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# THE IMPACT OF NEONATAL ANTIBIOTIC EXPOSURE ON ATOPIC SENSITISATION BY THE AGE OF 12 MONTHS

(VARHAISLAPSUUDEN ANTIBIOOTTIALTISTUKSEN VAIKUTUS ATOOPPISEEN HERKISTYMISEEN 12 KUUKAUDEN IKÄÄN MENNESSÄ)

Syventävien opintojen kirjallinen työ
Syyslukukausi 2014

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Bakteerien vaikutus terveyteen on merkittävä: ne ovat toisaalta hengenvaarallisten infektioiden aiheuttajia, mutta samanaikaisesti niiden läsnäolo on välttämätöntä terveen immuunipuolustuksen kehittymiseksi. Ensimmäisen elinvuoden aikana suoliston bakteerikanta on altis ulkoisten tekijöiden vaikutuksille. Varhaislapsuuden antibioottihoidolla voi olla tuhoisat seuraukset eri bakteerilajien suhteille, ja sen tiedetään altistavan erilaisille immuunivälitteisille sairauksille, kuten atopialle. Ei ole kuitenkaan selvitetty, onko tämä vaikutus johtunut käynnissä olevan infektion aiheuttamista muutoksista kehittyvään immuunijärjestelmään vai onko siihen ollut syynä infektioon aloitettu antibioottihoito.

Tässä tutkimuksessa selvitettiin eroja kahden, varhaista antibioottihoitoa saaneen ryhmän välillä ja päätetapahtumaksi katsottiin myöhemmin lapsuudessa ilmenevä atopiataipumus. Toinen ryhmä sai antibioottihoitoa kliinisesti todistettuun bakteeri-infektioon (varhaiseen sepsikseen). Toinen ryhmä taas sai antibioottihoidon pelkästään infektioepäilyyn eli ns. empiirisen hoidon, joka lopetettiin alle viiden vuorokauden kuluessa kun katsottiin, ettei oireiden taustalla ollutkaan bakteeri-infektiota. Mukana vertailussa oli lisäksi ryhmä, joka ei saanut lainkaan varhaista antibioottihoitoa. Antibioottihoidon pitkäaikaisvaikutusta arvioitiin ihon prick-testillä, joka kertoo atopiaan liittyvästä IgE-välitteisestä herkistymisestä. Varhaisella antibioottihoidolla tarkoitetaan tässä alle 72 tunnin sisällä syntymästä alkanutta hoitoa G-penisilliinin ja gentamysiinin yhdistelmällä.

Tutkimuksen aineisto koostui 755 vastasyntyneen lapsen seurantadatasta, joka saatiin neljän käynnissä olevan allergian ehkäisytutkimuksen aineistosta. Tutkimuksen mukaanottokriteereinä pidettiin sitä, että saatavilla oli tiedot sekä mahdollisen antibioottialtistuksen kestosta että ihon prick-testituloksesta joko 6 tai 12 kuukauden iässä. Tulokset analysoitiin logistisella regressioanalyysillä huomioiden mahdolliset sekoittavat tekijät: äidin allergia, ennenaikaisuus, tutkimusprobiootti, imetyksen kesto, äidin raskauden aikainen tupakointi ja synnytystapa.

Potilasryhmässä, joka sai antibioottihoitoa ilman samanaikaista infektiota, nähtiin tilastollisesti merkittävästi vähemmän positiivisia prick-testituloksia verrattuna lapsiin, jotka eivät altistuneet antibiooteille. Tulos on merkittävä, sillä se osoittaa, että varhaisella antibioottihoidolla on kauaskantoisia vaikutuksia kehittyvään immuunijärjestelmään.

Asiasanat: antibiootit, immuunijärjestelmä

**The Impact of Neonatal Antibiotic Exposure on Atopic Sensitization by the Age of 12 Months**

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**We investigated the immune consequences of early antibiotic exposure in 622 neonates with or without confirmed infection. The primary outcome was positive skin prick test by 12 months of age. Our results suggest that empirical neonatal antibiotic treatment without concomitant bacterial infection has a long-term impact on immune development.**

After the recognition of the profound impact indigenous bacteria have on human health, microbes are no longer considered merely potential pathogens.[1] Disturbances in indigenous intestinal microbiota composition may play a causal role in the development of non-communicable diseases such as atopic disease.[1,2] The delicate balance of host-microbe interactions is prone to disruptions by external factors such as antibiotics particularly early in life, when the immune responder phenotype is consolidated.[1,2] Consequently, early life antibiotic exposure may have far-reaching effects on gut microbiota composition and immune maturation, potentially predisposing to immune-mediated diseases including asthma, inflammatory bowel disease or obesity later in life.[3-6] Antibiotic exposure during infancy has previously been reported to increase the risk of atopic sensitization,[6] but little is known of the long-term immune consequences of neonatal antibiotic exposure.

Empirical antibiotic therapy is common in the neonatal period due to the difficulty of accurately and rapidly detecting early-onset sepsis. In neonates who receive antibiotics for a clinically confirmed bacterial infection both bacteria causing the infection and the antibiotic treatment may lead to immunological consequences. The majority of empirical treatments, however, are discontinued after the symptoms have resolved and no objective evidence of bacterial infection has been obtained. These cases enable investigating the specific effects of antibiotic therapy. The aim of this study was to evaluate the long-term immune

consequences of antibiotic therapy commenced during the first 72 hours of life in term and late-preterm infants with or without infection and evoke discussion about the safety of this brief empirical antibiotic exposure.

This retrospective study cohort consists of data gathered from four ongoing randomized double-blind prospective probiotic intervention trials.[8-11] All four studies are being conducted in the same single tertiary center in Turku, Finland. The study subjects have been born in the Turku region, in Southwest Finland. All four studies have been approved by the ethics committee of the Intermunicipal Hospital District of Southwest Finland.

Altogether 755 infants have been enrolled in the trials. Data on antibiotic exposure commenced during the first 72 hours of life and follow-up data until the age of 12 months was available from 626 neonates who were included in this study. The antibiotic regime used to treat suspected early-onset neonatal infection consisted of penicillin G and gentamycin according to the Turku University Hospital standard clinical protocol. Antibiotic therapy was categorized as follows: no exposure, brief empirical exposure (duration less than 5 days) or therapy for confirmed infection (duration ≥ 5 days). The primary outcome measure of the trial was positive skin prick test result either at the age of 6 or 12 months as an indicator of atopic sensitization. Skin prick testing was performed at these time points as previously described in detail using a panel of most common childhood allergens.[12]

The inclusion criteria varied for the four clinical trials with regard to family history of atopic disease and gestational age. In addition, the infants were subjected to different probiotic (or placebo) intervention protocols. The correlation between antibiotic exposure and positive prick test result was therefore analyzed by logistic regression and adjusted for maternal allergy, prematurity (defined as delivery before 37 weeks of gestation) and probiotic intervention. In addition, adjustment was performed for potential confounding factors including duration of breastfeeding, maternal smoking during pregnancy and mode of delivery. The statistical analyses were generated using SAS software (version 9.3 for Windows, SAS Institute Inc. Cary, NC, USA). The results are expressed as risk ratios (RR) with 95% confidence intervals. $P<0.05$ (two-sided) was considered statistically significant.

The clinical characteristics of the subjects are presented in Table 1. Altogether 4 subjects were excluded from the analyses due to lacking data on primary outcome. After adjusting for potential confounding factors, antibiotic exposure was statistically significantly associated with skin prick test positivity (Table 2). Surprisingly, brief neonatal antibiotic exposure without infection was associated with lower risk for prick test positivity. The prevalence of positive skin prick test was 25% (136/550) in infants without antibiotic exposure, 8% (4/52) in infants with brief antibiotic exposure and 29% (7/24) in infants who received antibiotic therapy for confirmed infection ($P=0.011$). Our results thus suggest that short-term early antibiotic exposure without concomitant infection modulates immune development in infancy. The effect is considerable for its long-term impact up to the age of 12 months and remained significant after adjusting for potential confounders. This phenomenon was not evident in neonates receiving antibiotics for confirmed infection.

Consistently with previous reports,[9,13,14] maternal allergy and prolonged breastfeeding increased the infant's risk for positive skin prick test result in this study (Table 2). The association between maternal allergy and infant skin prick test positivity is explicable by genetic predisposition for atopy. Genetic and immune factors in breast milk may cause the increased risk for positive prick test result associated with breastfeeding lasting 6 months or more. Probiotic intervention had no statistically significant effect on prick test positivity in this population.

It is notable that preterm infants received early antibiotic treatment more frequently than full term infants (Table 1). Preterm infants have previously been reported to exhibit reduced risk for prick test positivity.[15] However, no correlation between preterm birth and skin prick test positivity was observed in the present study and the association between antibiotic exposure and skin prick test positivity remained significant after adjusting for prematurity (Table 2).

Our findings suggest for the first time that antibiotic exposure in the neonatal period without concomitant infection may have far-reaching consequences on immune development. Such effects may be conveyed via disrupting intestinal bacterial diversity which may lead to disturbances in beneficial host microbe-interactions mediated by bacterial metabolites (e.g. short-chain fatty acids) and bacteria-induced immune tolerance.[1,2] In infants with infection, exogenous pathogens and endotoxins activating immune reactions may counteract these effects. The disruptive impact of antibiotics on gut

microbiota is well-established but little has been known about such effects in the neonatal period.[2] In order to prevent increased prevalence of atopic, metabolic and autoimmune diseases, further studies are needed to understand the immunological consequences of early antibiotic exposure in this vulnerable period.

| N=626 | No antibiotic exposure | Brief antibiotic exposure | Antibiotic therapy for infection |
|---|---|---|---|
| Gender, male | 298/549 (54 %) | 35/52 (67 %) | 14/23 (61 %) |
| Birth weight (grams) (mean with range) | 3535 (1865-4860) | 2686 (1740-4170) | 3192 (1610-4660) |
| Positive prick test result | 136/550 (25 %) | 4/52 (8 %) | 7/24 (29 %) |
| Prematurity | 36/548 (7 %) | 37/52 (71 %) | 9/24 (38 %) |
| Mode of delivery<br>vaginal<br>elective section<br>non-elective section | <br>467/544 (86 %)<br>42/544 (8 %)<br>35/544 (6 %) | <br>36/52 (71 %)<br>2/52 (4 %)<br>13/52 (25 %) | <br>17/23 (74 %)<br>1/23 (4 %)<br>5/23 (22 %) |
| Breastfeeding ≥ 6mo. | 331/545 (61 %) | 31/52 (60 %) | 13/24 (54 %) |
| Probiotic intervention | 273/550 (50 %) | 24/52 (46 %) | 12/24 (46 %) |
| Maternal allergy | 450/550 (82 %) | 33/52 (65 %) | 16/24 (67 %) |
| Maternal smoking during pregnancy | 28/549 (5 %) | 7/52 (13 %) | 8/23 (35 %) |

Table 1. Baseline characteristics of study subjects.

| | RR [95% CI] | p-value |
|---|---|---|
| Antibiotic exposure, total<br>brief antibiotic exposure<br>therapy for infection | -<br>0.31 [0.094-0.75]<br>1.44 [0.67-2.44] | 0.011<br>-<br>- |
| Maternal allergy | 1.55 [1.02-2.54] | 0.040 |
| Breastfeeding ≥ 6mo. | 1.39 [1.03-2.91] | 0.033 |
| Maternal smoking during pregnancy | 0.52 [0.19-1.11] | 0.10 |
| Prematurity | 1.18 [0.60-1.99] | 0.61 |
| Probiotic intervention | 1.02 [0.88-1.17] | 0.82 |
| Mode of delivery<br>elective section<br>non-elective section | -<br>1.03 [0.55-1.69]<br>0.90 [0.49-1.46] | 0.92<br>-<br>- |

Table 2. Logistic regression model for skin prick test positivity.

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