crude relative risk, 0.77; 95% CI 0.61–0.97; after adjustment for the stratification variables, adjusted odds ratio was 0.30; 95% CI 0.11–0.82, $P = 0.02$). Formal evaluation of effect modification using treatment by subgroup interaction terms in multivariable regression models, including the stratification variables, suggested insufficient evidence of heterogeneity of treatment effect between these subgroups (Supplementary Material Table S2).

Safety endpoints

In the safety population, more patients with at least 1 adverse event (AE) were seen in the levamisole group versus the placebo group (levamisole, 58% [29/50] vs. placebo, 38% [19/50], $p = 0.045$). However, the most clinically prominent AEs were similar in both groups (pyrexia, nasopharyngitis, cough, and mild neutropenia; 1000–1500 cells/μl in 3 patients of each group). None of the latter required study discontinuation (Table 4). Ten nonlethal serious AE occurred. They included 5 cases of moderate neutropenia (500–1000 cells/μl) (levamisole, 4/50 vs. placebo, 1/50) that were asymptomatic and reversible after levamisole discontinuation (2/4 levamisole group) or spontaneously (2/4 levamisole group); 3 hospitalizations (levamisole, 3/50 vs. placebo, 0/50), not related with neutropenia; 2 levamisole patients presented with reversible side effects after medication discontinuation, 1 with a reduced glomerular filtration rate and 1 with arthritis and antineutrophil cytoplasmic antibodies. With regard to the 4 levamisole patients with moderate neutropenia, in 2 of them, neutropenia resolved without terminating levamisole and both reached normal completion of the trial. The other 2 levamisole patients with neutropenia presented with a relapse at the same time, so their primary endpoint was reached and study medication was terminated. With respect to the 3 levamisole patients who required hospitalization (for high fever in 1, pulmonary infection in 1, and abdominal pain in 1), 2 presented with relapse at the same time, so trial medication was discontinued, whereas the third patient did not end trial medication but relapsed 8 months later (Table 4).

Comparison of laboratory values per visit (hemoglobin, platelets, neutrophils, albumin, aspartate aminotransferase,