

## Refusal to Participate in Blood Testing in a Study of Childhood Immunizations and Atopic Disorders: Characteristics of Non-Participants and Assessment of Possible Bias

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### Abstract

*Aim: Assessing characteristics of non-participation in epidemiological studies is often complicated by lacking information. The aim was to assess characteristics of non-participants in blood testing and possible non-participation bias in our previous study on the impact of vaccinations on atopic disorders.*

*Methods: In a previously conducted study on vaccinations and allergy we now used multivariable logistic regression to assess characteristics of non-participants in blood testing, an optional part of the study protocol. Possible bias*

*due to this non-participation was assessed by an analysis weighted with the inverse of the probability of being a participant and by a sensitivity analysis.*

*Results: Having refused consent to consult vaccination registration data (OR: 4.7, CI95%: 2.9-7.6), not having disclosed income, lower school class, lower birth order, not having a history of pertussis, and eating less vegetables were significant determinants of non-participation in blood testing. Weighted analysis and sensitivity analysis yielded results similar to those in the original study.*

*Conclusions: We found that refusal to participate in blood testing was related to reluctance to disclose private information in general and to sensitivity on the subject of vaccinations in particular. Also, parents of younger children with less older siblings, without a history of pertussis, and consuming less frequently vegetables, were more likely to be a non-participant. Selective participation in blood testing may have affected our assessment of the reliability of the reported vaccination status, but leaves our conclusion from the original study, that there is no positive association between the DTP-IPV vaccination and atopy, unaffected.*

**Keywords:** *Non-participation bias, childhood vaccinations, atopic disorders.*

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### Introduction

Non-participation should always be addressed in studies involving volunteers. Frequently participants and non-participants differ in aspects that are related to the outcome and/or to main determinants in the study and this could lead to biased estimates. For example, in a survey assessing the prevalence of Diabetes Mellitus in the United Arab Emirates (UAE), special techniques had to be applied to adjust for considerable non-response among se-

lected households<sup>1</sup>. Another cross-sectional study carried out in the UAE had a response rate of only 30%. The authors of this study, conducted to determine the prevalence of established cardiovascular risk factors, concluded that this low response rate did not affect their findings, as they had enough information on non-responders to assess that this group was similar to the responders in terms of variables relevant to cardiovascular disease<sup>2</sup>. However, in general such a detailed comparison between participants and non-participants is not possible because non-participants are by definition less visible to researchers than participants, and comparisons between these groups are limited to the (often few) characteristics available for both. Consequently, studies with low response rates are possibly subject to bias of an unknown extent and this phenomenon could be one of the explanations for heterogeneity in findings among studies investigating the same research question.

In a study<sup>3</sup> on the association between the diphtheria-tetanus-pertussis-(inactivated) poliomyelitis vaccination (DTP-IPV) in the first year of life and reported atopic disorders at age 8-12 years in Orthodox Reformed (Protestant) primary school children in the Netherlands, our main conclusion was that there was no association between childhood vaccinations and atopic symptoms and even a negative association with objective allergy. This study was mainly based on questionnaires but we also requested a blood sample for assessment of allergy by specific IgE and for validation of their vaccination status. Participation in this blood test was not a requirement for participation in the study. Therefore assessment of bias due to selective participation in blood testing is extremely important since the conclusion of no, or even a negative, association of vaccinations with reported symptoms and/or objective allergy heavily impacts the public attitude towards vaccinations and thus potentially the health of millions.

In the present study, our objective is to explore which characteristics were related to non-

participation in blood testing and their potential impact on possible participation bias.

## 2. Methods

### 2.1. Study Area, Population and Design

The original study was designed to assess the relationship between DTP-IPV vaccination and reported atopic disease<sup>3</sup>. Briefly, in 2003 and 2004, we sent questionnaires to 4480 children (aged 8-12 years) of 38 Orthodox Reformed (Protestant) primary schools in the Netherlands in three different regions (one in the eastern, one in the western, and one in the South-western part of the Netherlands). Many parents of children attending these schools refuse vaccinations for religious reasons, making this an appropriate group for exploring this relationship. A total of 1872 questionnaires (42%) were returned and suitable for analysis of which 671 pertained to reportedly DTP-IPV-unvaccinated children. A subsequent non-responder (to participation in the questionnaire part of the study) study found no evidence of selection bias: vaccination coverage in responders and non-responders was almost equal (63.1% and 63.8% respectively)<sup>3</sup>.

## 3. Data Collection in the Original Study

### 3.1 Questionnaire

The questionnaire asked questions on symptoms of atopic diseases (a Dutch translation of the ISAAC questionnaire<sup>4</sup>), whether the child had received childhood vaccinations, demographics and other relevant variables (see *Table 1*).

### 3.2 Blood Measurements

In order to get an objective measurement of allergy (specific IgE) and to validate the risk factor (DTP-IPV vaccination), we invited participants to give a blood sample. Because of the cost of blood tests and limited available funds we planned to collect blood samples from 100 children only. After first recruiting schools in the Western and Eastern parts of the Netherlands, we had already enrolled 948 participants of whom 683 (72%; 74% of the vaccinated, and 70% of the unvaccinated) had consented to blood collection. As we only needed 100 blood samples we omitted the

invitation for blood sampling from the questionnaire distributed in the third region (South-western part of the Netherlands). From these 683 children we selected a stratified random sample of 100 children from whom blood was taken. The sample was stratified by DTP-IPV vaccination (ratio 1:4 for vaccination yes/no), atopic symptoms (1:1 yes/no) and primary school class (equal distribution over 4 school classes).

### 3.2.1 Specific IgE, Objective Measurement of Allergy

We performed RAST tests using the Pharmacia® RIA method to determine specific IgE to five of the most common aero allergens in the Netherlands (house dust mite, cocksfoot pollen, common silver birch pollen, cat epithelium dander and dog dander) in the sera of the 100 children to obtain an objective measurement of allergy. Allergy was defined as at least one RAST class 2 or higher (i.e. IgE  $\geq 0.7$  IU/ml)<sup>3</sup>. This objective IgE based definition of allergy had a 66% agreement with reported asthma or hay fever (current symptoms or 'ever had'). On the basis of these objective allergy data of 100 children and their relationship to reported allergy as well as other relevant variables, we then imputed the objective allergy variable for the remaining 1772 children. Analysis of these imputed data yielded a statistically significant negative association between DTP-IPV vaccination and allergy<sup>3</sup>.

### 3.2.2 IgG Antibodies, Validation of the Risk Factor

Vaccination status was validated in the original study in two ways: by comparing the reported DTP-IPV vaccination status with the tetanus toxoid IgG and diphtheria IgG antibodies (in 80 reportedly unvaccinated children from the sample of 100 mentioned above) and by comparing the reported vaccination status with the official vaccination registry in a random sample of 120 children (drawn from those children in the total study population who gave consent to consult these data). Children with titres of at least 0.6 IU/ml

were considered as having been vaccinated for the pathogen concerned.

### *3.3 Statistical Analysis*

SPSS version 15.0 was used for all analyses. A two-sided p-value of 0.05 or less was considered significant. Different analyses were carried out. The aims of these analyses were to 1) determine characteristics of non-participants 2) assess whether adjustment for non-participation in blood testing would result in a similar agreement between atopic symptoms and objective allergy and 3) determine with a sensitivity analysis (by assuming that all subjects who refused participation to both blood testing and inspection of vaccination records had misrepresented their vaccination status) whether non-participation to blood testing might have affected the validity of the vaccination status.

#### 3.3.1 Factors Related to Non-Participation in Blood Testing

In order to determine which characteristics were related to non-participation in blood testing, we first determined which of the variables in *Table 1* were univariately related ( $p < 0.10$ ) to non-participation and then performed logistic regression (backward elimination, Wald method;  $p$  to remove = 0.10) with this subset of variables as independent variables (predictors) and non-participation in blood testing as the outcome variable. Using this logistic regression function we also calculated for each participant the predicted probability of non-participation given his/her values of predictors.

#### 3.3.2 Specific IgE, Reliability of Reported Symptoms

In order to assess whether selective participation in blood testing could have biased our original result of 66% agreement, we repeated the original analysis after weighting the data with the inverse of the predicted probability of being a participant in blood testing, using the logistic regression analysis described above (inverse probability weighting)<sup>5</sup>. We reasoned

Table 1. Possible determinants for non-participation in blood testing in a cross-sectional study on vaccinations and allergy in a religious group in the Netherlands

Variable
Received any childhood vaccination
Any atopic disorder (asthma, hay fever, eczema, food allergy)
*Birth order
Gender
Gestational age
Both parents born in the Netherlands
*Mother's age at the time of delivery
Exposure to smoking
Breast feeding for four months or more
Pet keeping (furry pets or birds)
Day care starting at age 6 months or less
*Current age
*School class (5, 6, 7, 8)
*Family history of asthma
Highest educational level of the parents
Family income
*Answered question on income
*Gave consent for consulting official vaccination data
Current level of urbanization (two levels)
Living on a farm
Region (eastern or western part of the Netherlands)
*Sibship size
Frequent (more than five days/week) consumption of:
- fruit
- *vegetables
- *anti-oxidants
- unskimmed dairy products
- whole meal bread
- meat
Frequent (at least once a week) consumption of:
- fish
- snacks
Being a member of a sporting club
Having a history of infection of:
- *pertussis
- mumps
- measles
- rubella

\*These variables were associated ( $p < 0.10$ ) with non-participation in blood testing in univariate analysis

that cases with a high predicted probability of non-participation effectively also represent several cases who did not participate. For example, if a subject had a probability of 90% of

being a non-participant, this category of subjects (with this particular set of predictors) had a probability of 10% of being a participant, and thus the participants among this category of subjects have to be weighted with a factor 10 to make up for the non-participants.

### 3.3.3 IgG Antibodies, Validation of Vaccination Status

In order to assess whether selective participation could have affected our validity assessment, we performed a sensitivity analysis (worst case scenario) by replacing the actual vaccination status of those children who refused to consent to both blood testing and consulting their vaccination records, with the

alternative value (i.e. we considered those who reported to be vaccinated as not vaccinated and vice versa).

### Results

A total of 948 participating children were invited to give consent for blood testing. Of these, 265 (28%) refused.

#### *Factors Related to Non-Participation in Blood Testing*

The variables marked with an asterix in *Table 1* were univariately related ( $p < 0.10$ ) with non-participation in blood testing. Including these variables in the logistic model, backward variable elimination yielded a model with refusing

*Table 2. Variables significantly related to non-participation in blood testing, adjusted odds ratios (aOR) and 95% confidence intervals (95%CI)*

	Non-Participation n/N (%)	aOR*	95%CI
<b>Refusing consent for consulting official vaccination data</b>			
No	212/860 (25)	-	-
Yes	53/88 (60)	4.7	2.9-7.6
<b>Refusing to answer the question on income</b>			
No	138/602 (23)	-	-
Yes	127/346 (38)	1.8	1.4-2.5
<b>School class</b>			
5	82/241 (34)	0.8**	0.7-1.0
6	68/254 (27)		
7	62/222 (28)		
8	53/231 (23)		
<b>Birth order</b>			
1	87/265 (33)	0.9**	0.8-1.0
2	57/200 (29)		
3	36/151 (24)		
4+	85/332 (26)		
<b>Frequent consumption of vegetables</b>			
no	128/400 (32)	-	-
yes	137/547 (25)	0.7	0.5-1.0
<b>Having a history of pertussis</b>			
no	211/700 (30)	-	-
yes	54/248 (22)	0.5	0.4-0.8

\* Odds ratios (OR) with 95% confidence intervals (95%CI) are adjusted for all other variables in the table

\*\* OR for increase of one position

consent to consult vaccination registration data, refusing to answer the question on income, being in a lower school class, having less older siblings, eating less vegetables, and not having a history of pertussis, being positively related to non-participation in blood testing. Adjusted odds ratios (OR) and 95% confidence intervals (95%CI) are shown in *Table 2*. The strongest association was with refusing consent to consult vaccination records: OR=4.7 (CI95%: 2.9-7.6).

#### *Objective Measurement (IgE) of Allergy, Reliability of Reported Symptoms*

Our weighted analysis to assess the agreement between reported symptoms of asthma/hay fever and allergy as defined by increased specific IgE to at least one out of five aero allergens resulted in a 2x2 table similar to the (unweighted) one in the original study with 64% agreement (66% in the original study).

#### *IgG Antibodies, Validation of Vaccination Status*

There were 53 participants (5.6%) without consent for both procedures (blood testing and disclosure of official vaccination records). Our sensitivity analysis resulted in an adjusted OR of any atopic disorder (vaccinated/unvaccinated) of 1.1 (CI95%: 0.9-1.4), in the original data this was 1.0 (0.8-1.2).

#### **Discussion**

This study found that non-participation to a non-obligatory nested sub-study of a cross-sectional study on vaccinations and atopic disorders was related to refusal of consent to consult official vaccination records, not answering a question on income, a lower school class, having fewer older siblings, infrequent consumption of vegetables and not having a history of pertussis. Non-participation in blood testing was not related to the main variables of the original study (vaccination and allergy). These results raise two questions, *viz.* 1) how to interpret the associations found and 2) whether selective participation might have biased results in the original study where blood measurements were involved.

#### *Interpretation of Factors Related to Non-Participation in Blood Testing*

Refusing consent for blood testing was clearly related to less openness and cooperativeness. Vaccinations are a sensitive issue in this religious group; both acceptors (parents who agree to vaccinate) and decliners (those who don't agree) are under pressure: acceptors deviate from the beliefs and practices of the more traditional segment of the Orthodox Reformed Church, while the decliners are often portrayed by the lay press as backward and gambling with the health of their children. Perhaps, although confidentiality was assured and participation was voluntary, some parents may even have misrepresented the vaccination status of their child. Also, non-participation was more common in younger children, who are in general more afraid of medical examinations, in children with lower birth order, who possibly don't have older siblings as role models and whose parents, being less experienced health care seekers, may be less willing to cooperate with physicians. Non-participation was also related to the child not having a history of pertussis. Maybe (as we believe to be the case with lower birth order) these parents lacked experience with physicians treating their child for a serious infection. Another predictor of non-participation was a lower consumption of vegetables, which is presumably an indicator of lower health awareness in parents. These parents may have motivated their children less to participate in blood testing.

#### *Biased Results in Original Study?*

##### Objective Measurement (IgE) of Allergy, Reliability of Reported Symptoms

Selective participation seems not to have biased the estimated agreement between symptoms of allergy and the objective measurement of allergy.

##### Validation of Vaccination Status

Our comparison of children's reported vaccination status with their IgG antibodies in our original study resulted in 4% disagreement (3 out of 80 reportedly unvaccinated children had IgG antibodies), while our comparison

with the official registry yielded an almost 100% match<sup>3</sup>. However, these comparisons include only children with parental consent to these validation procedures. If parents who refused consent for both validation procedures reported a false vaccination status, then a much larger percentage of reported vaccinations would have been misclassified. Clearly, such a misclassification could have biased several of our findings. However, large biases are unlikely because 1) there were only 53 participants (5.6%) without consent for either of the two procedures, and 2) the prevalence of any atopic disorder in these 53 children was similar to that in the study population, 3) 37 (70%) out of these 53 also did not answer the question on income, so these were presumably people who are just sensitive about disclosing private information, 4) our sensitivity analysis yielded an OR similar to the original result, 5) people who did not want to tell the true vaccination status of their child could have refused any participation in the study.

#### Imputation of IgE Positivity

Bias due to selective participation is not likely because all variables, including those that appeared in the present study to be predictors of non-participation to blood testing, were used to impute IgE positivity. Nevertheless, this assumes that non-participation to blood testing is completely determined by the available variables. This MAR (missing at random) assumption however can only be made plausible, never proven<sup>6</sup>.

#### **Conclusions**

In this study of characteristics of non-participants in blood testing we found that refusal to give a blood sample was related to reluctance to disclose private information in general and to sensitivity on the subject of vaccinations in particular. Additionally, parents of younger children with less older siblings, without a history of pertussis, (probably having less experience with health care for their children), and with a lower consumption of vegetables (maybe indicating less health awareness in the parents), were more likely to be non-participants. Selective participation in

blood testing may have affected our assessment of the reliability of the reported vaccination status, but leaves our conclusion from the original study, that there is no positive association between the DTP-IPV vaccination and atopy, unaffected.

#### **Abbreviations**

DTP-IPV	=	Diphtheria-tetanus-pertussis-(inactivated) poliomyelitis vaccination
UAE	=	United Arab Emirates
RAST	=	Radioallergosorbent test
RIA	=	Radioimmunoassay
SPSS	=	Statistical Package for Social Sciences
OR	=	Odds ratio
MAR	=	Missing at random

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The first author, who is independent of any commercial funder, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. None of the authors has any conflict of interest.

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