ICPSR | INTER-UNIVERSITY CONSORTIUM FOR POLITICAL AND SOCIAL RESEARCH

ICPSR 37226

Chinese Longitudinal Healthy Longevity Survey (CLHLS), Biomarkers Datasets, 2009, 2012, 2014

Yi Zeng
Duke University, and Peking University

James W. Vaupel
Max Planck Institutes, and Duke University

User Guide

Inter-university Consortium for
Political and Social Research
P.O. Box 1248
Ann Arbor, Michigan 48106
www.icpsr.umich.edu

Terms of Use

Information about Copyrighted Content

Chinese Longitudinal Healthy Longevity Study (CLHLS)
Biomarkers Datasets collected from the In-depth Studies of the Eight Longevity Areas in the CLHLS 5th, 6th and 7th Wave[1]

Shi Xiaoming[1], Yin Zhaoxue[1], Chen Huashuai[2,3], Lei Xiaoyan[2], Zheng Zhenzhen[2], Zeng Yi[2,3]

1. Chinese Center for Disease Control and Prevention; 2. Center for Healthy Aging and Development Studies (CHADS), National School of Development at Peking University; 3. Center for Study of Aging and Human Development, School of Medicine, Duke University

1. Introduction

China is an irreversible aging society in the 21st century. It is predicted that the Chinese elderly population (aged 65 and above) will consist of 16.2 percent of the total population in 2030, and the proportion will increase to more than 24 percent in 2050. The aging population has become an important national condition in socio-economic development in China.

In order to confront the serious challenge of population aging and to study the health conditions of different age groups and their determinants, the Chinese Longitudinal Healthy Longevity Study (CLHLS) collected the biomarkers datasets from the in-depth studies of the Longevity Areas. In 2009, as part of the CLHLS 5th wave, the Center for Healthy Aging and Development Studies (CHADS) of National School of Development at Peking University and Chinese Center for Disease Control and Prevention (CDC), initialized the in-depth study (including interviews, health examinations, blood and urine samples collections and biomarkers lab tests) in the seven "Longevity areas": Laizhou of Shandong Province, Xiayi of Henan Province, Zhongxiang of Hubei Province, Mayang of Hunan Province, Sanshui of Guangdong Province, Yongfu of Guangxi Autonomous Region and Chengmai of Hainan Province. Rudong of Jiangsu Province was included as an additional "in-depth study" site in the CLHLS 6th and 7th waves, conducted in 2012 and 2014 respectively. The eight "in-depth study" sites were selected from the "longevity areas" where the density of centenarians is exceptionally high and the environmental quality is very good, evaluated and officially designated by the experts committee of the Chinese Gerontology Association (http://www.zgcsx.net/content.asp?id=751).

During the in-depth study in the longevity areas in the CLHLS 5th, 6th and 7th waves in 2009, 2012 and 2014, in addition to the face-to-face interviews using the same questionnaires as used in the other study sites of CLHLS, China CDC local network medical doctors conducted physical examinations for the participants. The doctors also collected blood samples and urine samples from centenarians, the oldest-old aged 80-99 and other younger age groups. These data and blood/urine samples are very valuable research resources for interdisciplinary studies on healthy aging.

The on-site work conducted by the local CDC was highly valued and supported by

[1] The face-to-face interviews, health exams and blood/urine samples collections by the China CDC network medical doctors in the In-depth Studies of the Eight Longevity Areas of the CLHLS were jointly supported by the National Natural Science Foundation of China, the NIA/NIH and Duke-NUS Graduate Medical School in Singapore. We thank Dr. Huashuai Chen and Ms. Hanmo Yang very much for their outstanding work for the English translations of this document.

other relevant governmental departments at the county level, including the department of health, the department of civil administration, and the aging committee etc. The field work were successful, and the basic information of the respondents' sex and age distributions of the "in-depth studies" in the 8 longevity areas conducted in 2009, 2012 and 2014 are shown in Table 1.

The comprehensive and longitudinal follow-up survey helps to understand the health condition of the elderly and the impacting factors in a dynamic way. It is of great importance to the healthy aging policy-making, improvement of health intervention programs, promotion of health education, and achievement of health equity among the elderly.

Table 1. Age and sex distributions of the biomarkers datasets collected by the "In-depth studies" in the 8 longevity areas, as part of the CLHLS 5th, 6th and 7th waves

2009				2012				2014				Total			
Age	Male	Female	Total	Age	Male	Female	Total	Age	Male	Female	Total	Age	Male	Female	Total
35-64	272	301	573	35-64	65	20	85	35-64	65	19	84	35-64	402	340	742
65-79	210	127	337	65-79	483	266	749	65-79	467	265	732	65-79	1,160	658	1,818
80-89	165	176	341	80-89	281	293	574	80-89	342	357	699	80-89	788	826	1,614
90-99	116	286	402	90-99	183	292	475	90-99	213	359	572	90-99	512	937	1,449
100+	53	329	382	100+	84	472	556	100+	74	381	455	100+	211	1,182	1,393
Total	816	1219	2035	Total	1096	1343	2439	Total	1161	1381	2542	Total	3,073	3,943	7,016

2. Informed Consent

Before the interviews and health examination started, the well-trained interviewers notified the survey respondents and their family members about that the survey and blood/urine sample collections were approved by the relevant agencies at national level and we will keep all the personal information as strictly confidential. The interviewers read and explain the informed consent letter's contents to the respondents. Only after those who are willing to participate (or a family member of a willing respondent who understand the process of the informed consent) signed the consent form, the interviews, health exam and blood/urine samples collection would start.

3. Physical Examination

For each of the study subjects, a series of physical health examinations were conducted, including measures of blood pressure, heart rate, rhythm, height, weight, waist circumference, abilities of limb activity and picking up books on the ground, number of steps required for rotation in a circle, respiratory function, grip strength, nerve, hearing, chest examination, abdomen examination, and language ability, etc.

4. Collection, Processing, Storage and Testing of Blood and Urine Samples

4.1 Blood and urine samples collection

For all of the voluntary respondents, the blood collection tubes with heparin anticoagulant were used to collect 5ml of venous blood samples.

After centrifuging the venous blood samples, around 2.4 ml of plasma and 2.6 ml of blood cells were collected. 1.0-1.2 ml of plasma were separately absorbed and sub-packaged into two freezing tubes with a white and a blue lid, respectively. The leukocytic cream of the intermediate layer was absorbed, placed into one freezing tube with an orange lid.

The urine samples of all respondents were collected for the urine routine test. The urine samples were placed into 2ml yellow freezing tubes and preserved at a temperature of -20℃.

1. Requirements of blood collectors. The collectors of blood samples must be medical professionals with good experience of blood collection, familiar with using anti-coagulated vacuum blood-collection tube, and are capable for mastering the processing method of blood samples. Blood collectors could come from the local county level Center for Disease Control and Prevention (CDC) or from designated hospitals. The team leader of the survey at county level should train the blood collectors carefully to ensure that they clearly understand the amount, purpose, methods and operations related to blood collection.

2. Contact the study subjects to arrange blood collection. The survey team should make a good arrangement to notify the subjects about the collecting time, places and transportation arrangement, and make preparation for materials needed in blood collection. The bar codes should be pasted lengthwise along frozen tubes.

3. Blood sampling methods. The subjects were transported to places of sample collection via vehicles. The places of blood collection might be the town or community health service centers, local hospitals or the local CDC. The venous blood must be processed as soon as possible once collected.

1) Record the sample collection information. Blood collectors must record whether each subject is fasting or not before blood collection, and whether the venous blood and urine samples were collected, then fill in the form of Blood Collecting, Processing and Recording.

2) 5ml venous blood was collected with anti-coagulated vacuum blood-collection tubes and were put upside down for 10 times to sufficiently mix blood samples with anticoagulant. Then the tubes were placed on the tube racks. Blood samples in the blood-collecting hoses were used for blood routine test. The procedures are as follows: Pull out the blood-collecting needle, cut the blood-collecting hose near the needle, and put the hose downward perpendicularly as soon as possible to drop the blood on a slide. Use a glass capillary to absorb 20ul blood and then add it into blood routine test agent, which contain EDTAK2 anticoagulant.

3) The urine-collecting cups were distributed for collecting the midstream urine of the subjects. Then the samples were stored in low temperature, ready for urine routine test.

4.2. Processing, routine blood test, routine urine test, preservation and transportation of the samples

1. Sample processing. The blood samples should be centrifuged within one hour to separate plasma and blood cell. The blood samples were centrifuged at 3000 rpm under a room temperature of 18-25℃. It was estimated that 2.4 ml of plasma and 2.6 ml of blood cells could be obtained from 5 ml blood. Put plasma into freezing tubes with white or blue lids,1.0-1.2 ml each; the intermediate layers of leukocytic cream were transferred into the orange freezing tubes. The remaining plasma and the bottom layers of erythrocytes were allowed to mix when leukocytes were removed. Codes should be one-to-one correspondence.

2. Blood routine test and urine routine test. Local CDC was responsible for the

blood routine test and urine routine test. If the local CDC did not meet the requirements of laboratories, the county hospital could be entrusted for the tests.

Blood Routine test: The blood routine test was conducted using the pre-dilution method. The necessary items of blood routine test included counts of erythrocyte, leukocytes, blood platelets and hemoglobin, etc.

Urine routine test: The on-site urine specimens should be preserved at a low temperature, restored and determined within 2 hours. The urine routine test included 11 necessary items, i.e. specific gravity, pH, protein and glucose of urine, ketone body, bilirubin, urobilinogen, nitrite, leukocytes, erythrocytes and occult blood.

Quality control should be conducted before sample testing, and the quality control form should be filled in. The codes of survey objects should be correctly filed in during the process of sample testing; the printed results should be copied because the original printing paper is thermal sensitive. Finally, one copy was delivered to China CDC, and the other copy was kept as a backup and served as a basis for informing the results of the survey. Within one week after the survey, the results of blood routine test and urine routine tests would be notified to the subjects.

3. Sample preservation. After the urine routine test was completed, the sample was loaded into a yellow freezing tube of 2 ml and then preserved at a temperature of -20℃.

The codes of freezing tubes and labels of freezing boxes should be checked carefully. Then the separated 2 tubes of plasma, 1 tube of blood cell and 1 tube of urine samples were placed into the corresponding freezing boxes. The freezing tubes should be placed into the freezing box in strict accordance with the order of sample codes.

The Form of Information of Preserving Samples in Freezing Tubes was filled and put in the corresponding freezing box. The box was sealed with adhesive tapes. The label of the freezing box was filled out. The freezing box was preserved at a temperature of -20℃.

4. Sample transportation. After the field survey, samples in the shipping boxes provided by China CDC were shipped to Beijing at a low temperature by the designated persons. Damage and leakage should be avoided. The form of Sample Delivery should be filled out.

After all plasma was transported to Beijing, other indicators, including four testing items of blood lipid (total cholesterol, triglyceride, low density lipoprotein cholesterol and high density lipoprotein cholesterol), blood glucose, uric acid, serum creatinine and urea nitrogen and etc., would be tested.

4.3 Biomarkers used for further test and final incorporation into the database.

1. Blood Routine test: counts of erythrocytes, leukocytes, platelets and hemoglobin.

2. Urine Routine test: specific gravity (SG), pH, protein (PRO), glucose (GLU), ketone (KET), bilirubin (BIL), urobilinogen (UBG), nitrate (NIT), leukocytes (LEU) and occult blood (OBL).

3. Blood biochemistry: total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), fasting blood glucose, uric acid, serum

creatinine and urea nitrogen, Hypersensitive C-reactive protein, vitamin D, glycosylated albumin, superoxide dismutase (SOD) and malondialdehyde (MDA) (Only for part of the subjects due to the limited budget).

4. Microalbuminuria and urinary creatinine (Only for part of the subjects due to the limited budget).

Appendix: Precautions in the Process of Routine Urine and Blood Tests

A1. Blood routine Test

1. Subjects preparation. Many physiological factors can cause changes to the number of blood cells, such as emotional instability, tension and strenuous exercise, etc. Avoiding such factors is beneficial to the accurate results of blood routine tests.

2. Samples collection. Blood should not be drawn from the hand being transfused or the elbow on the same side.

3. Anticoagulation. Blood samples used for blood routine tests must be processed with anticoagulants. EDTA salt (EDTA2Na2 and EDTA2K2) is an anticoagulant with relatively small effects on leukocyte morphology and platelet, and hence very suitable for blood routine tests. The proportion of blood and anticoagulant should be appropriate. The personnel responsible for blood collecting and for blood routine tests should communicate well with each other on deciding the way of adding anticoagulants before blood collection.

4. Transport and storage of specimens. Minimize the time of transportation and storage so that the specimens can be tested as soon as possible, as the metabolism, evaporation and sublimation effects, chemical reactions of blood cells and microbial degradation of blood, etc. could directly affect the quality of specimens during storage. The placing duration of samples will affect the specimen quality, so it's better to complete the tests within 0.5-4 hours.

5. Dealing with testing results. In the process of sample testing, the codes of subjects should be input correctly. The codes of survey objects should be correctly input during the process of sample testing; create two copies of the printed results considering that the original printing paper is thermal sensitive. Finally, deliver one copy to China CDC, and keep the other copy as a backup and a basis for informing the results to the subjects.

6. Requirements of quality control in the laboratory

1) Technical professionals should be trained before the project started.
2) Normal storage, maintenance and proper usage of instruments.
3) Calibration of instruments: in order to ensure the accuracy of the test results, the routine calibration of instruments should be implemented, in addition to the regular maintenance.
4) Reagents must be used correctly and compatibly.
5) Blood samples should be blended well again in the initial way of mixing before they are ready to be tested.
6) The quality control should be carried out and records should be made before the tests.

A2. Urine routine test

1. Collect 5ml of urine.

2. The urine routine test should be completed within 2 hours, or completed within 6 hours if stored at a low temperature.

3. The instrument calibration and quality control check should be conducted before the test.

4. The samples should be transferred to the 5ml freezing tubes after the test.

5. Requirements of quality control in the laboratory.
The urine routine test is a common method to help determine whether a subject has diseases of urinary system, while quality control is a basic and critical component of the urine routine test.

1) Test strips. Use chemical analyzer together with test strips. The test strips should be kept sealed and away from moisture, lights, acid-base, ammonia, hydrochloric acid and other volatile gases and organic enzymes, etc. Hands or other pollutant are prohibited from touching the reaction block. Test strips should be used within the validity period.

2) Urine analyzer. Installation, usage and maintenance should be in strict accordance with the requirements of the manufacturer. The performance of the urine analyzer should be tested using a verification strip. The urine analyzer could not be used once the verification results do not meet the requirement. Urine analyzer should be calibrated before usage and the quality control procedures must be carried out before the tests of each day.