**Palivizumab prophylaxis in preterm infants and subsequent recurrent wheezing: 6 Year Follow up Study**

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**Abbreviations:**

CI ; confidence interval

GA ; gestational age

HR ; hazard ratio

LOCF ; the last observation carried forward

RSV ; respiratory syncytial virus

RR ; relative risk

**At a Glance Commentary**

**Scientific Knowledge on the Subject:** An association between RSV infection and subsequent asthma has been suggested by several studies, but refuted by others. Using passive prophylaxis that prevents severe RSV disease in infancy, a few studies from Europe and North America have addressed this causal association. None have followed children for 6 years.

**What This Study Adds to the Field:** In this prospective multicenter case-control study of 444 children, palivizumab prophylaxis was administered to preterm Japanese infants of 33-35 weeks gestation age in their first respiratory season. This did not reduce the incidence of atopic asthma, but significantly reduced subsequent physician-diagnosed recurrent wheezing up to 6 years.

**ABSTRACT**

**Rationale:** RSV (respiratory syncytial virus) induces not only infantile recurrent wheezing but also potentially atopic asthma.

**Objectives:** To test the effect of RSV infection, on development of subsequent atopic asthma, we evaluated whether palivizumab, an anti-RSV monoclonal antibody, by preventing severe RSV disease in the first year of life, could impact subsequent recurrent wheezing and atopic asthma, at 6 years of age.

**Methods:** During the 2007-2008 RSV season, the decision to administer palivizumab was made based on standard medical practice, and an observational prospective multicenter (n=52) case-control study in preterm infants with a gestational age between 33 and 35 weeks followed from 0 to 3 years (preceeding CREW study). The 52 investigators at hospitals now followed these subjects till 6 years of age, reported here. Parents of study subjects reported the infants’ physician’s assessment of recurrent wheezing, using a report card and a novel mobile phone-based reporting system using the Internet. The primary endpoint was the incidence of atopic asthma.

**Results:** Of 444 preterm infants enrolled, 349 received palivizumab during the first year of life. At 6 years, atopic asthma was not different in the groups, 15.3% and 18.2% of infants in the treated and untreated groups, respectively (p=0.57). On the other hand, physician-diagnosed recurrent wheezing was observed in 15.3% and 31.6% in the treated and untreated groups, respectively (p=0.003).

**Conclusion:** Palivizumab prophylaxis administered to preterm infants did not suppress the onset of atopic asthma, but resulted in a significantly lower incidence of recurrent wheezing during the first 6 years.

**Introduction**

Respiratory syncytial virus (RSV)-related lower respiratory tract infection (LRI) in preterm infants has been associated with an increased prevalence of early childhood wheezing and asthma in later childhood.(1-6)It has been proposed that RSV infection by influencing Th1/Th2 balance in early childhood might induce an atopic state.(7) In contrast, other studies suggest that the effect of RSV does not induce atopy, but rather causes recurrent wheezing that might last up to 13 years ~~but~~ which is not associated with an atopic phenotype.(4)

Palivizumab, a humanized anti-RSV monoclonal antibody that binds to the RSV fusion protein, neutralizes RSV and successfully inhibits viral replication.(8) Monthly administration of palivizumab to preterm infants reduces the incidence of hospitalization due to RSV LRI,(9) and recurrent wheezing, especially in non atopic young children.(10, 11)

 We have previously reported the effectiveness of palivizumab prophylaxis, in the first year of life, on preventing recurrent wheezing in children aged 1 to 3 years.(12)However, since the diagnosis of asthma is not entertained in this period, and the impact of palivizumab on the development of atopic asthma after RSV infection is still controversial, we followed the subjects of our previous trial, reported here. Childhood wheezing has been classified into several phenotypes (infantile wheezing, non atopic recurrent wheezing and atopic wheezing),(4, 13-15) but the impact of RSV on the various wheezing phenotypes is mostly unsettled. Here we use a controlled trial of palivizumab protection against RSV LRI, to understand the impact of RSV on recurrent wheezing and asthma.

**Methods**

**Study design and endpoints**

 The antecedent CREW study was a prospective, multicenter, observational cohort study to compare the incidence of physician-diagnosed recurrent wheezing up to 3 years in preterm children 33-35 wGA, who had and had not received palivizumab prophylaxis during their first RSV season.(12) The primary endpoint of this 6-year follow-up study, of the CREW study participants, was the suppression of the onset of atopic asthma in palivizumab recipients. The secondary endpoints were: the reduction in the rates of physician diagnosed recurrent wheezing, the mean number of respiratory-related outpatient visits and hospitalizations and growth of the children.

**Follow-up of children**

 The results of physician visits and monthly reports of illnesses, followed procedures reported in the CREW study, except that participants were followed up to 6 years of age.(12) Briefly, all parents of enrolled children were advised to visit a hospital/clinic when their children showed cold like symptoms. Physicians recorded the date of visit, presence or absence of wheeze, on a study card, with a QR code. This was photographed by the parent/caregiver with the mobile phone transmitted directly to the data center. Parents submitted monthly reports via a mobile phone-based automated answering system about the number of hospital/clinic visits and hospitalizations due to respiratory-related illness, and a diagnosis of allergic diseases. Allergic diseases were diagnosed by physicians and included atopic asthma, allergic rhinitis, allergic conjunctivitis and food allergy. The parents were reached by telephone if they failed to submit the monthly mobile phone report. In addition in the month of their 6th birthday a history, growth parameters and blood was obtained to measure serum total IgE level and specific IgE levels to *Dermatophagoides pteronyssinus*, using an *in vitro* diagnostic kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). Elevated total and specific IgE levels were >30 IU/mL and >0.35 IU/mL, respectively.

**Respiratory assessment**

 An episode of expiratory wheezing was defined as bronchial obstruction lasting for at least 24 hours preceded by at least ~~a~~ 1 week non-wheezing healthy period, as defined by a physician. Recurrent wheezing was defined as the occurrence of 3 or more episodes of expiratory wheezing diagnosed by a physician in a 12-month period.(10, 11). Children with a high serum total or specific IgE level were deemed atopic. Atopy and recurrent expiratory wheezing defined atopic asthma. We also examined these outcomes in those with and without a family history of allergy.(11) While these outcomes were prospectively defined in our study, subsequent to the end of our study, the Gina consortium suggests a probabilistic approach to defining asthma in this age group.(16) They suggest that several phenotypes, described in various studies,(1, 2, 13, 14, 17-19) lack clinical utility in predicting the need for asthma treatment and are not stable over time. They suggest a broader definition that includes atopy or a family history of asthma and suggests these children are more likely to be given a diagnosis of asthma or respond to regular controller treatment than children with episodes 2 to 3 times a year. Hence we did a secondary analysis based on this definition and defined probable asthma as “recurrent wheezing with a high serum total or specific IgE level OR a family history of allergy.”

**Statistical methods**

 Target sample sizes of 300 infants for the palivizumab-treated group and 150 infants for the untreated group for the CREW study were as described earlier.(12) Student’s t-test or Wilcoxon sign-rank test were used to test for significance of differences in groups for quantitative variables, and the chi-square test or Fisher’s exact test for categorical variables. Possible confounders (gestational age, smokers in the home, and a family history of allergy) based on their potential association with recurrent wheezing and significant background differences in groups were used for adjusting background risks in a logistic regression model. For time to event analyses, a Kaplan-Meier test with the log-rank test and an adjusted Cox regression model were used. Differences between the groups in numbers of hospital/clinic visits and hospitalizations due to respiratory-related diseases were compared using an adjusted Poisson regression model.

**Ethical approval**

 The institutional review board (IRB) at each center and the central IRB at Tokai University Hospital (No. 10R-133) approved the study, and written informed consent was obtained from each infant’s parent or legal guardian prior to enrollment. This study was registered with Clinical Trials.gov, number NCT01545245.

**Role of the Funding Source:**

The funding source played no role in study design, collection or analysis of data, or the decision to publish.

**RESULTS**

**Participants and follow-up**

 In 2007-2008, 349 infants that received at least 3 doses of palivizumab, and 95 infants who did not receive palivizumab were enrolled in the CREW study.(12) The number of the control infants was less than the target sample size because of more widespread use of palivizumab among preterm infants in Japan than expected. Of the 444 children enrolled in the study (Intention To Treat or ITT population), 328 children (75%) completed the 6 year follow up ending December 2013, (Per Protocol or PP population) 268 of whom were consented for the blood examination at 6 years of age (Atopic asthma sub-study population) (Figure 1).

**Demographics and baseline characteristics**

 Whereas there were statistically significant differences between the groups in gestational age (p = 0.008), smokers in home (p = 0.011) and family history of allergy (p = 0.005), in the ITT population, there were no significant differences in the PP or atopy sub-study populations (Table 1). In those who consented to have a blood examination at 6 years, the mean total IgE was 258.8 and 258.6 IU/mL in the palivizumab recipient and control groups, respectively (p=0.997), and the mean specific IgE was 21.7 and 16.3 IU/mL (p=0.245). Also, there was no difference in allergic diseases between the palivizumab recipient and control groups (40.1%, 44.3%, respectively, p=0.997).

**Primary Outcome**

 The prevalence of atopic asthma was similar, 31/202 (15.3%) in the palivizumab treated group, and in 12/ 66 (18.2%) in the untreated group (RR 0.82; 95%CI, 0.39 to 1.70, P=0.57; Table 2). There was no difference in this outcome, in those with and without a family history of allergy. The univariate time to event analysis (Figure 2) and the multivariable logistic regression and Cox regression analyses (Table 3) confirmed this finding. A sensitivity analysis using the revised Gina definition above of probable asthma in later life, showed no difference either [34/205 (15%) versus 16/66 (18.2% (RR 0.91 95%CI 0.50, 1.66; P=0.85] for the treated and untreated groups respectively.

**Secondary Outcomes**

 ***Physician Diagnosed Recurrent Wheezing***: On the other hand, there were significant differences in rates of physician-diagnosed recurrent wheezing between two groups. On univariate analysis there was a 2.64 fold relative reduction in the rate of recurrent wheezing, in the palivizumab treated group compared with the palivizumab un- treated group (P <0.001), in the ITT population. There was a 2.1-fold relative reduction in the PP population (P <0.001). Even in the smaller atopic population, there was a significant reduction in recurrent wheezing (P = 0.022) (Table 2). These significant differences were confirmed in the time to event analyses for all 3 populations examined (Figure 2).

 Multivariable logistic regression analysis and Cox regression analyses showed significant differences for physician diagnosed recurrent wheezing in the ITT, PP and atopic populations in both analyses (Table 3).

 These differences were demonstrated only in the subgroups of children with a family history of allergy, but not in those without. These results were consistent in univariate (Table 2) time to event (Figure 3) and multivariate analyses (Table 3) for all 3 populations (ITT, PP, atopic sub groups).

 ***Respiratory outpatient visits and hospitalizations***: In the ITT analysis, children who received palivizumab prophylaxis in infancy had a significant reduction in outpatient respiratory visits (19.0 versus 23.9 visits/person for the palivizumab recipients and the control infants, respectively, p=0.018, Wilcoxon test), whereas there was no difference in the number of hospitalizations due to respiratory disease (0.24 and 0.34 hospitalizations/person for the palivizumab recipients and the control infants, respectively, P=0.46). In the PP analysis there were no differences in outpatient visits, (21.8 and 28.1 visits/person for the palivizumab recipients and the control infants, respectively, P=0.10, Wilcoxon test), or in the number of respiratory hospitalizations (0.27 and 0.35 hospitalizations/person for the palivizumab recipients and the control infants, respectively, P=0.76). In the atopic sub-study population there were no differences in outpatient visits, (25.5 and 23.7 visits/person for the palivizumab recipients and the control infants, respectively, P=0.67, Wilcoxon test), or in the number of respiratory hospitalizations (0.21 and 0.23 hospitalizations/person for the palivizumab recipients and the control infants, respectively (P=0.217)

 ***Growth:*** At age 6 years, there were no significant differences in weight (19.4± 3.46 Kg versus 19.5± 2.66 Kg; P=0.83), height (112.0± 4.40 cm versus 112.7± 5.76 cm, P=0.33) or BMI (15.4± 1.85 versus 15.3± 1.26 P=0.75) in palivizumab recipients compared to controls respectively

**DISCUSSION**

 RSV is a leading cause of serious lower respiratory tract infection in infants, and induces persistent airway damage and bronchial hyper responsiveness. (20) It has been suggested that RSV infection not only exacerbates asthma attacks, but is also involved in the inception of asthma (13, 21). The observation that infantile RSV infection induces a predominantly Th2 response to airway allergens, supports this suggestion. (7, 22) However, the relationship between early RSV infection and allergic sensitization in the development of asthma at a later age is still hotly debated. Palivizumab, an anti-RSV-F monoclonal antibody, is used to prevent serious RSV disease in preterm infants and those with congenital heart disease.(23) Premature infants have airway prematurity, which induces persistent bronchial hyper-responsiveness.(24) A meta-analysis of the relationship between wheezing disorders and gestational age suggests that preterm birth, particularly very preterm birth, results in higher rates of wheezing than term infants.(25) Compared with full-term born infants, early preterm infants have the highest odds of developing later asthma, but late preterm infants have the second highest odds.(26) Using palivizumab, as a probe to identify its protective effect against later respiratory outcomes in preterm infants, previous studies have demonstrated a significant relationship between preventing RSV infection and preventing subsequent recurrent wheezing.(10-12, 27) However, the precise diagnosis of asthma and other allergic diseases is difficult to establish in infants. Thus, we planned a continuous investigation of late preterm infants recruited in the previous CREW study,(12) now followed up to 6 years of age to investigate the impact of preventing RSV LRI on the development of atopic asthma.

 The results of our 6-year follow up study, that atopic asthma was not significantly reduced in preterm palivizumab recipients suggests the possibility that preventing RSV LRI has no impact on the development of an atopic state. Supporting this observation we found no significant differences between the two groups in total serum IgE value, specific IgE antibody and the onset of other allergy diseases.

 Sigurs et al reported that RSV bronchiolitis in infancy is an important risk factor for the later development of asthma and allergy, (2) whereas Simões et al(11) reported that RSV prevention by palivizumab prophylaxis in nonatopic children decreased the relative risk of recurrent wheezing. They suggested that RSV predisposes to recurrent wheezing in an atopy-independent manner. The results of our investigation further this hypothesis, since we found no impact on atopic asthma.

 On the other hand, the secondary endpoints of physician-diagnosed recurrent wheezing in the intention-to-treat and per protocol analyses as well as in the atopy sub-study, were significantly reduced in the palivizumab recipient group. These results are consistent with that of our earlier CREW study, (12) as well as the earlier European studies (10, 11, 27) and strongly suggest a reduction of recurrent wheezing by RSV prophylaxis that persists up to at least 6 years.

 A recent placebo controlled trial of motavizumab (an efficacious anti RSV monoclonal antibody) in North American Aboriginal Peoples failed to demonstrate any impact on medically attended recurrent wheezing.(28) However this impact should be interpreted with caution since it was underpowered to show any impact on this outcome. Thus the rate of medically attended recurrent wheeze in the motavizumab study (28) was approximately 20-30 fold lower than in the other palivizumab studies. (10-12, 27)

 Considering the phenotypes of childhood asthma, Stein and coworkers have suggested three types based on a time trend classification:(4) transient wheeze (ending before 3 years), persistent wheeze (starting before 3 years, persisting beyond 6 years) and late onset wheeze (starting after 3 years). Persistent wheeze is induced by virus infection, and late onset wheeze is classic atopic asthma. In our study, palivizumab suppressed about half of the recurrent wheezing seen; 12.8% in palivizumab-treated group versus 28.4% in the palivizumab-untreated group. The ratio of virus-related non-atopic wheeze/asthma and atopic wheeze/asthma might be close to 50: 50 as in previous reports.(10, 21, 29) The ERS task force has proposed a symptom-based classification that defines episodic wheezing and multi-trigger wheeze.(30) Finally the GINA consortium have proposed a probability-based approach to diagnosis of wheezing children, suggesting that in preschool children with symptoms for >10 days during viral infections, severe or frequent episodes, interval symptoms during play, laughing or crying, and with atopy or a family history of asthma, are much more likely to be given a diagnosis of asthma later on.(16) We chose to use this recommendation to explore our population. Whether we used our definition or the Gina approach, we did not find that preventing RSV prevented current or probable future atopic asthma.

Different from our earlier study of palivizumab prophylaxis on wheeze outcomes in Europe/Canada,(11) the impact seen in our study was primarily in those with a family history of allergy/asthma. In contrast in the European/Canadian study the impact was primarily in those without this family history. The rates of the family history of allergy/atopy In the European/Canadian study 220/421 (52%) were not dis-similar to our in study in Japan, 250/440 (57%). The European/Canadian study followed children up to 3 years of age, and ours followed them to 6. However a review of the Kaplan-Meier curves in our study (Figures 2 and 3) shows that the differences in the atopic subgroup started prior to 3 years, suggesting a fundamental difference in the 2 study populations, related either to genetic makeup or the environment.

Maternal asthma confers a 5-6 fold higher risk for RSV hospitalization in Danish infants up to 18 months of age (31). While this risk is not as carefully studied in Japan (32), other factors including environmental, daycare and other socio-demographic factors might play a larger role in Japan on severe RSV disease, which might indirectly impact the role of RSV on subsequent recurrent wheezing. Currently ongoing studies in Holland (27) might throw some more light on this issue.

The limitations of our study include the potential biases due to the nonrandomized study design. The differences in demographics and baseline characteristics make interpretation of the results more complex. Although the differences in family history of asthma, birth weight, and gestational age were minimal, smokers in home and family members with a history of allergy were significantly more frequent in the untreated group, in the ITT population but there were no differences in the PP population and the atopic sub-study populations. Bias in the enrollment process were minimized because notification of this study to investigators and parents of eligible infants were purposefully started after the end of the infant’s first RSV season, so that the decision for prophylaxis was already made prior to enrollment. (12) The medical expenses related to the use of palivizumab for this infant population are completely reimbursed in Japan, which minimizes bias due to access to palivizumab. In Japan, RSV prophylaxis in preterm infants between 33 and 35 wGA is generally accepted as a standard of care, as reflected in our cohort, and was the reason for substantially fewer than expected infants in the untreated group. (33) Since an interventional study with a placebo control group was considered unethical, we employed a prospective observational study design. Given the differences in palivizumab in the 2 groups studied, it is possible that usage patterns of erythromycin like drugs and steroids, that could impact the recurrent wheezing phenotype, might have been different in the two study groups. We did not collect this information. However given the government-subsidized free availability of these drugs, this usage is unlikely to have been different in the two groups.

We chose a limited definition of atopy, on pragmatic grounds, which could potentially influence the interpretation of our results. House dust mite (HDM) allergy is strongly implicated in the pathogenesis of respiratory allergic diseases, a large proportion of patients with allergic rhinitis or allergic asthma being sensitized to *Dermatophagoides pteronyssinus or Dermatophagoides farinae*.(34) Thus a cohort of children with atopy to HDM had lower lung function compared to those who were unsensitized.(35) In a recent study from Japan, levels of serum IgE to *Dermatophagoides pteronyssinus* were highly predictive of an immediate allergic response to bronchoprovocation. (36) Several studies, assessing the association between atopy, viruses and subsequent asthma, have used varying definitions for atopy. These range from single tests for IgE to *Dermatophagoides pteronyssinus*, (37) to the inclusion of three measures of IgE sensitization(38) to those that include skin prick tests and very comprehensive measures, including up to 14 specific IgE tests.(1, 4, 13, 35, 39-43) We could not do skin prick tests in a standardized manner at 52 pediatric offices, however we do acknowledge that, while *Dermatophagoides pteronyssinus* might be the predominant aeroallergen testing to a wider range of aeroallergens, could have increased the number of children with an atopic diagnosis.

 The strength of our study is the high follow-up rate, which was achieved with our original method with the convenient mobile phone answering and photo submission system. (12) The most important advantages of this system include (1) parental follow-up reporting especially mothers; (2) minimum physician involvement in reporting findings during clinic visits, and conveniently (3) efficient data transfer directly to the research center, which seemingly attracted and motivated mothers.

 Palivizumab prophylaxis in preterm infants 33 to 35 wGA appears to have no impact on preventing subsequent atopic asthma, but effectively prevents recurrent wheezing up to 6 years. This study suggests two independent phenotypes of recurrent wheezing in young children: RSV LRI-dependent recurrent wheeze and RSV LRI-independent recurrent wheeze. Whether RSV impacts lung function in later life remains to be answered by this and other ongoing studies.

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**Conflict of Interest**:

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**FIGURE LEGENDS**

**Figure 1.** ***Flowchart of registration and outcome evaluation***. Infants were enrolled from July 1st to December 31st 2007.

**Figure 2.** ***Kaplan-Meier plot of time to the onset of physician diagnosed recurrent wheezing***

**Figure 2 A:** **Intention to treat analysis:** Percentage without recurrent wheezing shown for the treated group (n=345) and the untreated group (n=95). Infants’ age (in months) is indicated on the x-axis. The study infants were followed-up until 6 years of age. Analysis of the time to onset using a log-rank test showed a significant reduction of recurrent wheezing in the treated group (P<0.001)

**Figure 2 B:** **Per protocol analysis:** Percentage without recurrent wheezing shown for the treated group (n=249) and the untreated group (n=79). Infants’ age (in months) is indicated on the x-axis. The study infants were followed-up until 6 years of age. Analysis of the time to onset using a log-rank test showed a significant reduction of recurrent wheezing in the treated group (P<0.001)

**Figure 2 C:** **Atopic Asthma Population analysis:** Percentage without recurrent wheezing shown for the treated group (n=202) and the untreated group (n=66). Infants’ age (in months) is indicated on the x-axis. The study infants were followed-up until 6 years of age. Analysis of the time to onset using a log-rank test showed a significant reduction of recurrent wheezing in the treated group (P=0.014)

**Figure 3*. Kaplan-Meier plot of time to the onset of physician diagnosed recurrent wheezing, by family history of allergy.***

**Figure 3 A: Intention to treat analysis (with a family history of allergy)**: Percentage without recurrent wheezing shown for the treated group (n=184) and the untreated group (n=66). Infants’ age (in months) is indicated on the x-axis. The study infants were followed-up until 6 years of age. Analysis of the time to onset using a log-rank test showed a significant reduction of recurrent wheezing in the treated group (P<0.001)

**Figure 3 B: Intention to treat analysis (without a family history of allergy):** Percentage without recurrent wheezing shown for the treated group (n=161) and the untreated group (n=29). Infants’ age (in months) is indicated on the x-axis. The study infants were followed-up until 6 years of age. Analysis of the time to onset using a log-rank test showed no significant reduction of recurrent wheezing in the treated group (P=0.440)

**Figure 3 C: Per protocol analysis (with a family history of allergy):** Percentage without recurrent wheezing shown for the treated group (n=146) and the untreated group (n=54). Infants’ age (in months) is indicated on the x-axis. The study infants were followed-up until 6 years of age. Analysis of the time to onset using a log-rank test showed a significant reduction of recurrent wheezing in the treated group (P<0.001)

**Figure 3 D: Per protocol analysis (without a family history of allergy):** Percentage without recurrent wheezing shown for the treated group (n=103) and the untreated group (n=25). Infants’ age (in months) is indicated on the x-axis. The study infants were followed-up until 6 years of age. Analysis of the time to onset using a log-rank test showed no significant reduction of recurrent wheezing in the treated group (P=0.337)

**Figure 3 E: Atopic Asthma Population analysis (with a family history of allergy):** Percentage without recurrent wheezing shown for the treated group (n=124) and the untreated group (n=47). Infants’ age (in months) is indicated on the x-axis. The study infants were followed-up until 6 years of age. Analysis of the time to onset using a log-rank test showed a significant reduction of recurrent wheezing in the treated group (P<0.001)

**Figure 3 F: Atopic Asthma Population analysis (without a family history of allergy):** Percentage without recurrent wheezing shown for the treated group (n=80) and the untreated group (n=19). Infants’ age (in months) is indicated on the x-axis. The study infants were followed-up until 6 years of age. Analysis of the time to onset using a log-rank test showed no significant reduction of recurrent wheezing in the treated group (P=0.383)

**Table 1: Demographics of Study Populations**

a: Student’s t test. b: P < .01 (significant differences between-group). c: χ2 test. d: Fisher’s exact test. e: Birthday of the group of this study is between July and December. f: Wilcoxon rank sum test. g: P < .05 (significant differences between-group).

**Table 2: Univariate Comparison of Outcomes In the Study Populations**

**Table 3: Multivariate Comparison of Outcomes In the Study Populations**

Treatment effects adjusted for gestational age, smokers in the home, and a family history of allergy. N/A: Not applicable since HR could not be calculated, because the assessment was made only at 6 years of age.