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Wheezing and infantile colic are associated with neonatal antibiotic treatment

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Running title

Wheezing, colics and neonatal antibiotics

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Abstract

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Background Cohort studies have suggested that early life antibiotic treatment is associated with increased risk of atopy. We determined whether antibiotic treatment already in the first week of life increases the risk for atopic and non-atopic disorders.

Methods The INCA study is a prospective observational birth cohort study of 436 term infants, with follow-up of one year. 151 neonates received broad-spectrum antibiotics for suspected neonatal infection (AB+), versus a healthy untreated control group (N=285) (AB-). In the first year, parents recorded daily (non-) allergic symptoms. At 1 year, doctors-diagnoses were registered and a blood sample was taken (n=205).

Results Incidence of wheezing in the first year was higher in AB+ than AB- (41.0% vs. 30.5%, $p=0.026$; aOR 1.56 (95%CI 0.99-2.46, $p=0.06$). Infantile colics were more prevalent in AB+ compared to AB- (21.9% and 14.4% $p=0.048$), antibiotic treatment was an independent risk factor for infantile colics (aOR 1.66 (95%CI 1.00-2.77) $p=0.05$). Allergic sensitization (Phadiatop >0.70 kUA/L) showed a trend towards a higher risk in AB+ (aOR 3.26 (95%CI 0.95-11.13) $p=0.06$). Incidence of eczema, infections, and GP visits in the first year were similar in AB+ and AB-.

Conclusion Antibiotic treatment in the first week of life is associated with an increased risk for wheezing and infantile colics. This study may provide a rationale for early cessation of antibiotics in neonates without proven or probable infection.

Key words Antibiotics, Atopy, Neonate, Eczema, Infant, Infantile colic, Sensitization, Wheezing,

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Introduction

Broad-spectrum antibiotics (AB) is the most frequently prescribed drug type in neonates(1). Besides obvious beneficial short-term effects, there is mounting evidence of negative long-term side effects of antibiotics. Several clinical studies reported a relationship between the use of antibiotic courses in the first years of life and an increased incidence of atopic diseases, obesity, inflammatory bowel diseases and other immune mediated disorders(2). The primary hypothesis for this relationship is that antibiotics can disrupt the development of (gut-)microbiota and consequently lead to immune dysregulation(3).

Animal models suggest that a critical window exists in early life in which antibiotic treatment results in impaired immune maturation through a disturbed gut-microbiome development(4-6). It

can be assumed that a similar effect exists in humans, with neonatal antibiotic treatment resulting in the most profound long-term altered clinical outcomes.

However, most clinical studies, thus far, have investigated a long period of (repeated) AB exposure, up to 2 years. To date, no study has prospectively investigated the effect of neonatal antibiotics, given in the first week of life, when microbiome development and immune maturation are at their beginning and the AB effect may thus be very large, on health and the development of atopic disorders. Therefore, we performed an observational cohort study in term infants, with or without antibiotic treatment in the first week of life (7). The aims of this observational cohort study were to measure the effect of AB during the first week after birth, on the development of 1. signs of atopic disorders, as wheezing, allergic sensitization and eczema in the first year of life, and 2. infantile colics and other non-atopic disorders in the first year of life, and 3. to determine if the development of (non-)atopic disorders depends on the duration of AB treatment, as AB are often discontinued after 2-3 days if suspicion of bacterial infection has diminished, versus > 7 day treatment in neonates with a proven or probable early onset infection.

Methods

Study design

The INCA (INtestinal microbiota Composition after Antibiotic treatment in early life) study is a prospective birth-cohort study. Design, inclusion and exclusion criteria of our cohort have been published previously(7). Between August 2012 and January 2015, term born infants (≥ 36 weeks gestational age) were recruited from the maternity and neonatal wards of four teaching hospitals in the Netherlands.

Antibiotic treatment was at the pediatricians discretion, according to hospital protocol for suspected early onset neonatal infection and based on the Dutch guideline for early onset sepsis(8). In general, infants with suspicion of infection received broad-spectrum antibiotics., after a blood culture was taken. In case of a negative blood culture, combined with a low clinical suspicion of

infection and low c-reactive protein, antibiotics were discontinued after 2-3 days (AB2), otherwise antibiotics were continued for 7 days (AB7).

All term born infants staying in the hospital for at least 24 hours were eligible for inclusion. Exclusion criteria were severe congenital malformations, severe infection needing transfer to a neonatal intensive care unit, and insufficient knowledge of the Dutch language. Informed consent was obtained from both parents of all infants. The study was approved by the ethical board of the St. Antonius Hospital in Nieuwegein. The study was registered as NCT02536560.

Data collection

At inclusion, parents filled out a questionnaire concerning the background of the family (e.g. parental comorbidity, environmental factors, parental smoking habits, presence of siblings or pets).

Parents of all included children kept a daily checklist during the full study period (12 months), reporting presence of the following symptoms: cough, wheezing, fever $>38^{\circ}\text{C}$, runny nose, ear ache, rash, diarrhea and excessive crying ($>3\text{hr}/24\text{hr}$). Rash was defined as more than one day existing skin-redness on the face, arms, legs, or trunk. Wheezing was defined as a whistling, wheezy sound coming from the lower airways during expiration, but not when coming from or through the nose. Parents were instructed by written information and video files about how to recognize wheezing. Furthermore, a monthly questionnaire was filled out concerning nutrition, parent-reported eczema, daycare attendance, antibiotic treatment and general practitioner visits for respiratory or gastrointestinal symptoms.

An episode of wheezing was defined as present for at least 2 consecutive days. Infantile colics were defined according to the Rome III criteria with parent-reported excessive crying, ≥ 3 days within a week, within the first 3 months of life(9). Parent-reported eczema was recorded if the parents reported this in the monthly questionnaire in the first year.

The symptoms were verified at 1 year of age through doctors-diagnosis, via the General Practitioner electronic medical database using the International Classification of Primary Care

(ICPC)(10). Around the age of 1 year, a blood sample was obtained, if parents gave additional informed consent,

IgE analysis

Serum samples were analyzed for IgE antibodies using the Immucap® system according to the manufacturer's guidelines (Thermo Fisher Scientific, Uppsala, Sweden). All children were tested with Phadiatop® infant assays for the graded determination of atopy with semi-quantitative and -qualitative results. If total IgE concentration was $>0.35\text{kUA/L}$, further testing was performed for specific IgE levels of a predefined set of allergens: cat (e1), dog (e5), mite (d1), peanut (f13), milk (f2), egg (f1) and grass pollen mix (gx1). Specific IgE concentrations $>0.70\text{ kUA/l}$ were regarded as positive.

Power calculation

Expected incidence of eczema in AB- was 20%, and expected incidence in AB+ was 35%. To show this increase, assuming a dropout of 15%, 450 children (150 AB+, 300 AB-) needed to be included for a power of 80% and an alpha of 5% (7). Data of infants were included for analyses if at least 3 months of data were available, otherwise children were considered lost to follow-up. Inclusion continued until an expected cohort of 450 children was achieved.

Statistical analysis

Numerical data were analyzed with the Mann Whitney U test or Kruskal Wallis test, categorical or dichotomous data were analyzed with Chi-squared test as appropriate. A multivariable logistic regression analysis was performed to determine the effect of antibiotics on the incidence of wheezing, infantile colic, allergic sensitization and eczema expressed as aOR with 95% confidence intervals. Furthermore, potential confounders mentioned in table 1 were preselected and tested on a case by case basis. Confounders corrected for were; duration of breastfeeding,

delivery mode, tobacco exposure, day-care attendance, presence of siblings, familial history of atopy, household educational level.

Effect of duration of antibiotic treatment (AB2 vs AB7) was evaluated to assess possible differences on incidence of wheezing, infantile colic, allergic sensitization and eczema. Significance level was set at $P < 0.05$. All calculations were performed with SPSS 23.0 for Windows (Chicago, IL, USA).

Results

Baseline characteristics

In total, 608 children were included: 196 treated with antibiotics in the first week of life (AB+) and 412 healthy controls (AB-). As 172 children were excluded from analyses due to study withdrawal or lost to follow-up, figure 1), final analyses were performed in 436 children (AB+ (n=151), AB- (n=285)). In AB+, 42 children were treated for 2-3 days (AB2), and 109 children for 7 days (AB7) because of probable (104) or proven infection (5). Baseline characteristics were similar in both groups (table 1), except for higher gestational age and birthweight, and active maternal smoking during pregnancy in AB+.

Wheezing

The incidence of wheezing in the first year of life was significantly higher in AB7 (42.2%) but not in AB2 (38.1%) compared to AB- (30.5%) ($p=0.028$ and $p=0.324$, respectively; table 2). The episode of wheezing occurred significantly earlier in AB+ than in AB- (9.8 vs. 10.5 months; $p<0,001$). After correcting for confounders, AB+ was associated with a trend towards a higher risk for the development of at least one episode of wheezing (aOR 1.56; 95% CI 0.99-2.46; $p=0.055$). The association between wheezing and antibiotic treatment in children of atopic parents was higher than in children of non-atopic parents (aOR 2.19 (95%CI 1.24-3.87) vs. aOR 0.996 (95%CI 0.43-2.32 respectively).

Infantile colic

The incidence of parent-reported infantile colic was higher in AB7 compared to AB- and AB2 (24.8%, 14.4%, and 14.3%, $p=0.048$ and $p=0.015$ respectively). Incidence of doctors-diagnosed infantile colic was higher in AB+ than in AB- (4.0% versus 0.4%; $p=0.014$ respectively). Antibiotic treatment was an independent risk factor for infantile colic (aOR 1.66 (95%CI 1.00-2.77) $p=0.05$).

Allergic sensitization

Blood samples at 1 year of age were available from 205 children (AB+ $n=77$, AB- $n=128$; table 3). Allergic sensitization, total IgE ≥ 0.7 kUA/L, was found in 11.7% ($n=9$) of AB+ compared to 7.0% ($n=9$) of AB- ($p=0.254$). Duration of antibiotic treatment did not affect the results ($p=0.272$).

There was a trend towards contribution of antibiotic treatment to the risk for sensitization (aOR of 3.26 (95% CI 0.95-11.13), $p=0.06$). Sensitization for >one allergen occurred in 7/9 children in AB+ group and 0/9 children in AB- group. Incidence of a positive Phadiatop was not different in children from atopic and non-atopic parents ($p=0.376$).

Rash and Eczema

Incidence was not different in AB- and AB+ of both parental-reported eczema (37.7% vs. 34.7%, $p=0.533$) and doctors-diagnosed eczema (11.9% vs. 14.0%, $p=0.519$ respectively; table 2), nor between AB2 and AB7.

Although the incidence of eczema was similar, the median number of days with parent-reported rash was higher in AB+ than AB- (8.0 and 5.0 days respectively; $p=0.024$). Children in AB7 showed a trend towards most days with rash (9.0 days; $p=0.071$).

Other clinical outcomes

Incidence of gastro-intestinal tract, respiratory tract infections and mean number of days of fever, coughing, runny nose, ear infections, and diarrhea were not different between AB+ and AB- (data not shown).

Mean number of GP visits was not different between AB+ and AB- (2.24 and 2.3 times in the first year respectively). After the first week of life, infants in both groups had a similar chance during the first year of life of receiving one or more AB courses during the first year of life (AB+ 30.9% vs. AB- 30.5%) and number of courses was similar (mean number of courses AB+ 0.55 vs. AB- 0.50). Duration of neonatal AB treatment neither contributed to the incidence of GP visits nor the number of AB courses in the first year of life.

Discussion

This is the first prospective, observational birth-cohort study that evaluated the effects of antibiotic treatment in the first week of life on the subsequent risk for (non-)atopic disorders during the first year of life. Neonates receiving a 7-day course of antibiotics had an increased risk for an episode of wheezing and infantile colic compared to children treated for 2 days and healthy controls.

The incidence of wheezing in this study may seem high, but it is comparable to the range for the incidence of wheezing (37-56%) as reported in a recent review by Muglia *et al.* (11). The results of this study corroborate previously found associations between antibiotics in the first year of life and the development of wheezing or asthma. Alm *et al.* (2008) found an OR of 1.8 for neonatal antibiotics of at least one episode of wheezing during the first year of life(12). In the same cohort, Göksör *et al.* found that neonatal antibiotics were an independent risk factor for asthma at 8 years of age(13). However, the primary aim of the 'Infants of Western Sweden' cohort was to identify risk-factors contributing to atopic disorders and not to primarily investigate the role of neonatal antibiotics in the development of (non-)atopic disorders. In the systematic review of Murk *et al.* (2011), antibiotic treatment in the first two years increased the risk for childhood asthma with a

pooled OR of 1.52 (14). Reverse causation is a serious confounder that could not be excluded in many of the previous studies, with AB exposure up to the second birthday(14). However, reverse causation is unlikely to play a role in our study, as exposure to antibiotics was restricted to the first week of life when respiratory tract infections are a rare cause of infection.

The association between neonatal antibiotics and the increased risk for infantile colics has not been described previously. Several studies however, have shown an altered gut microbiome in children suffering from infantile colics, with an abundance of coliform bacteria and decreased count of lactobacilli(15-19). However, many of these studies evaluated gut microbiota at or after infantile colic diagnosis. It is therefore unclear whether dysbiosis predisposes a child to infantile colics or may be the result of, for example, stress associated with infantile colics. Stool sample analysis in our INCA cohort may help to elucidate this hypothesis. Subsequently, it may give opportunities for prevention or treatment of infantile colics by microbial modulation.

Although numbers were low, more children were sensitized after neonatal antibiotic treatment. Our results are in contrast with the Dutch birth cohort study KOALA, where no association was found. However, the KOALA study evaluated antibiotic use in the first 6 months of life(20). The previously described hypothesis of a critical window, which may be shorter than 6 months, may explain the difference with our results.

Mechanistic insights in the relationship between broad-spectrum antibiotics and higher IgE-concentrations were found in animal studies(21, 22), suggesting an important role for antibiotics in elevated IgE-concentrations which are in line with the results in our human cohort.

In contrast to the primary hypothesis, we did not find an increased risk for eczema after neonatal antibiotics, although we did find more days of parental-reported rash in the AB+ group. Whether this last finding suggests a more severe form of eczema in the AB+ group remains unclear. In 3 studies, an increased risk for eczema later in childhood after (repeated) antibiotics in the first year of life(23-25), while 3 other studies did not find such an association(20, 26, 27). The main difference however between our study and previously studies is the exposure period; we focused on

neonatal antibiotics whereas previous studies evaluated antibiotic exposure in the first 2 years of life. Furthermore, the definition of eczema is different in each study, with doctors diagnoses, questionnaire outcomes or parental-reported eczema. As shown in our study, there is a difference in incidence of parent-reported versus doctors-diagnosed eczema.

Wheezing, allergic sensitization and eczema are all thought to be clinical expression forms of a Th2-mediated allergic disorder. It is therefore remarkable that we found significant effects of neonatal antibiotics on wheezing and sensitization but not on eczema. Exposure of the mucosal immune system early in life to a diverse gut microbiota is required for proper development of the mucosal immune system, including development of tolerance to food allergens. The optimal timing, dosage and diversity of microbial stimulation required for tolerance induction is, to a large extent, unknown. Furthermore, differences may exist between T-regulatory cells and innate lymphoid cells in their dependency and sensitivity for these microbial regulatory elements. Our data don't allow definite conclusions, but it may suggest that immune-regulatory mechanisms that prevent development of eczema are more robust than similar mechanisms controlling wheezing and sensitization.

Neonatal antibiotic treatment was not associated with an increased risk for respiratory tract or gastro-intestinal infections, nor with use of additional antibiotic courses in the first year of life. Theoretically, dysbiosis due to neonatal antibiotics could be related with a higher risk of subsequent infections, but we did not find such association. Moreover, it is known that a healthy microbiota can protect against pathogens, a phenomenon referred to as colonization resistance(28). The very low number of bacterial infections in Dutch infants in general may have attributed to the absence of an association(29).

An important strength of this study is the prospective data collection in a relatively large group of healthy, term-born infants. Furthermore, infants were included from four different non-academic hospitals in the Netherlands, suggesting that these findings may also be applicable for

healthy term-born children in other Western European countries. Further studies in different countries are needed to confirm this assumption.

The study design may be its main limitation, being an observational cohort study rather than a randomized controlled trial. Therefore, potential confounders cannot be excluded, like the (suspicion of a) neonatal infection for which antibiotics were prescribed and unavoidable differences in baseline characteristics. Another limitation is the absence of consensus in the literature on the definition of wheezing and an episode of it. Even though parents were instructed by written information and video files, parent reported wheezing is less specific than doctors diagnosed wheezing(30). However, reported incidences of wheezing were similar to those reported in a recent review on wheezing (11).

Another important confounder is the fact that the AB+ group was admitted to the neonatal ward for two to seven days, whereas the control group stayed with their mothers at the maternity ward for one to three days before discharge. These different environments could result in different bacterial exposure contributing to the developing neonatal microbiota. Furthermore, it is reported that separation from parents can cause stress, which may also influence the developing gut microbiota and immune system in the AB+ group. Maternal separation has been reported to cause differences in gut-microbiome colonization in rodents (31), and might therefore contribute to the development of atopic disorders and infantile colic in this study. However, the fact that we found associations for 7 but not 2 days of antibiotic treatment in the first week of life suggests that antibiotic treatment may have a more pronounced contribution to the development of atopic disorders and infantile colic than parental separation and/or admission to the neonatal ward.

Conclusion

In the INCA study, we have shown that 7 days of antibiotic treatment in the first week of life is a contributing risk factor for wheezing and infantile colics in the first year of life. Allergic sensitization seemed to be more common in children treated with antibiotics, while no association

with development of eczema or infectious symptoms was indicated. Further follow-up of this cohort may elucidate whether neonatal antibiotic treatment may result in development of allergic symptoms, such as asthma and allergic rhinitis, later in life. The results of the INCA study imply that antibiotics during the first week of life, especially when administered for 7 days, can have negative long-term effects. Further optimization of the indications for starting antibiotics, early discontinuation if possible, or interventions with pre- or probiotics might limit these negative effects.

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		AB - N 285		AB + (total group) N 151		AB 7 days N 109		AB 2-3 days N 42	
		N	%	N	%	N	%	N	%
Gestational age mean (SD)		39.56 (+/- 1.49)		40.0 (+/- 1.29)*		40.27 (+/-1.16)*†		39.41 (+/-1.41)	
Birthweight mean (SD)		3444 (537)		3681 (533)*		3717 (558)*		3588 (457)	
Male		152	53.5	85	56.3	66	60.6	19	45.2
Delivery mode									
	Vaginal	187	65.6	112	74.2	83	76.1	29	69.0
	C-section	98	34.4	39	25.8	26*	23.9	13	31.0
Maternal antibiotics									
	No	200	70.2	88	58.3	66	60.0	22	52.4
	Yes	24	8.4	47*	31.1	32	29.1	15	35.7
Breastfeeding									
	Mean mo (SD)	4.6 (4.1)		4.1 (4.0)		4.1 (3.9)		4.0 (4.2)	
	Mean mo exclusive (SD)	2.8 (3.6)		2.5 (3.6)		2.5 (3.6)		2.6 (3.9)	
Tobacco exposure									
	Pregnancy active	12	4.2	16*	10.6	10*	9.2	6*	14.3
	Pregnancy passive	32	11.2	23	15.2	20	18.3	3	7.1
	Home	25	8.8	14	9.3	11	10.1	3	7.1
Pets									
	Cat	72	25.3	42	27.8	31	28.4	11	26.2
	Dog	47	16.5	24	15.9	18	16.5	6	14.3
	Cat+dog	27	9.5	12	7.9	8	7.3	4	9.5
	Other	28	9.8	13	8.6	8	7.3	5	11.9
Daycare									
	Yes. <3 mo	27	9.5	28	18.5	23	21.1	5	11.9
	Yes. 3-6 mo	132	46.3	44	29.1	33	30.3	11	26.2

	Yes. >6 mo	35	12.3	26	17.2	18	16.5	8	19.1
	No	91	31.9	53	35.1	35	32.1	18	42.9
Siblings									
	No siblings	147	51.6	91	60.3	66	60.6	25	59.5
	≥1 sibling. not atopic	80	28.1	30	19.9	19	17.5	11	26.2
	≥1 sibling. atopic	38	13.4	17	11.2	15	13.8	2	4.8
Parental atopic disease									
	No	95	33.3	54	35.8	42	38.5	12	28.6
	Mother	70	24.6	29	19.2	23	21.1	6	14.3
	Father	59	20.7	34	22.5	19	17.4	15	35.7
	Both	41	14.4	20	13.2	16	14.7	4	9.5
Household education									
	Low	6	2.1	2	1.3	1	0.9	1	2.4
	Middle	49	17.2	35	23.2	24	22.0	11	26.2
	High	208	73.0	97	64.2	74	67.9	23	54.8

Table 1 Baseline characteristics; AB- (healthy controls); AB+ (antibiotic exposed); AB2 (antibiotic treatment 2-3 days); AB7(antibiotic treatment 7 days). SD (standard deviation). Mo (months).

Gestational age is in weeks. Due to missing data, numbers not always add up to the total of the group.*p<0.05 as compared to healthy controls †p<0.05 as compared to 2-3 days AB

	AB- N=285	AB+			aOR (95%CI)
		Total N=151	2 days N=42	7 days N=109	
Wheezing <i>Parental-reported</i>	87 (30.5%)	62 (41.1%) P=0.027*	16 (38.1%) P=0.324	46 (42.2%) P=0.028*	1.56 (0.99-2.46) P=0.055
Wheezing <i>Median days (range)</i>	0.0 (0-163)	0.0 (0-48)	0.0 (0-20)	0.0 (0-48)	
Infantile Colics <i>Parental-reported</i>	41 (14.4%)	33 (21.9%) P=0.048*	6 (14.3%) P=0.986	27 (24.8%) P=0.015*	1.66 (1.00-2.77) P=0.050*
Infantile Colics <i>Doctors-diagnosed</i>	1 (0.4%)	6 (4.0%) P=0.014*	3 (7.1%) P<0.001*	3 (2.8%) P=0.034*	N too small
Eczema <i>Parental-reported</i>	99 (34.7%)	57 (37.7%) P=0.533	17 (40.5%) P=0.468	40 (36.7%) P=0.716	1.09 (0.7-1.7) P=0.698
Eczema <i>Doctors-diagnosed</i>	40 (14.0%)	18 (11.9%) P=0.519	5 (11.9%) P=0.696	13 (11.9%) P=0.567	0.85 (0.45-1.61) P=0.625
Rash <i>Median days (range)</i>	5.0 (0-326)	8.0 (0-312) P=0.024*	5.5 (0-191) P=0.308	9.0 (0-312) P=0.026*	

Table 2 AB and (non-)atopic outcomes. AB- (healthy controls); AB+ (antibiotic exposed); AB2 (antibiotic treatment 2-3 days); AB7 (antibiotic treatment 7 days). aOR was calculated between AB+ and AB-. *p<0.050 as compared to healthy controls

	AB- N=128	AB+			aOR (95%CI)
		Total N=77	2 days N=18	7 days N=59	
Allergic sensitization $\geq 0.35kUA/L$	14 (10.9%)	13 (16.9%) P=0.223	2 (11.1%) P=0.982	11 (18.6%) P=0.150	2.504 (0.99-6.32) P=0.052
Allergic sensitization $\geq 0.70kUA/L$	9 (7.0%)	9 (11.7%) P=0.254	2 (11.1%) P=0.539	7 (11.9%) P=0.272	3.26 (0.95-11.13) P=0.06

Table 3 AB and allergic sensitization. AB- (healthy controls); AB+ (antibiotic exposed); AB2 (antibiotic treatment 2 days); AB7 (antibiotic treatment 7 days). aOR was calculated between AB+ and AB-.

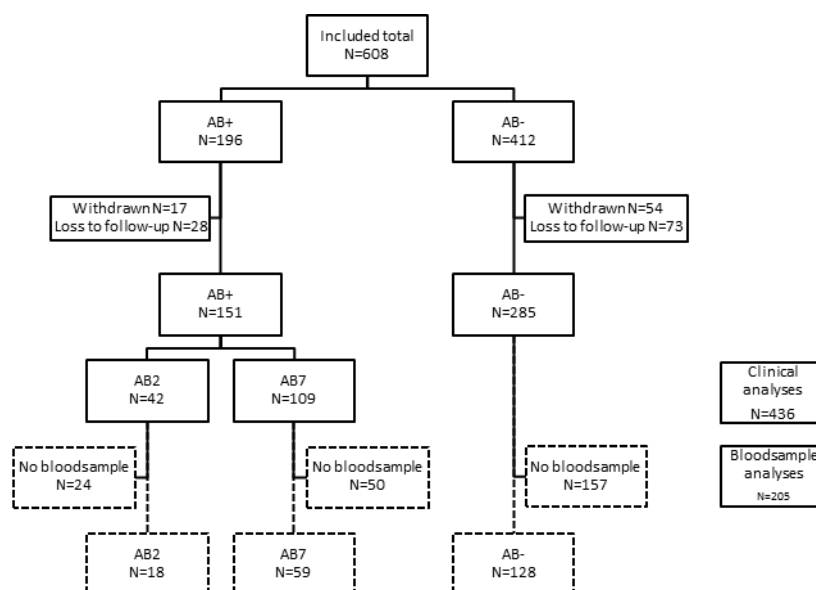


Figure 1; Participant flow diagram. AB+ (antibiotic exposed); AB2 (antibiotic treatment 2 days); AB7 (antibiotic treatment 7 days); AB- (healthy controls). The dotted blocks show a subpopulation of the cohort in which blood sample analysis was done. Children were considered lost-to-follow up whenever there were less than 3 months of data available. Children withdrawn were considered as such when parents actively withdrawn participation in the study.