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Higher risk of allergies at 4-6 years of age after systemic antibiotics in the first week of life

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To the editor,

In humans, the first 100 days appear to be a "critical window" of colonization during which microbial communities shape immune maturation^{1,2}. The use of antibiotics early in life may disrupt the normal maturation process leading to adverse health outcomes such as atopic disorders^{1,3-5}. The effects of antibiotic exposure immediately in the first week of life have rarely been investigated, nor the differences between treatment of 2-3 days and a prolonged treatment of 5-7 days. In the INCA study, a prospective birth cohort study of 151 infants receiving broad-spectrum antibiotics in their first week of life (AB+), and 285 healthy controls (AB-), we previously showed that antibiotic treatment in the first week of life was associated with an increased risk of wheezing, infantile colic and a trend towards more allergic sensitization in the first year of life⁶. The aim of this follow-up study in 418 eligible children was to determine if antibiotic treatment in the first week of life in term-born children was associated with an increase in atopic disorders at 4-6 years of age, using ISAAC questionnaires filled out by parents, ICPC codes derived from general physicians, and pharmaceutical records from local pharmacies. Detailed information regarding the subjects and methods is described in the online Appendix.

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In total, 341 of 418 (82%) questionnaires were filled out (114 AB+ and 227 AB-, Table 1). Parental reported allergy was significantly higher in AB+ vs AB- children (23% vs 11% respectively, $p=0.003$) as was doctor-diagnosed allergy (12% vs 4% respectively, $p=0.008$). Confirmed food allergies were more common in AB+ children compared with AB- children (10 vs. 4% respectively, $p=0.03$). After correcting for sex, age, daycare attendance, and parental level of education, parental-reported allergy was clearly associated with antibiotics use in the first week of life (aOR 2.40 [95%CI 1.22-4.72, table S1]). Additional analyses showed that only 5-7 and not 2-3 days AB treatment was associated with a higher risk of parental reported allergy (aOR 2.85 [95%CI 1.37-5.91, aOR 1.47 [95%CI 0.46, 4.75], respectively]). More importantly, this effect was independent of exposure to additional antibiotics in the first two years of life (table S2). The prevalence of eczema, wheezing/asthma, or allergic rhinitis was not different between AB+ and AB- children (Table 2).

These results suggest that very early exposure to AB in the first week of life has a higher impact on immune development than when administered later in childhood. It also emphasizes the need for judicious use of AB in neonates, especially prolonged treatment of 5-7 days. As AB in early life have a major impact on microbiota development which may lead to aberrant immune maturation,², our findings accentuate the need for finding strategies to modify microbiome development after AB exposure to decrease the risk of allergies.

Strengths of this study are the prospective design, the high response rate (82%), and the combined information collected from doctors and pharmacists, contributing to the reliability of the reported results, which allowed us to distinguish between the effect of antibiotics within and after the first week of life. A limitation of the study is the 4-6-year follow-up, which may have been too short for diagnosing asthma and allergic rhinitis.

In conclusion, the risk of having an allergy at 4-6 years of age increased nearly 3-fold in children after antibiotic treatment for 5-7 days in their first week of life, independent of later AB treatment. These long-term adverse health effects of neonatal antibiotic use emphasize the need to implement AB stewardship programs to avoid AB overuse and reduce the duration of AB treatment where possible in the first week of life.

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Conflict of interest

The authors declare no conflict of interest in relation to this work.

Key words: epidemiology, food allergy, pediatrics, prevention

Authorship

Kim Kamphorst: conception and design, acquisition of data, analysis and interpretation of data; drafting the manuscript, approval of final version of the manuscript

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Ruurd M. van Elburg: conception and design, analysis and interpretation of data; drafting the manuscript, approval of final version of the manuscript

Table 1: baseline characteristics

	AB- N=227	AB+ N=114	AB2 N=32	AB7 N=82
Age median (IQR)*	5 (4.6-5.9)	4.7 (4.4-5.0)	4.7 (4.4-5.3)	4.7 (4.4-5.0)
Sex (male n,%)	122 (54)	65 (57)	14 (44)	51 (62)
BMI mean (SD)	15.6 (1.5)	15.6 (1.4)	15.7 (1.4)	15.5 (1.4)
Delivery mode* n (%)				
Vaginal	146 (64)	86 (75)	22 (69)	64 (78)
C-Section	81 (36)	28 (25)	10 (31)	18 (22)
Breastfeeding				
Median duration (IQR)	4 (1-8)	2.5 (1-7)	2 (0-7)	3.5 (1-6)
Median duration exclusive (IQR)	2 (0-5)	0 (0-4)	0 (0-6)	0.5 (0-4)
Pets n (%)				
No	84 (37)	45 (39)	11 (34)	34 (42)
Cat	59 (26)	34 (30)	9 (28)	25 (31)
Dog	37 (16)	18 (16)	6 (19)	12 (15)
Cat + dog	23 (10)	7 (6)	3 (9)	4 (5)
Other	24 (11)	10 (9)	3 (9)	7 (9)
Daycare attendance n (%)				
No	73 (32)	32 (28)	11 (34)	21 (26)
<3 months	25 (11)	24 (21)	4 (13)	20 (24)
3-6 months	99 (44)	38 (33)	10 (31)	28 (34)
>6 months	30 (13)	20 (18)	7 (22)	13 (16)
Siblings n (%)				
Yes	120 (53)	69 (61)	18 (56)	51 (62)
No	100 (44)	41 (36)	12 (38)	29 (35)
Missing	7 (3)	4 (4)	2 (6)	2 (2)
Parental atopic disease n (%)				
Yes	141 (62)	64 (56)	21 (66)	43 (52)
No	79 (35)	46 (40)	9 (28)	37 (45)
Missing	7 (3)	4 (4)	2 (6)	2 (2)
Highest level of education n (%)				
Low/middle	44 (19)	28 (25)	10 (31)	18 (22)

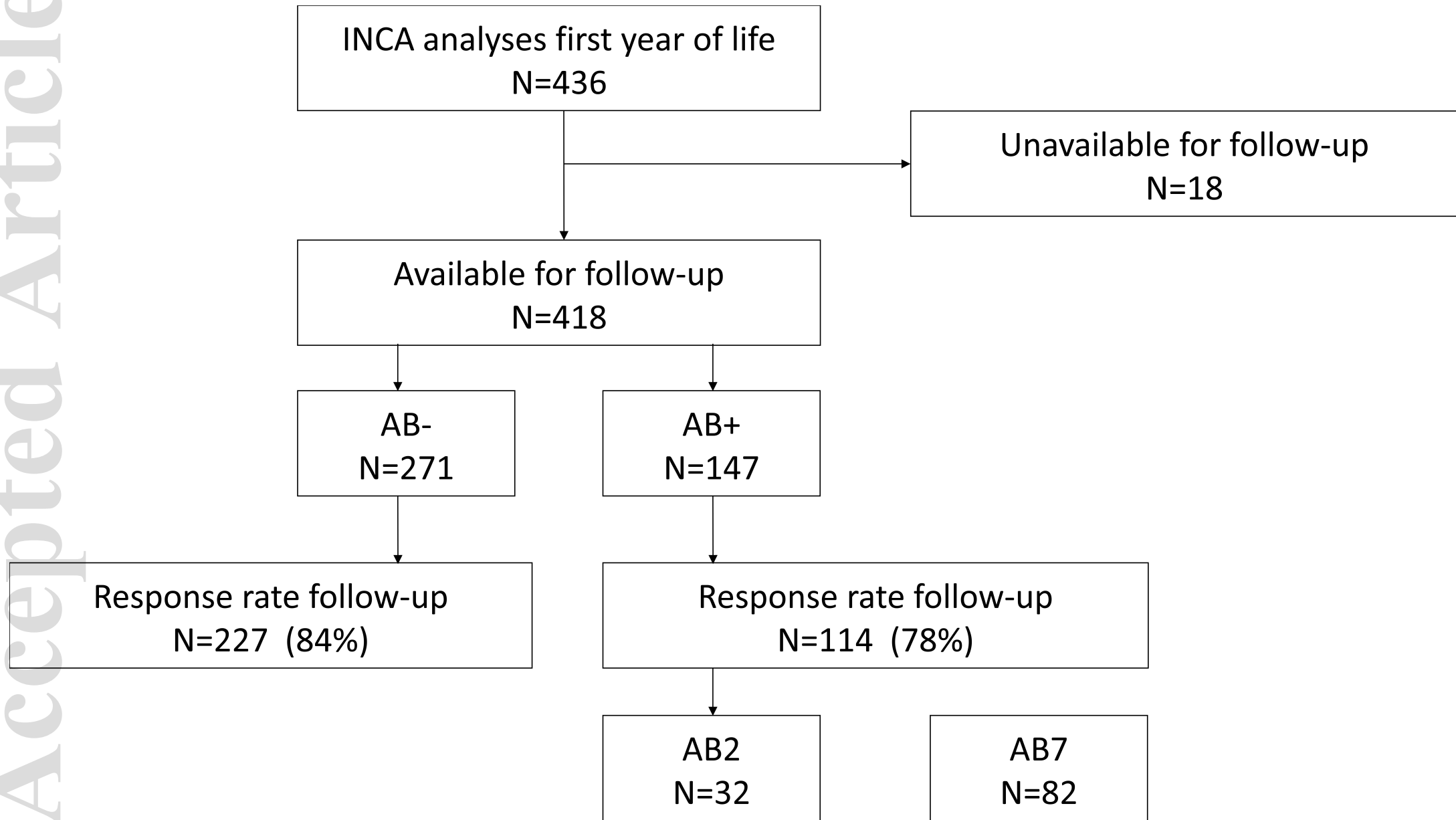
High	174 (77)	80 (70)	19 (59)	61 (74)
Unknown	9 (4)	6 (5)	3 (9)	3 (4)
Number of courses in the first year of life (first week excluded) median (IQR) ¹	0 (0-1)	0 (0-1)	0 (0-0)	0 (0-1)
AB free months in the first year of life* ¹ (first week excluded) median (IQR)	9 (7-11)	7 (2-9)	8 (7-10.5)	7 (2-9)
Number of courses after first week in the first 2 years ² median (IQR)	1 (1-2)	1 (1-2)	1 (1-4)	1 (1-2)
Eczema first year of life n (%)				
Yes	79 (35)	40 (35)	12 (38)	28 (34)
No	148 (65)	74 (65)	20 (63)	54 (66)

AB-: no antibiotics in the first week of life; AB+: antibiotics in the first week of life, for 2-3 days (AB2) or 5-7 days (AB7). *Significant difference between AB+ and AB- by independent t-test and non-parametric test as appropriate¹ Parental reporting, collected prospectively every month during the first year of life. ² Parental reporting, collected retrospectively during the follow-up.

Table 2: incidence of atopic disorders

	AB- N = 227 N, %	AB+ N = 114 N, %	Significance
Parental reported allergy	24 (11)	26 (23)	P=0.003
Wheezing/ Asthma	51 (22)	33 (29)	P=0.206
Eczema	66 (29)	33 (29)	P=0.94
Allergic rhinitis	48 (21)	25 (22)	P=0.90
Doctor diagnosed allergy*	10 (4)**	14 (12)**	P=0.008
Inhalation allergy	4 (2)	6 (5)	P=0.10
Drug allergy	0 (0)	1 (1)	P=0.35
Food allergy	8 (4)	11 (10)	P=0.03
Hazelnut	1 (0.5)	5 (4)	P=0.02
Walnut	1 (0.5)	5 (4)	P=0.02

AB-: no antibiotics in the first week of life; AB+: antibiotics in the first week of life. Significance determined by independent t-test. *Confirmed through skin prick test, blood test and/or provocation test; **6 children have multiple allergies (4 in AB+ and 2 in AB-)



Subjects and Method

Study design

The study design of the prospective birth-cohort INCA study has been described previously ¹. In short, between 2012 and 2015, 436 infants born at term were recruited from maternity and neonatal wards of four teaching hospitals in the Netherlands. Both infants with suspicion of infection, receiving a combination of broad-spectrum antibiotics (n=151) in their first week of life (AB+), and 285 healthy controls (AB-) were included. Blood cultures were taken before AB treatment was started. In case of a negative blood culture, combined with a low clinical suspicion of infection and low C-reactive protein, antibiotics were discontinued after 2 to 3 days (n=42) (AB2), otherwise antibiotics were continued for 7 days (n=109) (AB7).

Data collection in the first week and first year of life

Data collection in the first week and first year of life has been described previously ^{1,2}. In short, at inclusion, parents filled out an online questionnaire concerning the background of the family (e.g., environmental factors, parental smoking habits, family history of atopy and presence of siblings or pets). In addition, perinatal data were collected such as mode of delivery, gestational age, birthweight and type of feeding. During the first year of life, a daily checklist on the health of the child was kept combined with a monthly questionnaire concerning nutrition, parent-reported eczema and wheezing, day care attendance, antibiotic treatment, and general practitioner visits for respiratory or gastrointestinal symptoms. These symptoms were verified at 1 year of age

by checking doctors' diagnoses using the general practitioner electronic medical database based on the International Classification of Primary Care (ICPC) ³.

Follow-up study

Of the 436 children, 18 children were unavailable for follow-up after the first year of life. Therefore, 418 children (271 AB- and 147 AB+) were eligible for follow-up (figure S1). Between May 2018 and May 2019, the parents of these 418 children were approached to complete an online questionnaire. The children were 4-6 years of age depending on the year of inclusion. Data regarding eczema, wheezing/asthma, and allergic rhinitis were collected using the validated ISAAC questionnaire ⁴. Furthermore, information about additional antibiotic use during the second year of life and the presence of allergies (food, drug, insect venom, inhalation or contact allergy) was collected. If parents reported an allergy, additional information was collected whether this was confirmed through a skin prick test, blood test and/or provocation test. If allergy was confirmed, this was defined as a doctor diagnosed allergy. Upper and lower respiratory disorders, gastrointestinal disorders, and allergies were verified through doctors' diagnoses using ICPC, subject to obtaining informed parental consent for requesting this information ³. Furthermore, with additional informed parental consent, the pharmacy was approached to collect information about medication use from birth until 4-6 years of age.

Statistical analysis

Baseline characteristics and differences between AB+ and AB- were analyzed by using the independent t-test and Chi-squared test or non-parametric test as appropriate. Multiple logistic regression analysis was used to assess the association between antibiotic treatment in the first week of life and allergic disorders. The model was

adjusted for sex and age at follow-up, other confounders (BMI, delivery mode, duration of breastfeeding, day-care attendance, presence of pets, siblings, family history of atopy, highest parental level of education, additional antibiotic courses in the first two years of life, and eczema in the first year of life) were included if beta change was $\geq 10\%$ (table S1). The effect of duration of antibiotic treatment (AB2 vs AB7) was also analyzed. Furthermore, we performed a sensitivity analysis in the group with medication history from the pharmacies, to determine the effect of antibiotic exposure after the first week but within the first 2 years of life on parental reported allergy at 4-6 years of age (Table S2)

A p-value < 0.05 was considered statistically significant. Analyses were performed with IBM SPSS Statistics for Windows version 26 (Armonk, NY, USA).

Ethics

Informed consent was obtained from both parents of all participating children. The study was approved by the ethical board of the St. Antonius Hospital in Nieuwegein, the Netherlands and was registered in the clinical trials register as NCT02536560.

Additional results

Antibiotics administered between the first week and two years of age were similar in the AB+ and AB- group (36% and 35% respectively). Furthermore, family history of atopy was similar in both groups (approximately 60%) and did not influence the statistical analyses and therefore was not included in the final models (tables S1 and S2).

Additional Discussion

An important point of the study is the generalizability. The positive family history of around 60% in both the AB group and the control group, and is comparable to other

large cohort studies in the Netherlands such as the PIAMA study ⁵, which makes the study results applicable for the general population, not specific for a high-risk population. However in our logistic regression analysis, family history of atopy did not influence the association between AB in the first week of life and risk of parental reported allergy and therefore was not included in the final model. We cannot comment on the question whether the proportion of children exposed to antibiotics in the first week of life was comparable to the Dutch population or other studies, because of the study design: a prospective birth cohort study of 151 infants receiving broad-spectrum antibiotics in their first week of life (AB+), and 285 healthy controls (AB-). This design with 1/3 of the children exposed to AB in the first week of life and 2/3 of the children not exposed was specifically chosen to prospectively evaluate the effect of AB in the first week of life. However, the percentage of children exposed to antibiotics in the first 2 years of life in another recent Dutch study was similar to that in our study ⁶. Overall, except for the percentage of infants exposed to antibiotics in the first week of life, the results of our study are fairly comparable to other studies.

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Figure S1: Flowchart of response rate. AB+ are treated with antibiotics in the first week of life and AB- are the healthy controls. AB2 = antibiotic treatment for 2-3 days, AB7 = antibiotic treatment for 7 days.

Table S1. Likelihood for parental-reported allergy at 4-6 years of age after antibiotic treatment in the first week of life (n=341)

		Model 1 aOR (95%CI)	Model 2 aOR (95%CI)	Model 3 aOR (95%CI)
AB-		Ref	Ref	Ref
AB+	All	2.15 (1.14, 4.06)	2.09 (1.09, 4.02)	2.40 (1.22, 4.72)
	AB2	1.34 (0.43, 4.20)	1.44 (0.45, 4.58)	1.47 (0.46, 4.75)
	AB7	2.77 (1.25, 4.89)	2.37 (1.18, 4.77)	2.84 (1.36, 5.95)

Results of logistic regression analyses for allergies at 4-6 years of age. Model 1: AB+; Model 2: model 1 + sex+ age; Model 3: model 2 + day-care attendance + highest parental level of education. AB-: no antibiotics in the first week of life; AB+: antibiotics in the first week of life; AB2: 2-3 days antibiotics in the first week of life; AB7: 5-7 days antibiotics in the first week of life.

Table S2. Sensitivity analysis: In group with medication data (n=296), likelihood for parental-reported allergy at 4-6 years of age after antibiotic treatment in the first week of life.

		Model 1 aOR (95%CI)	Model 2 aOR (95%CI)	Model 3 aOR (95%CI)	Model 4 aOR (95%CI)
AB-		Ref	Ref	Ref	Ref
AB+	All	1.99 (1.02, 3.86)	1.95 (0.99, 3.84)	2.26 (1.11, 4.58)	2.29 (1.13, 4.65)
	AB2	1.51 (0.47, 4.82)	1.64 (0.50, 5.38)	1.67 (0.51, 5.52)	1.69 (0.51, 5.58)
	AB7	2.16 (1.06, 4.39)	2.08 (1.00, 4.32)	2.51 (1.16, 5.42)	2.60 (1.20, 5.64)

Model 1: AB+

Model 2: model 1 + sex + age

Model 3: model 2 + day-care attendance + highest parental level of education

Model 4: model 3 + antibiotics in first 2 years