

POLISH MOTHER AND CHILD COHORT STUDY (REPRO_PL) — METHODOLOGY OF FOLLOW-UP OF THE CHILDREN

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Abstract

Background: A prospective cohort study design gives the opportunity for identification, update of different exposures and their verification by biomarker measurements. The aim of The Polish Mother and Child Cohort Study (REPRO_PL) is to evaluate the impact of exposure to different environmental factors during pregnancy and, after birth, on the pregnancy outcome, children's health and neurodevelopment. **Materials and Methods:** REPRO_PL cohort was established in 2007. From the cohort of 1300 mother-child pairs, 300 children are followed-up until they are two years old to assess the exposure, health status and neurodevelopment. Children are examined twice: at one and two years of age by a pediatrician and a psychologist/child development specialist. During each visit, detailed questionnaire is conducted with the mothers and supplemented with the information from the medical charts to have appropriate recognition of the child's health and development. Additionally, the current health status of the child is checked and his/her biometry is performed. A urine sample is collected from each child for the verification of environmental tobacco smoke (ETS) and polycyclic aromatic hydrocarbons (PAH) exposure. Some children have a blood sample collected for the assessment of the lead and cadmium levels. Child's neurodevelopment is assessed based on Bayley Scales for Infant and Toddler Development (Bayley-III). **Results and Conclusions:** The results of the study will become available within the next few years and will help to determine the impact of the environmental exposures on children's health and neurodevelopment. REPRO_PL cohort is a middle-sized cohort, very much focused on specific research questions with the potential for future extension and cooperation.

Key words:

Birth cohort, Follow-up of children, Child's health, Neurodevelopment

INTRODUCTION

It is well understood that different environmental exposures during pregnancy, or even in the preconceptional period, have significant impact on the pregnancy outcome and children's health. Additionally, postnatal exposures

can significantly influence child's health and development. For appropriate risk assessment it is crucial to have valid assessment of the exposures. A prospective study design gives opportunity for identification, update (notification of any changes in the exposure level) of

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such exposures and their verification by biomarker measurements. Taking into account such advantages, many European countries have established birth cohorts with the shared purpose to examine early determinants of health by following the subjects from the prenatal period, throughout childhood to adolescence or even adulthood. Those cohorts include more than 350 000 mother-child pairs across Europe. Some of them are very large cohorts developed in 1990s (such as: DNBC — Danish National Birth Cohort [1], or MoBa — The Norwegian Mother and Child Cohort [2] with about 100 000 participants in each cohort), others are smaller (a few thousands of subjects) with a more specified exposure or disease of interest. The enrollment of mothers into the study is commenced during their pregnancy or at the delivery and follow the children up to different time points after birth. For instance, the children from the MoBa cohort are examined a few times in the first years of life (at 6, 18 months, 3, 5, 7 and 8 years) with the main focus on child's health, nutrition, well-being and mental development [2]. As regards the exposure, most cohorts collect the information about the outdoor air pollution, active and passive smoking, maternal occupation (for pregnancy exposure) and allergens. Other specific exposures such as: water contamination, metals, noise, POPs and radiation are less frequently examined, but also studied in some cohorts. Outcome information focused mostly on the birth outcome (a newborn's anthropometric parameters at birth), and a child's health and development; including the incidence of asthma, allergy, respiratory diseases, childhood growth and neurodevelopment.

To improve the exchange of information, collaboration between researchers, and effective use of the existing data, as well as to indicate the direction for future research in the field of children's health, three international projects founded by the European Union's Seventh Framework Program have been performed. The ENRIECO Project (Environmental Health Risks in European Birth Cohorts)

has created an inventory of all existing birth cohorts in Europe with the data on environmental exposures [3,4]. The other two projects: CHICOS (Developing a Child Cohort Research Strategy for Europe) and RICHE (A Platform and Inventory for Child Health Research in Europe) are focused mostly on the identification of gaps and needs in the current knowledge, and the development of recommendations for research action at a European level [5,6]. Full inventory of birth cohorts is available in the form of a searchable database on www.birthcohortsenrieco.net or www.birthcohorts.net [7,8].

In Poland, two cohorts have been created. The Cracow cohort is focused mostly on the assessment of impact of air pollution and mercury on the birth outcome and children's health and development [9–11]. The Polish Mother and Child Cohort (REPRO_PL) is a multicenter study on different exposures in the pre/postnatal periods and the outcomes including anthropometric measurements at birth and health status, as well as the neurodevelopment within the first years of life [12,13].

This paper summarizes the aim and methodology used for the follow-up of the children included in the REPRO_PL Cohort.

OBJECTIVES

The general objective of REPRO_PL is to evaluate the impact of exposure to different environmental factors during pregnancy and, after birth, on the pregnancy outcome and children's health. Specific research hypotheses refer to the role of heavy metals, exposure to polycyclic aromatic hydrocarbons (PAH) and environmental tobacco smoke (ETS) in the etiology of intrauterine growth retardation (IUGR) and preterm delivery (PD). It is also intended to explain the role of oxidative stress and the nutritional status of pregnant women. The impact of occupational exposures and stressful situations on the pregnancy outcome is also analyzed within REPRO_PL.

As regards the children's health within the first years of life, the study is intended to provide the answers to the six important questions stated below:

1. What is the influence of prenatal and postnatal ETS exposure on children's neurodevelopment, the risk of upper and lower respiratory infections, allergy and middle ear diseases?
2. What is the role of prenatal exposure to polycyclic aromatic hydrocarbons (PAH) in the risk of neurobehavioral disorders?
3. What is the children indoor air exposure for microbial levels, endotoxins and glucans?
4. What is the role of low intakes of dietary antioxidants during pregnancy in the development of children allergy?
5. Is the prenatal exposure to lead at the level below 10 µg/dl a risk of delayed child's mental development?
6. Does mercury exposure due to fish consumption during pregnancy affect the mental and psychomotor development?

The list of research questions is not final. Furthermore, additional specific research questions may be formed and answered as a result of the REPRO_PL study.

The information about the prenatal exposure needed to answer some of the above questions is already collected and analyzed (the detail description of the methodology related to the pregnancy period was published in a previous paper [12]). This article describes the methodology for the follow-up of the examined children at one and two years of age.

MATERIALS AND METHODS

Study design and population

The Polish Mother and Child Cohort Study (REPRO_PL) was established in 2007 under the project "Epidemiology of reproductive hazards in Poland — multicentre,

prospective cohort study — Repro-PL" founded by the National Center for Research and Development. The complete description of the pregnant women cohort was published elsewhere [12].

The follow-up of the children from the REPRO_PL cohort is carried out in cooperation with The Norwegian Institute of Public Health (NIPH) — the main coordinator of the MoBA cohort. The Norwegian Institute of Public Health, as the partner within the project, has mostly the advisory role. This part of the study is performed under the project entitled "Prenatal and postnatal exposure to tobacco smoke, PAH and heavy metals and the risk of respiratory diseases, allergy and poor mental and physical development" supported by the grant PNRF-218-AI-1/07 from Norway through the Norwegian Financial Mechanism within the Polish-Norwegian Research Fund.

Under the current project and the current financial resources, from the REPRO_PL cohort consisting of 1300 mother-child pairs, 300 children are followed-up up to two years of age in order to assess their exposure, health status and neurodevelopment. The children come from two regions of Poland: Łódź and Legnica. The follow-up of 300 children began in 2009 and it is planned to be finished by the end of 2011.

The study was approved by the Ethical Committee of the Nofer Institute of Occupational Medicine, Łódź, Poland (Decision No. 3/2008). All information collected during the study is confidential and used only for the research purpose. All mothers of the children from the REPRO_PL cohort are informed about the aims, purpose and data/biological samples intended to be collected in the course of the study and are asked to sign the informed consent form. Children born into the cohort participate on the basis of their mothers' consents until they are able to decide for themselves. The biological material collected for the purpose of the study is transported, stored and processed with caution and can be identified with its code number only. The participants have the right to participate in a part of the study (i.e., they

can refuse their consent to have a blood sample collected from their children) and to have their data removed from the cohort database at any time and not be the subject to any more interviews or sampling.

Power calculation / Sample size

The required sample size is calculated for a two-sided test at the significance level equaling to 0.05, and the power for the selected alternative hypothesis equals 0.9. The calculation is performed for two kinds of outcomes: 1) continuous variables (results Bayley-III, biometric indicators), and 2) dichotomous variables (allergy, respiratory diseases, middle ear diseases). The exposure variables (based on the biological samples measurements) are continuous variables. Taking this into consideration, the sample size is calculated as the example for 1) Bayley-III and the exposure to environmental tobacco smoke (ETS), and 2) the risk of allergy and ETS. The association between the Bayley-III test and ETS is evaluated using linear regression. We assumed that the mean difference in the Bayley-III test between the most extreme groups with respect to exposure is equal to 0.7 SD of Bayley-III. Under the above assumptions, the sample size should be 224. The calculations are carried out using the PS software [14,15]

The association between the risk of allergy and ETS is evaluated using the logistic regression model. We assumed the prevalence of asthma at the level of 11%, and that there is a two-fold risk increase for one SD increase in the ETS exposure. The required sample size for this model is 230 [16,17].

Taking into account the above calculations, about 300 children from the REPRO_PL cohort are followed-up until they are two years of age under this part of the project. The study is still ongoing and up till now we have received the response (participation) rate on the level of 65%.

The project is not yet fully financed and the data collection period as well the sample size may be extended in the future.

Follow-up of the children

An invitation letter is sent to all mothers participating in the REPRO_PL cohort (from the designated areas of Poland) one year after the child's birth with the proposal of having the child's exposure, health status and neurodevelopment examined. Within the next two weeks a phone call is made to schedule their (mother and child) visit at the clinic involving child's examination by a pediatrician and a psychologist/child development specialist. The same procedure is repeated when the child is 18 or 24 months old.

Questionnaires

During each visit (at the age of 12 and 18 or 24 months) a questionnaire is conducted by a pediatrician with a child's mother. The first part of the questionnaire covers certain socio-demographic information (i.e., family size, place of living, material status of the family) and includes details questions in order to obtain the data about home conditions (e.g., some visible damp and mold, house dust, the frequency and type of house cleaning, fuel used for cooking or heating, furred pets at home). The information about the proximity to heavy traffic and any sources of industrial exposure is inquired. The first part of the questionnaire is also collecting the information about the nutrition of the small child (including any restrictions in the diet), his/her home environment and contacts with other children. The smoking status of the parents and other potential sources of children's ETS exposure are identified. The children's ETS exposure is assessed based on the number of the smoking persons at home, the number of cigarettes they smoke and the time spent by a child in places where someone smokes cigarettes.

The second part of the questionnaire investigates the children's health and development condition within the first years of life. The incidence of the upper and lower respiratory tract infections (tonsillitis, laryngitis, bronchitis and pneumonia), middle ear diseases and any symptoms of allergy to food and inhalant allergens are noted. The data

given by the mothers is supplemented with the use of the information from individual medical charts of each child. The duration of each infection and disease, medications taken and hospitalizations, if any, are identified. The information about the results of the biometric measurements within the first years of life is noted. This covers the child's weight, height, as well as the head and chest circumference.

Special attention is paid to the identification of any signs and symptoms of allergy and asthma. This part of the questionnaire has been developed by an allergologist based on recommendations from the International Study of Asthma and Allergies in Childhood (ISAAC) [18]. It covers two general modules: 1) skin symptoms: including eczema and itchy rash, and 2) nose or airway symptoms: including rhinitis (sneezing, or a runny, or a blocked nose), wheezing, cough, dyspnea and medically diagnosed asthma. In addition to this, the occurrence of allergy among the family members is noted.

The questionnaire which is conducted with the mothers when the child is two years old is meant to update any changes in the information provided during the first questionnaire.

Assessment of children's exposure

Postnatal children's exposure to ETS at 12, 18 and 24 months after birth is assessed. As it was stated above, a detailed questionnaire to identify any sources and times of children's exposure to ETS is conducted with the mothers. Additionally, urine samples from all children, and saliva samples from the mothers are collected. The saliva and urine cotinine levels are analyzed using high performance liquid chromatography (HPLC) coupled with tandem mass spectrometry/positive electrospray ionization (LC-ESI⁺MS/MS) and the isotope dilution method.

Postnatal exposure to PAH is examined in 300 children. The HPLC method is used for the analysis of the level of 1-hydroksypyrene in urine as the biomarker of PAH exposure.

Part of the children (whose mothers agree to it) have a blood sample collected for the assessment of the lead and cadmium levels. Those heavy metals concentrations in blood are determined by graphite-furnace atomic-absorption spectrometry.

For the assessment of exposure to microbial levels, endotoxins and glucans, approx. 50 family homes of children are visited. The settled dust is collected from the floors in the living rooms and from the children's beds (mattresses and blankets). To determine the allergens concentrations in dust samples, the commercially available enzyme-linked immunosorbent assay (ELISA) kits are used according to the protocol for Der p1 (5H8/4C1), Can f 1, Fel d 1 (6F9/3E4) and Bla g 2 (Indoor Biotechnologies, Charlottesville, VA, USA). To determine endotoxins and (1→3)-β-D-glucans concentrations, the LAL test is applied.

Determination of the children's health and development

As it was stated above, a detailed questionnaire with the mothers is conducted by pediatricians and supplemented with the information from individual medical charts of each child to have appropriate recognition of children's health and development. In addition to this, during the clinical visit, the current health status and children's biometry (height, weight) are examined. Due to low validity and reliability in small children, skin prick tests are not performed, but any signs and symptoms of allergy are identified.

Determination of the neurodevelopment of the children

Children's neurodevelopment is assessed by a psychologist or child development specialist based on Bayley Scales for Infant and Toddler Development (Bayley-III). This test is performed during each clinical visit (when the child is 12 and 18 or 24 months of age). Bayley-III is an individually applied examination that assesses the developmental functioning of infants and children aged from 1 to 42 months. Bayley-III presents children with situations and tasks designed to produce an observable set of behavioral

responses. The test assesses five developmental areas: cognitive, motor (fine and gross motor), language (receptive, expressive), social-emotional and adaptive behavior. Bayley-III measures cognitive development using age-appropriate activities such as counting, puzzle completion, matching colors and pretending to play. It evaluates fine motor skills, such as visual tracking, reaching and grasping, as well as gross motor skills, such as sitting, crawling, standing, jumping, and walking up and down the stairs. In the area of language, Bayley-III assesses the development of communication skills including babbling, gesturing, naming objects, vocabulary, and the use of plurals and verb tenses. The adaptive behavior assessment includes questions about child's communication, home living, self-direction and other topics. The social-emotional assessment indicates how a child uses all the capacities to meet the needs, deal with feelings, think and communicate.

The strengths and weaknesses of the REPRO_PL cohort study

The REPRO_PL cohort is a middle size cohort compared to other European cohorts (such as DNBC or MoBa). The biggest cohorts mostly have the strategy to collect data on as many exposures and health outcomes as feasible and in the future to state and test the specific hypothesis [1,2]. On the other hand, the advantage of the Polish cohort is that at the phase of planning and development of the cohort all research hypotheses (taking into account the specific exposures and diseases) are clearly stated, which determines the data and sample collection and analysis. Thanks to this, in the course of the study, or a few years after the study, we will be able to answer the specific questions relating to the level of exposures (specific to our country) and the associations between the exposures and diseases. The sample size determination was calculated assuming rather strong associations and prevalent diseases. We have assumed that at least one of the variables (exposure or outcome) will be on the continuous scale, when both variables are dichotomous the power will

be low except for very strong associations. For rare diseases, the power of the study is low (sample size cannot be sufficient to detect some associations), so we will provide our data to conduct joint analyses with other mother-child cohorts.

The strength of REPRO_PL is that measures of both genes and the environmental factors are available in the same study for a high proportion of the study subjects. Very detailed questionnaires (with sub questionnaires such as: Food Frequency Questionnaire, Subjective Work Characteristics Questionnaire, Cohen Questionnaire, a detailed questionnaire for identification of symptoms of allergy among small children) are filled by trained interviewers (mostly nurses) and, where appropriate, by gynecologists/obstetricians, neonatologists, pediatricians, allergologist, psychologists/child development specialists. Consequently, we are able to avoid some bias resulting from misunderstanding or missing some of the questions. The questionnaires are conducted three times during pregnancy and at 12 and 24 months after the delivery, which allows for the update and/or verification of some information.

For the valid assessment of exposure, during each visit a broad spectrum of biological samples is collected (from mothers: saliva, urine, hair, blood, cord blood; from children: urine, blood). In collected biological samples, as many as possible (and relevant to our country) biomarkers of exposure are analyzed (cotinine, 1-hydroxypirene, heavy metals, microelements, dietary antioxidants, antioxidant enzyme activities, TBARS, GPx1, GPx4, PCDD/PCDF/PCB), and their associations with the health outcomes are assessed. A very interesting example from our cohort can be the cotinine level for the assessment of active and passive smoking exposure. It can be recognized as the risk factor for many diseases (i.e., poor pregnancy outcome, respiratory diseases, neurodevelopment) and also as the confounding factor for the other studied associations.

The REPRO_PL cohort covers many geographically different areas of Poland (with different exposures). It cannot be seen as representative for the whole population of

Poland, but it seems to be representative for the designated regions. In the process of selecting different regions for the study, we tried to cover an area of the country with different exposures. For instance, we choose Łódź — a big city with air pollution exposure coming mostly from road traffic and old buildings in the city centre heated by solid fuel (mainly coal). The Copper Basin was selected because of high exposure to heavy metals. We also included in our research less polluted areas.

The weakness of the REPRO_PL cohort can be a possible selection bias related to low recruitment or losses to follow-up. It is likely that there is a socioeconomic gradient that influences the prevalence estimates. However, the aim of the REPRO_PL is to provide valid estimates of association between putative causal factors and diseases, and the prevalence of the exposures and diseases may be different from that what is found in the total population. Nevertheless, the estimates of the associations can still be valid [2].

The majority of limitations can be related to the children's exposure, health and neurodevelopment assessment. Particularly important confounders which should be considered while analyzing the association between exposures and children's development are: parental intelligence (to account for the genetic background), and the quality of the home environment (to account for the differences in the environmental stimulation during the development). Other confounders are as follows: age, sex, parental education, drugs and vitamins intake during pregnancy, and other environmental contaminants. In our cohort, we tried to avoid as many limitations as possible by careful development of the questionnaire (i.e., identification of confounding factors: socio-economic determinants, proxy to parental intelligence, place of residence, social network, nutrition) and valid assessment of the exposure by biomarkers levels (i.e., cotinine, 1-hydroxypirene, lead, cadmium). There is always the risk in assessing children's neurodevelopment that the assessment tools may not be sensitive enough to identify the outcome, which refers to

the younger age group. To find the suitable tests to measure the differences and points is a very challenging task. In REPRO_PL, we chose the Bayley-III test — the most commonly used one for evaluating the neurodevelopment effect of environmental exposure among small children. Such test was adapted to Polish conditions. The children's health and development status can be determined by many factors acting not only in postnatal, but also in prenatal period. Due to the small sample size of our REPRO_PL cohort, it may be not possible to distinguish such exposures and judge which of these periods are the most important. The advantage of our cohort is that the health and biometric status of the children is determined by a detailed interview and current examination made by a pediatrician. This information is also supplemented with the data from individual medical charts of each child. Although skin prick tests are not performed (due to their low validity and reliability in small children), we tried to identify any signs and symptoms of allergy. To achieve this, a detailed sub questionnaire was developed by an allergologist based on the ISAAC recommendations [18].

Summing up, the REPRO_PL cohort is a middle size cohort, very much focused on specific research questions with the potential for future extension and cooperation.

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