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Population Cohort Study of Wroclaw Citizens (PICTURE) – study protocol

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ABSTRACT

The objective of the PICTURE project is to conduct a comprehensive survey study and laboratory analyses in children and their parents concerning their health status with observation of environmental and social factors influencing lifestyle and occurrence of risk factors. The randomly selected participants (1250 children aged 7-14 years and 1250 parents) were invited to enroll in the study, which started in 2019. Every participant of the study undergoes a questionnaire study, including nutritional and physical activity assessment, and medical examination: anthropometric measurements, ECG, spirometry, audiometry, tympanometry, blood pressure measurement, hand grip strength and body composition. Children are also examined for posture defects using a Podoscan and a scoliometer. Data are obtained in the standardized way, periodically once every two years with annual phone contact. For the purpose of future analyses, the specimens (blood, urine, feces and saliva) are stored in the first integrated biobank in Poland with an ISO 9001:2015 certificate and positive quality assessment in BBMRI-ERIC. Our study aims to create a Wroclaw cohort, which gives an opportunity of longitudinal observation of the impact of societal, behavioral and familial factors and environmental exposure on health outcomes, which will be the basis for targeted preventive programs.

KEY WORDS: health status, environmental factors, social factors, lifestyle, children, parents.

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INTRODUCTION

Longitudinal cohort studies are a unique source of knowledge that can advance a population's health. Their contribution to the development of preventive medicine is invaluable. One of the first longitudinal studies, the Framingham Heart Study, brought us essential knowledge on modifiable and nonmodifiable risk factors of cardiovascular diseases (CVDs) [1]. Although CVDs are still a leading cause of death worldwide, the awareness of the risk factors contributed to a decrease in morbidity and mortality in many countries. The significant decline of mortality due to coronary heart disease in Poland observed since the 1990s can be predominantly attributed to the reductions in major risk factors [2]. Thus the exploration of the origins of many chronic diseases in lifetime exposure to societal, environmental and behavioral factors has become the main target of biomedical research. Therefore, cohort studies targeting early childhood and adolescence are of significant importance. A comprehensive and detailed database of population health is an essential first step to implement targeted preventive programs properly and efficiently. Several children's cohort studies have been organized worldwide [3-12]. Some of them are birth cohorts, following the children's development and exposure to environmental factors since birth [3-6, 13] or even the prenatal period [8-12].

In Poland, despite rapid economic growth and socioeconomic changes, a discrepancy in health status and life expectancy in comparison to western European countries is still observed. The high rate of premature deaths, as well as health inequalities between social groups, is especially worrisome. Although precise data on the prevalence of pediatric obesity in Poland are sparse, the available studies indicate an alarmingly increasing trend in overweight and obesity in Polish children [14]. According to the estimates of the World Obesity Federation, by 2030, about 20% of 5-9-year-olds and 13.9% of 10-19-year-olds will be obese [15]. There is an urgent need to understand factors underlying mentioned phenomena.

The PICTURE (Population Cohort Study of Wroclaw Citizens) project fits into a long-term public health policy of Wroclaw agglomeration. The primary objective of the PICTURE study is to conduct a comprehensive survey study and laboratory analysis in children and their parents regarding their health status and lifestyle. The secondary aim is to observe environmental and social factors influencing lifestyle and occurrence of risk factors. The final aim is to broaden our understanding of morbidity and mortality factors in the population of Lower Silesia and to determine factors contributing to health inequalities in the population of Wroclaw. Concomitantly, the project will create a cohort, which will be prospectively observed and invited for the follow-up every two years. Considering a broad range of analyzed factors, it is one of the most comprehensive longitudinal cohort studies in a population of children in Poland. The project will benefit participants by the opportunity to take a "picture (selfie) of their health" - to check important blood parameters, hearing capacity, pulmonary function and take anthropometric measurements. The novelty of the study includes focusing on the household, instead of merely on an individual, which enables complex socio-economic and environmental factors underlying lifestyle to be investigated. Furthermore, laryngological assessment will shed a new light on the link between the occurrence of otolaryngologic diseases and environmental and lifestyle factors, especially obesity [16]. The study has been conducted during the COVID-19 pandemic, which is believed to profoundly impact the level of physical activity and adiposity in children and adults [17]. The collected dataset of health parameters and close cooperation with the Municipality of Wrocław will provide the basis for the implementation of targeted preventive programs.

MATERIAL AND METHODS

OVERVIEW

The PICTURE project was created as a complementary study for the PURE Poland cohort study followed by the "Let's get the kids moving [18]" campaign. The study was created and organized by the employees of Wroclaw Medical University and the "Run for Health" foundation. The study involved pupils from Wroclaw's primary schools aged 7-14 years along with their parents/ legal guardians. The total of 43 983 children/adolescents (22 439 boys and 21 544 girls) lived in Wroclaw in 2018. An application to the Polish Ministry of Digital Affairs for a random selection by a PESEL identification number of 3750 participants born between 2005 and 2012 (1905 boys and 1845 girls) was submitted. Selected children were invited to participate in the study with one of their parents/guardians, creating household child-guardian pairs. The selected participants were asked to enroll in the study by traditional letter invitation. The project started in November 2019. The response rate was estimated at approximately 30%. The project aims to conduct a longitudinal analysis, creating Wroclaw cohort (1250 children and 1250 parents in the first two years of the study). Data are obtained in the standardized way, periodically once every two years with annual phone contact. Currently, the baseline phase of the study has ended and participants, who were enrolled in November 2019, will be invited for a first follow-up with a consistent protocol in 2022. The participants are also contacted annually by phone to gather additional information regarding medical events, changes in anthropometric data and hospitalizations. At every stage the participant provides samples of blood, urine, feces and saliva, which are stored in Wroclaw Medical University Biobank for further analyses (except for a partial blood sample, which is used to provide laboratory results for the participant). In the PICTURE project all biological material and associated data are collected and stored in the Wroclaw Medical University Biobank (WMU Biobank), the first integrated biobank in Poland with the ISO 9001:2015 certificate. Moreover, WMU Biobank as a BBMRI-ERIC member is the first biobank with a Q-mark in BBMRI-ERIC Directory integrated with the international standard for biobanks: ISO 20387:2018.

The questionnaire study consists of eight questionnaires which are presented in Table 1 along with the summary of variables investigated in the study. The questionnaire study is conducted by a trained researcher using a tablet, which enables instant digitalization of reported data. The questionnaires contain several sections, including, but not limited to, health status, medical history, bedtime, behavioral factors, physical activity and nutritional assessment. The nutritional assessment is a semi-qualitative questionnaire concerning the frequency of consumption of 27 groups of products. Participants can choose

Questionnaire study	 Family questionnaire Household questionnaire – 175 parameters Health questionnaire (adult) – 310 parameters Health questionnaire (child) – 203 parameters International Physical Activity Questionnaire (IPAQ) for adults International Physical Activity Questionnaire (IPAQ) for children SARS-CoV-2 questionnaire – 43 parameters
Blood	 Blood smear/Morphology Glucose Lipids (total cholesterol, LDL, HDL, TG) Creatinine TSH Potassium Sodium HbA_{1c}
Urine	• Urinalysis
Anthropometric measurements	 Body mass Body mass composition Body height Waist circumference Hip circumference Handgrip strength
Posture defects	PodoscanScoliometer
Otolaryngologic assessment	 Otolaryngologic examination Pure tone audiometry Tympanometry Otoacoustic emissions (OAE)
Other measurements	ECGSpirometrySmokerlyzer

TABLE 1. The parameters collected in the PICTURE cohort study

between 8 categories of frequency from "never" to "more than 4 times a day". The questionnaires, though adjusted especially for this study, were based on questionnaires already used by the authors in the Prospective Urban and Rural Epidemiological Study (adult and household surveys) [19, 20] and questionnaires used in the "Let's get the kids moving" project (children health status and lifestyle questionnaires) [18].

Every participant of the study undergoes medical examination: ECG, spirometry, audiometry, tympanometry, blood pressure measurement, anthropometric measurements of waist and hips circumference, hand grip strength, body height, body mass and body composition using Tanita Scale. Additionally the amount of CO in the exhaled breath is measured in all participants using the Smokerlyzer. Children participating in the study are also examined for posture defects using a Podoscan and a scoliometer. Participants are also encouraged to bring a typically filled schoolbag (textbooks, notebooks, other school equipment) to perform a weight assessment. The equipment used for analysis of posture defects in children as well as the Tanita Scale have been provided by the Sensoria Foundation. Upon receiving the results, the participants are informed by the physician about any disorders or alarming deviations from the norms – including abnormal laboratory results, anthropometric measurements or laryngological assessment.

OTOLARYNGOLOGIC ASSESSMENT

Every participant of the study undergoes otolaryngologic examination, pure tone audiometry, tympanometry, and otoacoustic emissions (OAE). A detailed case history is collected, with particular attention paid to the current ailments, and previous ENT diseases. In each pair of participants examination of the ear (otoscopy), nose (anterior rhinoscopy), and oral cavity is done. Additionally, in adults, indirect laryngoscopy to assess larynx condition is performed. To assess palatine tonsils Brodsky's grading scale is used, classified into 5 grades as follows: grade 0 indicated the previous tonsillectomy; grade 1 indicated that the tonsils were hidden in the pillars; grade 2 indicated that the tonsils were beyond the anterior pillar and between 25 and 50% of the pharyngeal space; grade 3 indicated that the tonsils were beyond the pillars but not to the middle and occupied > 50% and up to 75% of the pharyngeal space; grade 4 indicated that the tonsils occupied > 75% of the pharyngeal space.

To estimate the prevalence of possible hearing loss, hearing tests are done. Tympanometry is performed to provide quantitative information about the presence of fluid in the middle ear, mobility of the middle ear system, and ear canal volume. An audiometric evaluation is performed by means of conventional audiometric methods in a soundproof room to determine if the patient's hearing levels fall within normal limits. The collected data included air conduction and bone conduction thresholds in each ear at frequencies of 0.5 kHz, 1 kHz, 2 kHz, and 4 kHz. The pure-tone average for bone conduction in each ear is calculated for each patient as the average of bone conduction thresholds at 0.5 kHz, 1 kHz, 2 kHz, and 4 kHz. The air-bone gap in the affected ear was calculated as the difference between air conduction and bone conduction thresholds at 0.5 kHz, 1 kHz, 2 kHz, and 4 kHz. OAE is a physiological test specifically measuring cochlear (outer hair cell) response to the presentation of a stimulus. It is an effective screening tool for inner and middle ear abnormalities by assessing the mechanical efficiency of the hearing receptor to detect cochlear hearing loss.

NEPHROLOGY

In our study, a survey study includes questions aimed at detecting any signs of kidney diseases, including chronic kidney disease (CKD), its complications, or factors that increase the risk of CKD (for example obesity and hypertension). Laboratory tests include the following parameters: serum creatinine and estimated glomerular filtration rate (eGFR), basic urinalysis. It is planned to perform the additional tests in the urine retrospectively to detect early tubular injury (urinary neutrophil gelatinase-associated lipocalin-uNGAL, netrin-1).

ACQUISITION OF SPECIMENS

Biological material (BM) – blood and saliva – is collected during the participant's first visit, in a fasting state. The procedure is performed by qualified and trained staff in a dedicated place. The following biological material is protected from each participant for biobanking purposes: blood (5 ml into a tube with clotting activator and gel barrier; 4 ml EDTA tube), saliva (2 ml for the Isohelix Genefix kit with DNA stabilization buffer at room temperature (RT); 3 ml native into Eppendorf type tube without any additives). The amount of BM collected in the project is determined by the amount of blood collected for diagnostic tests. At the second visit, when the medical results are transferred to the participant, the stool and ~50 ml of urine are delivered to the biobank.

TRANSPORT, QUALIFICATION OF SPECIMENS

Samples are transported in an isothermal container at RT (18-25°C; monitored and documented conditions) to the biobank Lab within 30 min from the acquisition with informed consent, consignment note and collection report. The qualification process is performed according to the internal procedures, where formal and technical aspects are checked. After qualification BM is accepted for further processing.

PREPARATION AND PRESERVATION OF SPECIMENS

- 1. Blood from tube with clotting activator and gel barrier: after 30-60 min needed for complete clotting, blood is centrifuged (2000 g, 10 min). Aliquot scheme: 2×0.3 ml, 2×0.7 ml. The separation allows the BM to be preserved for a possible epidemic situation (e.g. serological status assessment 2×0.3 ml). The remaining 2×0.7 ml samples are secured for future purposes, which can be performed from the serum with a backup sample.
- 2. Blood tube with EDTA: aliquots 2×0.4 ml of EDTA whole blood and 0.4 ml of RNA stabilization buffer are secured; the remaining material is centrifuged (2200 g, 15 min). Plasma is divided into 2×0.8 ml for future purposes with a backup sample. Furthermore, the cell pellet is vortexed and separated into 2×0.7 ml aliquots (e.g. for future DNA isolation).
- 3. Saliva into Isohelix Genefix kit with DNA stabilization buffer: thanks to stabilization saliva can be preserved in RT and the DNA isolated will be characterized by high quality and recovery.
- 4. Saliva collected without any additives is gently vortexed before separation. Then 3 aliquots are prepared, where several analyses can be performed with backups.
- 5. Urine: after mixing a portion is transferred into 3×1.5 ml cryotubes. It is followed by a urine strip test with ComboStik R-700. The following parameters are examined: specific gravity, pH, glucose, ketone bodies, bilirubin, blood, urobilinogen, proteins, nitrites, leukocytes, microalbumin, creatinine and microalbumin/creatinine ratio. In a further step the urine sample is centrifuged and the supernatant is divided into 3×1.5 ml aliquots.
- 6. Stool is divided into 3 portions and placed into cryotubes.

All prepared aliquots of each BM are placed in ultralow temperature freezers –80°C (except Isohelix Genefix saliva samples, which are stored at RT). At each step of biobanking, required documentation is prepared and approved.

ETHICAL ASPECTS

Before the examination, from each participant informed consent in written form is obtained. The informed consent and information about the project are obligatory forms which are given to the participant. The informed consent allows the donor to determine the scope of consent and respect the participant's right to know/not know about the clinically significant results for his/her health and life. The PICTURE study has been reviewed and accepted by the Bioethics Committee of the Wro-claw Medical University in Poland (positive opinion no. KB-667/2019).

All ethical aspects are accomplished according to the Quality Standards for Polish Biobanks Chapter 5 ELSI (Ethical, legal and societal issues) [21]. The requirements for the right protection to privacy and the confidential information of participants, recipients and users, particularly during storage and transmission of data, are sustained. Moreover, the information and declaration form in informed consent that the collected samples and associated data will not be shared with third parties for purposes other than scientific are assured. Further usage of collected biological samples and associated data for scientific purposes is possible only after ethical approvals with impartiality safeguarding also with a transparent policy conduction. Material and data can be shared in an anonymized form. Surveys, interviews and questionnaires are performed with respect for privacy of the surveyed person, protection of personal data, comfort and security. Biosamples and associated data are processed, stored and are not enabled for the identification of the participant for anybody besides the dedicated biobank.

DISCUSSION

The PICTURE cohort gives a unique opportunity to observe a large sample of the population of children and their parents over the years. Familial factors, including genetic, behavioral, and socio-economic, impact the development of noncommunicable diseases over the lifespan. The study protocol enables us to assess biological samples retrospectively and prospectively. If disorders are revealed in any results, the patient is immediately informed, receives the results as soon as possible and is advised to contact the specialist. Every participant receives the following laboratory results: blood smear/ morphology, glucose, lipids (total cholesterol, LDL, HDL, TG), creatinine, TSH, potassium, sodium, glycated hemoglobin, basic urinalysis (for scientific purposes). If necessary, participants receive information regarding improving their lifestyle.

The comprehensive study of children's and parents' health is an effective tool to examine presented problems. In Poland, longitudinal data on health and lifestyle of children and adolescents are limited. One of the most comprehensive cross-sectional studies, Health Behavior in School-Aged Children (HBSC), led by the World Health Organization (WHO) and Institute of Mother and Child (IMID) in Poland, is conducted repetitively every several years in the group of 11- to 15-year-olds [22]. Other cross-sectional studies on children's health included the OLAF study [23], the Childhood Obesity Surveillance Initiative (COSI) study [24], the "Wise Nutrition-Healthy Generation" project [25], "Your Child's Healthy Life" program [26] and the SOPKARD-Junior program [27]. To our knowledge one of the few longitudinal cohort studies was the REPRO_PL study [28, 29], which followed the development of children from the prenatal period to the age of 7. The aim of the study was to track the impact of environmental exposure on children's development. Another prospective cohort study, PREMATURIAS, aimed to observe the development of prematurely born children to the age of 2 [30]. There was also a study which investigated cardiovascular risk factors in 24-29-year-olds in relation to their birth weight investigated in the previous study [31]. Our study adds to the literature by involving school-aged children and their parents in longitudinal observation of development of noncommunicable diseases and their risk factors. The study group consisting of child-guardian pairs enables us to observe important socio-economic, environmental and familial impacts on lifestyle and behavior.

Biological samples with well described associated data are essential raw material for biomedical research including translational medicine. Biobanking infrastructure operates in the background of the research but also provides specific analysis as core facilities in projects. Regarding this, biobanks handle their roles by taking a unique position to preserve valuable biological material with associated data not only for ongoing studies but for future purposes as well [32]. Quality is a prerequisite condition to develop comparable specimens and data collections. Moreover, pre-analytical procedures and international standards, which are essential to improving analytical data reliability and reproducibility, as well as the increasing importance of data management, are highly important. It is necessary to document all the pre-analytical and analytical phases and methods. Thus, origin information should have sufficient technical details to demonstrate compliance of the biospecimens with common quality standards (ISO, CEN standards [33], Quality Standards for Polish Biobanks [34], ISBER Best Practices [35], IARC Minimum Technical Standards [36]).

There are some limitations of our project which must be considered. Firstly, the possibility to expand the cohort and continuation of the study depend on the stability of the financial conditions. Despite the COVID-19 pandemic and temporary suspension of the project due to lockdowns, the study is currently continued under the specific conditions of the sanitary regime. All actions are strictly monitored and supervised. Considering implemented preventive solutions, the baseline investigation of the whole study group was completed at the end of 2021. The study focuses on school-aged children and aims to observe their development prospectively. The age of the study group was chosen complementarily to the "Let's get the kids moving" project. The age range is suitable to investigate the development in the prepubertal and pubertal age and the impact of school education on behavioral and lifestyle factors.

In our view, the benefits of the project overcome the possible limitations. The number of cohort studies in Poland is limited, especially those focusing on children. Our study gives a unique opportunity of longitudinal observation of the impact of societal, behavioral and familial factors, and environmental exposure on health outcomes. Thus the project shall be continued and developed. Another strength of the study is the focus on laryngological assessment, which is not a common approach in cohort studies and available data are sparse. The biobanking of specimens enables detailed expanded analysis in the future. An additional aspect of the study, which has not been included in the initial protocol, but was later forced by the epidemiological situation, includes the possibility to assess the prevalence of COVID-19 in the cohort, hospitalization due to COVID-19, and prevalence of symptoms both in children and adults. Close cooperation with the Municipality of Wroclaw enables application of the study results to shape the public health policy of the region. The results of the study are regularly updated and disseminated on the project website: http:// www.picture.umed.wroc.pl/.

CONCLUSIONS

The cohort will be a source of a unique dataset which can be utilized in comparative international studies. The specimens stored in the biobank enable detailed retrospective analysis in the future. The authors are open for cooperation regarding the collected dataset and biological material. The collected dataset of health parameters will provide the basis for the implementation of targeted preventive programs.

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DISCLOSURE

The authors report no conflicts of interest.

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AUTHORS' CONTRIBUTIONS

AMW, TZ, KZ, KKP, KPZ prepared the concept of the study and the paper. AMW, ML, TZ, KZ, KKP, KPZ, ABR, JN, KPazdro contributed to methodology and investigation; AMW, ML – biobanking and urine analysis, TZ – principal investigator, KZ/KPZ/ ABR – epidemiology, internal diseases, nutrition, KKP – pediatry, TZ/JN/KPazdro – laryngology. AMW, ML, KPZ were responsible for data curation. AMW, ML, TZ, KZ, KPZ, ABR, KKP, TZ, JN, KPazdro wrote the original draft. AMW, ABR were responsible for review and editing of the manuscript. AMW, TZ, KZ supervised the project. AMW, TZ, KZ, KPZ were involved in funding acquisition. All authors took part in preparation of the final version of the manuscript.