Study on the Detection and Follow-up of Cardiovascular Disease and Risk Factors in the Southern Cone of Latin America

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Study summary

This is an observational study that was designed with the goal of investigating the prevalence and distribution of risk factors, as well as the incidence of cardiovascular and chronic obstructive pulmonary disease (COPD) in the general population. To our knowledge, this is the first demographic study that has been developed with this methodology in Latin America. Its principal strengths reside in the size and representiveness of the sample (multistage random sampling), in the length of follow-up, and in the selected variables, which will be discussed.

This study will include a probabilistic sample of 8,000 non-institutionalized adult men and women between the ages of 35 and 74 years old (2000 per site) coming from Bariloche and Marcos Paz (Argentina), Temuco (Chile) and Canelones (Uruguay). In the first stage of the study, a specially trained interviewer will conduct a household survey to uncover information about lifestyle (diet, physical activity, quality of life, smoking, alcohol consumption), socio-demographic data (age, sex, occupation, conditions of life), access to and utilization of health services (consultations, laboratory analysis, hospitalizations, etc.), risk factors and illnesses (high blood pressure, diabetes mellitus, and cardiovascular problems, among others). Once the questionnaire is finished, the interviewer will invite the participant to attend a health center to complete the evaluations (physical exam, blood test, ECG and spirometry).

Follow-up monitoring will be conducted annually via telephone, and, after two years from the survey and baseline measurements, measuring the same factors at from the initial clinical visit, except for the spirometry, which only will be repeated at the end of follow-up period. The duration of follow-up for the cohort of this study will be four years in total.

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Background and Rationale

Global burden of cardiovascular disease in developing countries:
Non-infectious chronic diseases are increasing throughout the world, and in this context, cardiovascular diseases (CVD) are the cause of almost 18 million deaths each year, representing 11% of the global burden of disease. Furthermore, in middle-income countries and transitioning economies, CVD represents the fastest growing burden of disease. In fact, CVD, cancer, chronic respiratory diseases and diabetes account for around 60% of these deaths, of which 80% occur in low and middle-income countries. It has been projected that in 2015, 41 million people will die of chronic disease if effective concerted actions are not put into place for their prevention and treatment. What is more, in low and middle-income countries, almost half of these deaths will occur in people younger than 70 years old, compared with only 27% of corresponding age groups in high-income countries. Due to this, the World Health Organization (WHO) recently commented on the significance of chronic diseases as a global health problem and called upon governments and the entire international community to promote effective actions to reduce the mortality from these causes[i]. The global goal is to reduce the mortality rate from chronic diseases by an additional 2% each year, which would mean avoiding 36 million deaths between 2005 and 2015[ii]. Furthermore, comparing the GDP of each country (as recommended by the WHO) shows that these countries will spare almost 10% of the expected loss of income in the long term[iii]. Cardiovascular disease is the principal cause of death, responsible for 30% of all deaths in the world, as well as the loss of disability adjusted life years. Although age-adjusted rates for cardiovascular mortality have diminished in developed countries, rates have increased in low and middle-income countries reaching 80% of the global burden of disease[iv].

Burden of cardiovascular disease in Latin America and the Southern Cone:
It is estimated that from 1990 until 2020, death from ischemic cardiomyopathy and CVD will increase by approximately 145% in Latin America (for both men and women), compared with an increase of 28% for women and an increase of 50% for men in developed countries during the same period[v].
The strategies to manage cardiovascular diseases have largely been developed for high-income countries, but these strategies cannot be implemented in the majority of developing countries. However, there is strong evidence of a possibility of reducing cardiovascular mortality by 50% by modifying only three risk factors: smoking, arterial hypertension and high cholesterol. Moreover, at least 75% of cardiovascular disease can be explained by other more proximal risk factors such as unhealthy diet, low physical activity and tobacco[vi]. The majority of these conditions are avoidable, and there is evidence about the efficacy of

interventions directed at reducing the burden of disease through controlling risk factors. In the 2002 World Health Report (WHO), 26 risk factors were evaluated and classified according to their importance. The principal risk factors that were identified for the majority of Latin American countries were arterial hypertension, elevated body mass index, alcohol, and smoking[vii]. Furthermore, the majority of cardiovascular risk factors in the Southern Cone could be explained by tobacco consumption, abnormal lipids and arterial hypertension. This was demonstrated by the INTERHEART study that included 3125 cases and controls from different Latin American countries[viii].

The available data about cardiovascular risk factors in Argentina, Chile and Uruguay come predominantly from cross-sectional studies that are principally based on self-reporting or studies conducted with small convenience samples, which do not give reliable estimates. Although these three countries have conducted surveys about risk factors at the national level (Argentina in 2005[ix], Chile in 2004[x], and Uruguay in 2006[xi]), these studies, although useful in principle for surveillance, have significant limitations. In Argentina, all of the risk factors were evaluated by self-reporting, not including evaluation of cardiovascular diseases. In Uruguay, two thirds of the participants did not undergo physical and/or biomedical measurements. In Chile, although they measured some risk factors, the cardiovascular pathologies were self-communicated. None of these studies utilized prospective follow-up. These methodological differences make it impossible to compare the data. A recent study involved seven Latin American cities (including Buenos Aires and Santiago in Chile), has distributed data on prevalence of risk factors, but again, it is based on small samples and does not include participants from Uruguay[xii]. Some retrospective studies, such as INTERHEART(8) in Latin America, have given some estimations about the effects of cardiovascular risk factors. To date, no longitudinal study has been conducted with entire populations or regions, which could offer an estimate of the impact of these risk factors on the incidence of cardiovascular events. Therefore, the lack of studies with follow-up impedes our countries from understanding local needs and the data necessary to determine the burden of cardiovascular disease, as well as the stratification of risk factors and the identification of management strategies on a population level. As a consequence, due to the limited and imprecise evidence available, a strong need exists to develop a population cohort study in order to confront the gaps in knowledge that still exist and the formation of policies regarding the impact of cardiovascular disease in our countries. This study will generate exact estimates of prevalence, distribution, and secular tendency of CVD and its risk factors in this region. These data will give way to new knowledge about avoidable risk factors for the development of CVD. This information will contribute to the improvement of public health strategies based on the application of primary care interventions, thus helping to improve cardiovascular health in Latin America.

Chronic obstructive pulmonary disease

The burden of COPD is increasing, in particular in economically developing countries, largely because of tobacco use and exposure to second-hand smoke, but also because of poor indoor and outdoor air quality. COPD is the fourth most common cause of death worldwide and is predicted to overtake lower respiratory tract diseases to become third after ischemic heart and cerebrovascular diseases by 2030. Argentina, Uruguay and Chile share similar socio-demographic characteristics. Vital statistics data from the three countries show that the epidemiological transition has already occurred in this region where more than 60% of total deaths can be attributed to chronic diseases. Moreover, limited and preliminary data on chronic disease risk factors also indicate that the levels of risk factors are high and increasing locally. It is also known that forced expiratory volume in 1 second (FEV_1) declines normally with aging by approximately 30 ml/yr. In susceptible smokers though, a greater decline of about 60 ml/yr has been reported, resulting in a higher risk for development of COPD. Furthermore, FEV_1 is central to the definition and severity classification of COPD and, correlates with survival and quality of life as well. There are no longitudinal data related to the incidence of chronic diseases and risk factors in the region of Latin America. This prospective cohort study population in the region will provide valuable information related to the incidence and risk factors for the development of COPD and decreased lung function, quality of life and healthcare resource utilization associated with this condition.
This study will assess the prevalence, incidence, and the role of risk factors associated with the development of COPD in the Southern Cone of Latin America.

1. Methodology

1.1 Study Design

This is an observational prospective cohort study with 4 years of follow-up. The study will consist of two phases. In the first phase, baseline data will be collected regarding exposure to risk factors and prevalence of cardiovascular and pulmonary disease. In the second phase, follow-up data will be obtained on the incidence rate of cardiovascular and pulmonary disease and the association between exposure and the event.

2. Study Population

2.1 Population Characteristics

This study will include a probabilistic sample of 8000 non-institutionalized adult men and women between the ages of 35 and 74 years old (2000 per site) coming from Bariloche and Marcos Paz (Argentina), Temuco (Chile) and Canelones (Uruguay).

The characteristics of the selected locations are the following:
a) City of Bariloche: Located in the north of Patagonia, the city and surrounding areas have 120,000 inhabitants. There is a strong connection between researchers and local health authorities. Two years ago, the population participated in a small-scale project, which got an excellent response. The site is home to multiple research projects, including national registries and clinical protocols.

b) City of Marcos Paz: Located in the province of Buenos Aires (60 kilometers away from Buenos Aires), the city and surrounding areas have 50,000 inhabitants. The local health authorities and provincial authorities have demonstrated a strong commitment to the proposed research project. The city is included in a health municipalities program that is being implemented all around the country. Numerous primary care programs have been organized in the community through health promoters, some of which have focused on lifestyles and prevention of cardiovascular risk factors.

c) City of Temuco: This city is located in the south of Chile and has 240,000 inhabitants (including the surrounding areas). The main local university is the Universidad de la Frontera (UFRO), which is very committed to the project. The principal researcher and other researchers conducted various community and intervention programs in Temuco about cardiovascular disease and its risk factors.

d) Department of Canelones: Adjacent to Montevideo, the capital of Uruguay, Canelones is the department with the second largest population in the country. The study will be conducted in Pando and Barros Blancos, which were selected

due to demographic characteristics and the presence of the university headquarters which will serve as a referral institution for this project. The leadership of the project in Uruguay will be in the charge of the Faculty of Medicine (Unversidad de la República), through coordination with three departments: Family and Community Medicine, Preventive and Social Medicine and Cardiology.

The following three characteristics were taken into account for the selection of these locations:
1. Population: the population characteristics of these cities follows the country population pyramid model. Added to this, the four selected cities demonstrated a stable population in the last ten years, with a migration rate less than 10%.
2. Infrastructure: Necessary in order to conduct quality research (adequate data collection and follow-up).
3. Local commitment: Local and institutional authorities are strongly committed to the study.

2.2 Inclusion and exclusion criteria

a) Inclusion Criteria

- Permanent resident of the location at least 6 months out of the year
- Men and women between the ages of 35 and 74 years old
- Offer written consent to participate

b) Exclusion Criteria

- Expressed intention to relocate within the next two years
- Not in condition to respond autonomously to the questionnaire (cognitive deterioration, language problems)

2.3 Sampling methodology

A randomized multi-stage stratified cluster sampling strategy will be conducted, with the goal of selected a representative sample of the general population between 35 and 74 years of age in Marcos Paz Bariloche, Canleones, and Temuco. The sampling will consist of three stages. The first stage will consist of randomly sampling the census radii of each location, which are stratified by socio-economic level. In the second stage, the selection will be conducted at the level of households contained in each radius. In the third stage, an individual between the ages of 35 and 74 will be selected. The final sample will be stratified by gender with 50% women and 50% men with an age distribution according to the following categories: 35-44, 45-54, 55-64, and 65-74 years of age.

2.4 Recruitment Plan

The households selected by the sampling procedure will be invited to participate through a letter from CESCAS and the local institution, endorsed by local authorities, which explains the objectives and characteristics of the study. The first contact with the participant will be made by an interviewer, who will visit the selected household and will then proceed to randomize household members who meet the study's inclusion criteria. When possible, the survey will be conducted at this time, and when not, the interviewer and participant will agree upon an appointment for the survey. During the household visit, the interviewer will collect information through a questionnaire and will arrange the visit to the health center well the physical measurements, an electrocardiogram and blood sample will be obtained.

In order to increase participation during the recruitment phase, the following measures will be taken:

- The health centers will be located in the same location as the selected participant.
- For those participants who cannot transport themselves to the health centers, adequate transportation will be provided or a control clinic will be coordinated in the home.
- Diverse schedules will be offered for appointments with the goal of adapting to the needs of community members.

3. Measurements

3.1 Baseline Measurements

Baseline measurements will be conducted in two stages (household and health center), in which the following information will be collected:

Data obtained through the survey (see Appendix A)

Socio-demographic data: Age, gender, education, race, occupation, family income and satisfaction of basic needs.

Resource utilization: Type of health coverage and degree of health service utilization.

Smoking:

-Current smoker: a person who smokes at least one cigarette per day at the time of survey

-Ex-smoker: a person who, having been a smoker, does not smoke at the time of survey

-Passive smoker: involuntarily breathes air that is contaminated by tobacco smoke

Other types of tobacco use (pipe, cigar), age started smoking, years of exposure and daily quantity of cigarettes will also be determined

Alcohol: Level of consumption (daily quantity, frequency, type of alcoholic beverage)
Physical Activity: Type of activity, frequency and intensity, along with physical activity done in free time, activity related to type of work and comparison of current exercise level with previous levels. For this the International Physical Activity Questionnaire (IPAQ) will be used, which has been approved in Argentina and is used in the National Cardiovascular Risk Factor Survey.
Eating habits: Types of foods, quantity and frequency
Self-report of:
a) Coronary disease, cerebrovascular accident, peripheral vascular disease, cardiac insufficiency, chronic obstructive pulmonary disease (COPD), tuberculosis, arterial hypertension, dyslipidemia, diabetes mellitus and chronic kidney disease
b) Pharmacologic and non-pharmacologic treatment received for arterial hypertension, dyslipidemia, COPD, diabetes, or cardiovascular disease
c) Family history of myocardial infarction, cerebrovascular accident, arterial hypertension, hypercholesterolemia and premature diabetes
d) Other chronic diseases (cancer)
e) History of hospital admissions due to pulmonary problems in childhood
f) Indoor air pollution: heating and cooking with biomass in poorly vented dwellings

Quality of life: The evaluation of the stat of global health and quality of life will be conducted through SF-12 and EQ5D questionnaires, which have both been tested in the Argentinean population.

Physical and laboratory measurements
a) Arterial tension: Trained and certified observers will measure arterial tension during the health center visit. Given this, procedure will be conducted following recommendations from the American Heart Association (AHA—see references). The study requires that, before taking blood pressure, the participant remain seated and at rest for 5 minutes and is not permitted to ingest tea, mate or coffee, to smoke, or to exercise in the 30 minutes prior to the test. A standardized sphygmomanometer (mercury or aneroid) with an adequate cuff size will be used. The cuff will be placed on the right arm of the participant, inflated to 10 mmHg, the cuff must then be insufflated until reaching a pressure of 30 mmHg above the level at which the radial pulse stops palpitating. Three measurements will be obtained, with two-minute intervals between them. The first measurement will be thrown out, and an average will be taken of the second two. In order to determine the systolic arterial pressure, the first and fifth Korotkoff sound will be considered, respectively.

b) Anthropometrical measurements:

Trained observers will measure:
Body mass: The measurement will be conducted with undergarments and without shoes. The weight will be recorded in kilograms to one decimal place, using standing scales supported on a steady surface.
Height: This measurement will be recorded without shoes, in centimeters to one decimal place, on a Frankfort plane positioned at a 90-degree angle against metallic metric tape mounted on the wall.
Abdominal circumference: This measurement will employ a centimeter-based type of anthropometric tape. The abdominal circumference will be recorded in centimeters to one decimal place, on a horizontal plane at 1 cm above the belly button that generally coincides with the narrowest circumference and has the advantage of being easily reproducible.
Hip circumference: This will be measured at the major trochanters, which generally coincide with the symphysis pubis. The subject must be standing, with relaxed buttocks and feet together. The measurement will be recorded in centimeters to one decimal.

The following formulas will be calculated:
BMI: weight in Kgs/height^2 (meters)
Waist-hip Ratio (WHR)=Waist circumference (in centimeters)
Hip circumference (in centimeters)

c) Biochemical Measurements
-Measurement of serum lipids: This measurement will be taken through venipuncture, extracting 8 mL of blood. The participant will have to fast for at least twelve hours for the measurement of lipids in the blood. The samples will be processed and temporarily stored in the laboratory at the extraction site in order to be sent later to the central laboratory (Central Laboratory of the Hospital Italiano in Buenos Aires), where they will be stored and analyzed.

The levels of total cholesterol, HDL cholesterol, and triglycerides will be determined using enzymes, utilizing commercially available reagents. LDL cholesterol levels will be calculated utilizing the Friedewald equation for the participants who have <400 mg/dL. According to this equation, total LDL cholesterol is equal to: total cholesterol – HDL cholesterol – triglycerides/5

CHOLESTEROL Cholesterol reagents are used to measure the concentration of cholesterol using a kinetic end-point method in a fixed interval of time. 1, 2, 3 In the reaction, esterase cholesterol (EC) hydrolyzes the cholesterol esters into free cholesterol and fatty acids. The free cholesterol oxidizes into cholestan-3-one and hydrogen peroxide through cholesterol oxidase. The peroxidase catalyzes the reaction of the hydrogen peroxide with 4-aminoantipyridine (4-AAP) and

phenol to produce a colored quinoneimine product. The SYNCHRON LX System automatically provides the correct volumes for samples and reagents in a cuvette. The proportion utilized is 1 part sample to 100 parts reagent. The system controls the change in absorbency to 520 nanometers. This change in absorbency is directly proportional to cholesterol concentration in the sample and is used by the SYNCHRON LX System to calculate and express cholesterol concentration.

HDL CHOLESTEROL This direct method for HDL cholesterol is a homogenous test that does not require either centrifugation or any previous offline treatment. The method depends on a special detergent that solubilizes only HDL lipoproteins and frees HDLS cholesterol. The detergent reacts with cholesterol esterase and the choleserol oxidase in the presence of chromogens to produce a colored product. The same detergent also inhabits the reaction of the cholesterol enzymes with the LDL, VLDL and chylomicron lipoproteins through adsorption to their surfaces. A polyanion contained in the reagent improves the selectivity of the test for HDL cholesterol, establishing bonds with LDL, VLDL and chylomicrons.

The HDL Cholesterol Reagent is used to measure cholesterol concentration through a timed end-point method. 1,2 The SYNCHRON LX System automatically dispenses the appropriate volumes of the sample of HDL cholesterol and the reagent in a cuvette. The proportion utilized is 1 part sample to 93 parts reagent. The system controls the change in absorbency to 560 nanometers. This change in absorbency is directly proportional to cholesterol concentration in the sample and is used by the SYNCHRON LX System to calculate and express the concentration of HDL cholesterol.

TRIGLYCERIDES The GPO Triglycerides Reagent is utilized to measure the triglyceride concentration through an end-point method, in a fixed interval of time. 1,2 The triglycerides in the sample hydrolyze into glycerol and free fatty acids by virtue of lipase activity. A sequence of three coupled enzymatic steps that utilize glycerol kinase (GK), glycophosphate oxidase (GPO) and horseradish peroxidase (HPO) provokes the oxidative coupling of the 3.5-dichloride-2-hydroxybenzene sulfonic acid (DHBS) with 4-aminoantipyrine to form a red colored quinoneimine. The SYNCHRON LX System automatically dispenses the appropriate volumes of the sample l and the reagent in a cuvette. The proportion utilized is 1 part sample to 100 parts reagent. The system controls the change in absorbency to 520 nanometers. This change in absorbency is directly proportional to the concentration of triglycerides in the sample and is used by the SYNCHRON LX System to calculate and express the concentration of triglycerides.

- Measurement of plasma glucose: In order to analyze glucose, it is necessary to keep the extracted sample in vacuum tubes containing sodium fluoride. The measurement will be conducted with a modified hexokinase enzymatic method. The SYNCHRON LX System determines the concentration of glucose through an oxygen kinetic method that utilizes a Beckman oxygen electrode. 1,2 A precise volume of sample (10 µL) is injected into a cup of reagent that contains a glucose oxidase solution. The proportion that utilized is 1 part sample to 76 parts reagent. The maximum speed of oxygen consumption is directly proportional to the concentration of glucose in the sample. 3

- Measurement of serum creatinine: This measurement will be conducted through a standardized (IDMS-TRACEABLE) calorimetric method (JAFFE) in mg/dL.

d) **Electrocardiogram**
The study will employ an electrocardiogram with 12 derivations that is standardized at 25 mm/sec and at 1 mV of amplitude.

e) Spirometry:
Spirometry is the most frequently used pulmonary function test and enables health professionals to make an objective measurement of airflow obstruction and assess the degree to which it is reversible. As a diagnostic test for COPD, spirometry is a reliable, simple, non-invasive, safe, and inexpensive procedure.. Participants will do up to 8 forced expiratory maneuvers to obtain 3 American Thoracic Society (ATS) acceptable maneuvers, with forced vital capacity (FVC) and forced expiratory volume in the first second (FEV_1) reproducible within 150 mL, according to the ATS recommendations. Then, albuterol 200 µg will be administered by inhalation and the test will be repeated 15 min later.
Participants with any of the following conditions will be excluded from the performance of spirometry:
- Thoracic or abdominal surgery within the last 3 months
- Myocardial infarction within the last 3 months
- Eye surgery or retinal detachment within the last 3 months
- Active tuberculosis
- Pregnancy

3.2 Strategies to reduce subject loss at the baseline stage
To minimize absenteeism at the clinical consultations, the following measures will be taken:

- During the home interview, the interviewer will solicit detailed contact information from the participant (land and cellular phone lines, email

and postal addresses) and the name of at least one family member (parents, partner, siblings, children), friend and/or neighbor who can locate the participant.

- Efforts will be made to contact individuals who do not show up to the programmed baseline clinical visit in order for them to complete it either in the health center or in the home, if necessary.

3.3 Follow-up measures

Follow-up data will be gathered as follows:

a) Telephone interview: The participants and/or someone close to the participant will be contacted annually to gather following information:

- Updated contact information
- Medical background regarding risk factors and occurrence of events during the follow-up period.
- Socio-demographic, habit and lifestyle (smoking, alcohol, physical activity, diet, quality of life, and medical background) information
- Health service utilization (Appendix, follow-up survey)

b) Clinical visit: Two years from the initial visit participants will be brought to undergo a visit to the clinic in their assigned health center. During the visit the following activities will be conducted:

- Physical measurements: arterial tension, height, weight, waist and hip circumference
- Electrocardiogram
- Laboratory measurements: lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides), glucose and plasma creatinine

After four years of the initial visit, spirometry will be repeated to assess changes in lung function over time and the development of COPD on the basis of established criteria.

c) Information regarding clinical events during the follow-up period

3.4 Plan for reducing loss to follow-up

To reduce non-attendance at clinical consultations, the following measures will be taken:

- For those who have moved to another city, telephone, mail or e-mail will be used to obtain the information
- Efforts will be made to contact patients who do not show up to the programmed clinical visit so they can complete it either in the health center or in the home, if necessary.
- An informative note about progress and achievements of the study will be sent periodically to the researchers at each site. An informative

bulletin and laboratory results for the participants enrolled at each site will also be sent, and there will be a publicity campaign on local radio stations and in local newspapers. Community participation will be stimulated through health-promoting activities. The leaders of each community will be brought together to participate and lend support to our study.

4. Definition and classification of events

4.1.1 Death

The event of death will be discovered through a telephone interview, absence to the clinical visit, or notification from someone close to the participant. This will generate an alert in the system that will inform the principal research and the CESCAS center coordinator.

In the case of hospital death, the hospital must concur in order to obtain copies of the source documents (clinical history, autopsy, studies conducted) and information about possible causes of death that appear on the death certificate.

In the case of non-hospital deaths (external clinics, home, deaths occurring during transportation to the hospital or those admitted without vital signs), the coordinator will attempt to contact the doctor in charge in order to obtain the information required for the study. When it is not possible to obtain the information, family members or those close to the decease will be contacted to determine the date, time and cause of death. Later, the form for events will be completed with the information available and copies will be sent to the CESCAS event adjudication committee.

***CLASSIFICATION according to CAUSE of DEATH**

A. Death by atherosclerotic coronary disease (ACD)

A1. Death by AMI

Definitive diagnosis of infarction at 4 weeks from the death.

Note: Deaths from one underlying non-coronary cause when the terminal event is a myocardial infarction, the death is attributed to the underlying cause, not the MI.

A2. Death defined by ACD

Must meet ALL of the following criteria:

1. Lack of sufficient tests to diagnose "definitive AMI" (page 20 of protocol)
2. Absence of "non-atherosclerotic" or "non-coronary atherosclerotic" as a potential cause of death
3. Presence of one or both of the following results:

a) History of precordial pain within 72 hours before the death

b) History of chronic ischemic cardiopathy such as defined or possible MI, coronary insufficiency or chest angina, in the absence of valvular disease or other non-ischemic cardiomyopathy

A3. Possible death by ACD
Must meet ALL of the following three criteria:
1. Lack of sufficient tests to diagnose "Death by AMI" or "Definitive death by ACD"
2. Absence of non-atherosclerotic or non-coronary atherosclerotic disease as a potential cause of death
3. Death certificate with underlying cause combatable with atherosclerotic coronary disease

B) Death by cerebrovascular accident (CVA)
C) Death by other atherosclerotic disease (non-coronary/non-CVA)
Aortic dissections or aneurisms of all varieties are found in this section.
D) Death by non-atherosclerotic cardiac disease
Valvular diseases and mycardiopathies with the exception of ischemic myocardiopathies will belong in this category
E) Death by non-cardiac disease or unknown cause

***SUB-CLASSIFICATIONS of DEATH**
Categories A,C and D will be sub-classified by:
- Time
Time elapsed between the initiation of symptoms of the causal event until death. The categories will be divided in: < 1 hour; 1 to 24 hours; unknown.
- Mechanism
Discriminate the most important mechanism/s causing the death (more than one can apply):

a. Arrhythmic death: Death secondary to a cardiac arrhythmia; death is not a consequence of a final event from a state of low output or shock

b. Death by cardiac insufficiency: Death secondary to low output or shock

c. Revascularization proceedings: Death during an angioplasty or coronary bypass

d. Hemorrhage: Death secondary to thrombolytic/anticoagulant/antiaplatelet therapy

e. Unknown or uncertain: Does not qualify for any of the categories mentioned above

4.1.2 Acute myocardial infarction
The following criteria will be considered for the diagnosis of acute myocardial infarction:

a) Precordial pain

Any pain that is squeezing, burning, or discomfort in the precordium, epigastrium, neck, back, or arm that exceeds 20 minutes is considered positive criteria. Anginal equivalents or atypical pain that is interpreted as ischemic also qualify. The final interpretation of the etiology of the pain will be based on the clinical judgment of the doctor who is treating the patient.

b) Enzymatic criteria (positive-doubtful-normal)

Algorithm for enzyme classification

	Without cardiac surgery PTCA, muscular trauma or hemolysis	**With cardiac surgery PTCA, muscular trauma or hemolysis**
CK-MB ≥ then MLN in two successive samples ó CK-MB ≥ 2x MLN	Positive	Normal
CK-MB ≥ 10% CK Total, if there is no MLN*	Positive	Normal
CK Total ≥ 2x MLN y LDH ≥ 2x MLN	Positive	Normal
LDH1: LDH2 > 1	Positive	Normal
LDH1 ≥ 2x LMN if LDH2 is not available	Positive	Normal
CK Total ≥ 2x MLN or LDH ≥ 2x MLN	Doubtful	Normal
Total CK > normal y < 2x MLN and LDH > normal < 2x MLN	Doubtful	Normal
CK-MB ≥5% y < 9% de CK Total* or CK-MB slightly elevated	Doubtful	Normal
CK-MB > normal < 2x MLN	Doubtful	Normal
LDH1> normal < 2x MLN	Doubtful	Normal
Data that do not qualify with at least two of the descriptors above	Incomplete	Incomplete
Other results	Normal	Normal

MLN=maximum normal limit

Total CK and MB must be reported in the same units for this criterion

c) Electrocardiographic criteria

The following will be considered positive electrocardiographic criteria:

1) Presence of new Q waves in at least 2 derivations in the same vascular territory, whose depth are at least 25% of the amplitude of the R wave and are 0.03 sec.

2) ST segment elevation ≥ 1 mm, in at least 2 segments belonging to the same coronary territory
3) ST segment depression ≥ 1 mm, in at least 2 segments belonging to the same coronary territory
4) T wave inversion of at least 2 segments belonging to the same coronary territory
5) New Q waves in at least two derivations in the same vascular territory that do not meet criterion 1

Classification of infarction based on ECG, Enzymes and precordial pain

Cardiac Pain Present **Cardiac Enzymes**

Electrocardiographic pattern	**Abnormal**	**Doubtful**	**Incomplete**	**Normal**
New higher Q wave [1]	Definitive AMI	Definitive AMI	Definitive AMI	Definitive AMI
ST elevation[2] with or without Q wave or Left bundle branch block (LBBB)	Definitive AMI	Probable AMI	Probable AMI	No AMI
ST-T depresión or inversion of T wave only[3] or Evolution of lower Q wave only[4]	Definitive AMI	Probable AMI	No AMI	No AMI
Only one ECG with higher Q wave or Only one ECG with LBBB	Definitive AMI	Probable AMI	No AMI	No AMI
Other ECG, absent, not classifiable	Probable AMI	No AMI	No AMI	No AMI

Cardiac pain absent **Cardiac enzymes**

Electrocardiographic Pattern	**Abnormal**	**Doubtful**	**Incomplete**	**Normal**
New higher Q wave [1]	Definitive AMI	Definitive AMI	Definitive AMI	Definitive AMI
ST elevation[2] with or without Q wave or Left bundle branch block (LBBB)	Definitive AMI	Probable AMI	No AMI	No AMI
ST-T depresión or inversion of T wave only[3] or Evolution of lower Q wave only[4]	Probable AMI	No AMI	No AMI	No AMI
Only one ECG with higher Q wave or Only one ECG with LBBB	Probable AMI	No AMI	No AMI	No AMI
Other ECG, absent, not classifiable	Probable AMI	No AMI	No AMI	No AMI

4.1.3 Angina

Angina is a symptom that can manifest itself as a squeezing or burning pain or discomfort located in the precordium, epigastrium, neck or back. It sometimes can manifest itself in an atypical form such as dyspnea or discomfort with exercise. Pain generally lasts less than 20 minutes.

A) **Diagnosis of "Angina"** will be determined exclusively by the clinical criteria of the physician, and the following are required to qualify it as such:

- Medical diagnosis of "Angina" where the source document clearly describes the diagnosis
- Absence of "non-cardiac' cause as a differential diagnosis of the symptoms
- Having been treated with anti-anginal medications for the present symptom (beta blockers, nitrates/nitrites, calcium channel blockers)

B) Other criteria will also be considered:

b.1 Electrocardiogram

ST segment rectification, depression or elevation ≥ 1 mm in at least 2 derivations of the same vascular territory or change in T wave shown in an ECG that were not present in previous or later ECGs along with precordial pain.

b.2 Cardiac stress test with imaging (eco-stress, Spect)

It will be considered positive criteria when a cardiac stress test detects any grade of myocardial ischemia.

b.3 Cardiac stress test without imagine (ergometry)

ST segment rectification, depression or elevation ≥ 1 mm associated with 1 anginous precordial pain.

b.4 Cinecoronarography

Obstructions of ≥70% of any coronary artery are considered positive criteria.

b.5 Revascularization

History of angioplasty or revascularization surgery are considered positive criteria.

In general, to control for the criteria for angina, the researcher will collect information from a clinical history and from the original study reports. In the case of not being able to access these documents, the researcher will use the summary from the hospital discharge (epicrisis).

Judging an event as **definitive Angina** requires:

- A medical diagnosis of angina (A) + at least one positive (B) criterion

Judging an event as **probable Angina** requires:

- Medical diagnosis of Angina

Judging an event as **No Angina:**

- Does not meet any of the criteria described above

It is important to note that in the case of infarction, the event of angina should not be judged as associated with the event of myocardial infarction.
There is not a special section for unstable angina, which will qualify as angina.

4.1.4 Cardiac insufficiency
Congestive heart failure is defined as a combination of symptoms (such as difficulty breathing, fatigue, orthopnea, paroxysmal nocturnal dyspnea) associated with physical signs (such as edema, rales, tachycardia, gallop rhythm) that are produced when cardiac output is not sufficient for metabolic necessities, despite adequate pressure in the filling of the left ventricle.
Judging an event as **cardiac insufficiency** requires:

A) Clinical diagnosis of cardiac insufficiency by the physician in charge
and
B) Being under medical treatment for congestive heart failure
(ie diuretics, vasodilators, beta blockers, digital, ACEI)

It will be considered very important (although not obligatory) to obtain information regarding:
-The presence of cardiomegaly and pulmonary edema in a chest x-ray
-Evidence of ventricular dilation or contractility problems that are either continuous or segmented, along with diminishment in systolic function, seen either by ECG or contrast ventriculography.
-Determine ventricular function seen by means of ejection fraction (one data point or the range of data that are found on the registries). In the case it cannot be counted by this mean the categorical value will be registered (normal, light, moderate, or severely diminished).
-In the case of valvular heart disease (stenosis of aortic and or mitral insufficiency) they will be classified as light, moderate, or severe on the basis of echocardiogram or diagnostic imaging studies. All that is defined as significant will be classified as severe. All that is defined as trivial will be classified as light.
-In the event there is a discrepancy between the numeric results of the echo and the dictation of the reporter, the dictation will be considered the final decision.
-In the case the echo is not available the data from a recent echo (no greater than 3 months before or after) can be used as a data source. In the case of more than one echo during the hospitalization, the echo with the greatest grade of ventricular deterioration (the lowest EF and or values with greatest deterioration of vavular heart disease)
In the case of no available echo, the data can be obtained from a cardiac catheterization or other image study can be used in its place.

If it is available from various forms, like cardiac catheterization and an echo, the echo is the first choice of this information.
-When information from an echocardiograph Doppler is relied upon (exclusively), information from the ventricular diastolic phase should be substituted. The same will be classified as: absent or light diastolic dysfunction, moderate or severe (independent of the type of dysfunction).

In general to control for the criteria of cardiac insufficiency, the investigator will collect the history of the clinical visit reports from the studies completed. In the case of no access to those studies the hospital discharge(epicrisis) will be used.

4.1.5. Cebrovascular events
All potential cerebrovascular events will be classified as: cerbrovascular accident (CVA), transient ischemic attack (TIA), or "No cebrovascular event". All events classified as CVA will be classified according to type: hemorrhagic (subarachnoid, intraparenchymatous or other hemorrhage) cerebral infarct and unknown. The criteria for TIA, cebrovascular accident and the type of cebrovascular accident will be proportioned on continuation.

A) Transient ischemic attack (TIA)

To classify an event as a TIA the following criteria in totality must be met:

- One or more neurological deficits with duration > 30 seconds.
- Complete clinical resolution within 24 hours
- Maximum deficit with a duration < then 5 minutes.
- Absence of significant lesions in images (CT/MRI)*

or
If there is no image, all of the above as described plus:

- Absence of other causes like clonic tremors, uneven eye tracking, cephalalgia, nausea, vomiting, scotoma, prolonged Jacksoniana gait, traumatic

B) cerbrovascular accident (CVA)
To classify an evet as CVA the following criteria in totality must be met:

- Rapid instalation of neurological focal, cafaleans or meningism deficits

- Significant lesions in images *
- Absence of secondary causes like cerebral trauma, tumor, infection (for example encephalitis or meningitis) o other non-vascular cause.
- Duration of > 24 hrs. or death related to the event.

*Signifigant lesion on the brain image: image that is considered compatible with the signs and symptoms, independently of the time between the onset of symptoms and the cerebral images(that is, less then or greater than 24 hrs), independently of the type of CVA (with or without hemorrhage), and independent of the technique used(computed tomography) or magnetic resonance [MRI])

Subtypes of CVA

b.1) Subarachnoid hemorrhage (SAH)

For the diagnosis of SAH the following criteria will be considered:

A) Headache, meningism, coma (rare focal neurlogical deficit).
B) CT/MRI with blood in the subarachnoid space (basal cistern or convexness) or isolated intraventricular hemorrhage
C) Presence of blood or xanochromatic color in the cerebrospinal fluid (CSF) in context of a non-traumatic event
D) Surgery or autopsy with subarachnoid hemorrhage.

To classify the event of SAH it is required that:

- Criteria A+B

or

- If no images: Criteria A, without defined sign of focus + C

or

- Criteria D

b.2) Intraparenchymal hemorrhage (IPH)

For the diagnosis of IPH the following criteria will be considered:

A) Focal neurological deficit with or without comatose state
B) Image (CAT/MRI) compatible with dense intraparenchymal hemorrhage
C) Presence of blood or xanochromatic color in the cerebrospinal fluid (CSF) in context of a non-traumatic event
D) Surgery or autopsy with intraparenchymal hemorrhage

To classify an IPH event it is required that:

- Criteria A+B

or

- There is no image to rely on: Criteria A+C

or

- Criteria D

b.3 Other Hemorrhages (OH)

For the diagnosis of IPH the following criteria will be considered:

A) There is lacking data to classify the hemorrhage as a **s**ubarachnoid or intraparencnymal
B) Images with blood in the cerebral parenchyma, the subarachnoid space or in both
C) Presence of blood or xanochromatic color in the cerebrospinal fluid (CSF) in context of a non-traumatic event
D) Surgery or autopsy with Intraparenchymal, subarachnoid or both types of hemorrhages

To classify an event as OH the following must be met:

- Criteria A+B

or

- If there is no image to rely on: Criteria A+C

or

- Criteria D

b.4) Acute cerebral infarct (ACI)

For the diagnosis of an ACI the following criteria will be considered:

A) The criteria for an SAH, IPH or OH are not fulfilled
B) Focal neurological deficit with or without comatose state.
C) Image consistent with ACI (speckled or focused hypodense in locations compatible with clinical presentation, no bleeding or hemorrhage that can be interpreted as a hemorrhagic transformation from a infarct).
D) Surgery or autopsy with diagnosed ACI

To classify an event as ACI the following criteria are required to be met:

- Criteria A+B+C

or

- Criteria D

b.5) Other type of CVA (OCVA)

For the diagnosis of OCVA the following criteria will be considered:

The criteria for SAH, IPH, OH or ACI are not met.

[Example: a venous thrombosis with hemorrhage, an arterial dissection.]

b.6) Unknown CVA (UNK)
The criteria for SAH, IPH, OH , CVA or OCVA are not met.
[Ex. There is no gathered data on the case or is insufficient.]

4.1.6 Peripheral vascular disease
Intermittent claudication is defined as pain in an inferior limb/s and is generally associated with physical effort, secondary of peripheral ischemic vascular disease. The criteria of evaluation to classify the event as "**claudication**" are:

A) Presence of pain in the leg with exercise and alleviation with rest
B) Medical diagnosis of claudication
C) Arterial pressure of ankle-brachial ratio ≤ then .08

To declare and event as "Claudication" it is required that:

Criteria A+Criteria B or C are met

Additionally, whichever of the following criteria are considered sufficient, but not necessary to validate a diagnosis of claudication:

- Doppler- Ultrasound shows an arterial obstruction ≥ 75% of transversal area of the artery or presence of an ulcerated plaque.
- Absence of a Doppler pulse in a major vessel.
- Positive test result of physical effort test of claudication
- Bypass surgery, angioplasty or thrombolysis for peripheral vascular disease.

In general to control for the criteria for peripheral vascular disease, the investigator will collect the information from clinical history and original reports of completed studies. In the case there is no access to these reports the hospital discharge will be used (epicrisis).
The committee of events of the IECS will do the final classification of peripheral vascular disease.

4.1.7. Revascularization

Will be considered as surgery as well as any endovascular procedures in any vascular territory (coronary and not coronary). It will be documented as a separate category of other events.

4.1.8. COPD

We will use the definition of COPD proposed by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) that is a ratio of the post-bronchodilator FEV_1 to FVC below 0.70. This definition is consistent with guidelines from the European Respiratory Society and ATS recommendations.
According to GOLD, the spirometry classification of severity of COPD includes four stages:

- Stage I: mild $FEV_1/FVC < 70\%$, $FEV_1 \geq 80\%$ predicted
- Stage II: moderate $FEV_1/FVC < 70\%$, $50\% \leq FEV_1 < 80\%$ predicted
- Stage III: severe $FEV_1/FVC < 70\%$, $30\% \leq FEV_1 < 50\%$ predicted
- Stage IV: very severe. $FEV_1/FVC < 70\%$, $FEV_1 \leq 30\%$ predicted or FEV_1 <50% predicted plus chronic respiratory failure.

4.1.9. Lung function decline

A decrease of 12 percentages or more in FEV_1 with respect to the predicted FEV_1 in the first spirometry (equivalent to 150 mL/year) will be considered an accelerated loss of pulmonary function[10].

4.2 Other definitions:

4.2.1 Arterial hypertension: Arterial systolic pressure ≥ 140mmHg, and or diastolic pressure ≥ 90mmHg or the participant claims to be under current pharmacolic treatment with antihypertensive medications.
4.2.2 Obesity: Overweight will be considered as BMI ≥ 25kg/m^2 and obese with BMI is ≥ 30 kg/m^2
Normal values of hip to waist ratio are .80 in women and 1 in men, values greater indicate abdominalvisceral obesity.
4.2.3 Dislipedima: Dislipidima will be considered as total cholesterol ≥ 200mg/dL or 11.1 mmol/l, or LDL cholesterol ≥130mg/dL or 7.2 mmol/l or HDL cholesterol <40 mg/dL or 2.2mmol/L.
4.2.4 Diabetes Mellitus: Fasting glucose is ≥126 mg/dL or 7 mmol/l
4.2.5 Glucose intolerence: Fasting glucose levels are found between 110mg/dL-125 mg/dL or 6.1 mmol/1-6.9 mmol/L.

5. Identification of events

5.1 Information sources

a) Telephone identification: Participants will be contacted annually by telephone interview, in which they will complete a questionnaire regarding clinical events.

These contacts make up the initial information source for potential cardiovascular events.
Personnel who have specialized in OpenClínica (web-based software that will be utilized in centralized data storage and management) will put the results of the telephone survey into the system, completing the corresponding forms. When an event cines up, the system will send an automated query to CESCAS and the site's principal researcher to inform them of the event.

b) Identification in the clinical visit: Every two years, the patients will have an appointment to visit the health center. In this visit, information described in Section 3.3 of this document will be attained. Personnel who have specialized in OpenClínica (web-based software that will be utilized in centralized data storage and management) will put the results of the telephone survey into the system, completing the corresponding forms. When an event cines up, the system will send an automated query to CESCAS and the site's principal researcher to inform them of the event.

c) Identification through notification from the patient or someone close to the patient:
The participant will be requested to instruct close family or friends (those whose contact information was initially given) about the importance of notifying either the principal researcher or CESCAS in the case of death or hospitalization. The participant will also have to notify the same contacts in the case of receiving a diagnosis of a cardiovascular event from his or her personal physician. This request will be reiterated to the participants during each visit to the clinic, telephone follow-up and in the informative bulletins that the study will regularly send to participants. In the case of a potential event, the principal researcher will go to the necessary location to obtain copies of the available source documents. Later, the researcher will complete the event for in OpenClínica and will send the copies of the clinical case to CESCAS.

6. Event investigation
6.1 Non-fatal event investigation

a) Hospitalization events
In the case of possible non-fatal cardiovascular events that end in hospitalization, the site's principal researcher will go to the location in which the participant was held. The researcher will obtain precise information regarding the date of admission (entrance and exit) as well as the hospital contact information (name, address, telephone number). The researcher will look for clinical history information pertaining to:
Exit diagnosis, treatment received, daily progress, studies conducted (cardiac catheterization, echocardiogram, cardiac perfusion or radioisotopic VTG, Echoes,

laboratory tests, etc.). The researcher will obtain copies of the source documents to send to the Event Adjudication Committee. The center's researcher will later complete the event form in OpenClínica.

b) Non-hospitalization events

For events that do not end in hospitalization (including consultations conducted exclusively with an on-call provider service), the researcher will obtain the information from the participant his or herself or from someone close to him or her who can give precise information regarding the location in which the participant received treatment (date, telephone, address, name of physician). The site researcher must contact the physician and go to the location in which all of the documentation regarding the potential event is found in order to audit it. The researcher will obtain copies of the treatment received, medical summary of the source document, studies conducted (cardiac catheterization, echocardiogram, cardiac perfusion or radioisotopic VTG, Echoes, laboratory tests, etc.). The researcher will later send all available documentation to the Event Adjudication Committee. The center's researcher will later complete the event form in OpenClínica.

6.2 Fatal event investigation

a) Hospital deaths

This is defined as all deaths that occur during hospitalization. This will not account for deaths that occur in the emergency room. The site's principal researcher will go to the place of the event to obtain information about the date and time and to determine the cause of death.
The researcher will later send copies of all of the available documentation (clinical history, studies, ECGs, laboratory tests, death certificate) to the Event Adjudication Committee and will complete the final notification form in OpenClínica.

b) Non-hospital deaths

Deaths occurring in the home, emergency room (without having been admitted into the hospital), arrival at the hospital without vital signs, or in transit are considered non-hospital deaths. The principal researcher must contact the family members and/or physician to obtain information regarding the death (date, time, location, possible cause). The researcher will collect the available information regarding previous hospitalizations in order to obtain a clinical history or any document that contains information referring to the participant's medical background. The researcher will then send copies of all available documentation (clinical history, studies, ECGs, laboratory tests, death certificate) to the Event Adjudication Committee and will complete the final notification form in OpenClínica.

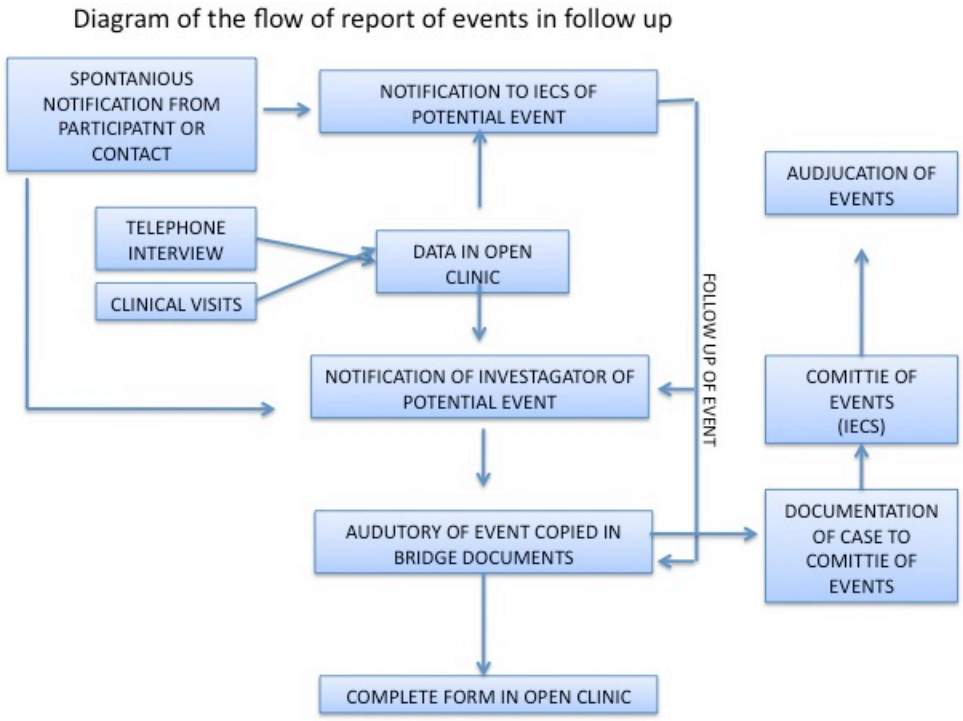
7. Data reporting methodology

The information about a potential event will come from telephone surveys or clinical visits. The system (OpenClínica) will generate a query to notify the event committee and the site's principal researcher about the event. This will aid in coordinating the obtainment of all of the available information about the event and in conducting follow-up regarding the event in a centralized fashion. The researcher must go to the location where the event occurred to audit it. The researcher will later send copies of the source document to the CESCAS event committee and complete the system's corresponding form (event or death form).

8. Revision and final judgment of event

The study's event committee will be made up of three researchers. Each one of the members will independently analyze the case documentation that has been sent in order to conclude a diagnosis of the event. In the case of disagreement, the majority opinion will determine the final judgment of the event.
(See Figure 1.)

Figure 1



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9. Field work quality control

9.1 Repetition of measurements

Between 5 and 10% of participants will have repeated measurements of arterial pressure, height, weight and waist circumference. The motive is to adjust the effect of measurement errors on selected variables and quantify the variability between the variables (variation coefficient).

9.2 Standardization

Standardization of collected data will be conducted through:

a) An operations manual which describes the procedures that must be conducted and the questionnaire terms.

b) An intense training program for interviewers and study personnel that must be successfully completed. This program will provide specific tools and methodology for data collection. The study's team coordinator (IECS) will be in

charge of the personnel training, which will be conducted periodically to reinforce basic concepts.
c) Certification of equipment using international standardization norms.

9.3 Training program
All of the researchers and staff (interviewers, technicians) on-site will be required to successfully complete a rigorous training program, at either a central or local level. They will undergo the training annually during the first 3 years, in agreement with the protocol timeline. The goal of this training is to standardize the data collection in the sites, through both the equipment and the data collection forms.
The observers in charge of recording the participant's arterial pressure will be required to complete a special training session on controlling arterial pressure measurements through a standardized procedure. To obtain a certification, they are first required to have a satisfactory performance on the written exam, which examines theoretic knowledge related to the procedures. Second, they must watch a video that teaches the standardized technique. Finally, they must take consistent measurements in the presence of an instructor. The observers in charge of anthropometric measurements must also participate in specialized trainings. For certification, they must participate in a training session and take consistent measurements of height, weight and waist and hip diameter in the presence of an instructor.
Technicians who will conduct lung function studies will be required to successfully complete a rigorous training program. Standardization of data collection across local field centers will be achieved through intensive training and periodic retraining and certification according to a standard protocol. Spirometric studies will be sent weekly to the coordinating center, where they will be analyzed. Periodic reports will be generated for quality assurance.

9.4 Monitoring procedures
Quality control will be done during the collection of information both at the sites and the central level. The coordinators of every site will monitor the personal of the study daily to assure that the collection of data is in accordance with the quality standards required for the study. Every questionnaire will be checked for completeness previous to participant departure of medical center, to correct errors or omissions during the process of filling in answers.
The equipment of the study, which includes the mercury sphygmomanometer and scale, will be calibrated regularly throughout the course of the study; the spirometer will be calibrated daily, following standard procedures.
Between 5% and 10% of the participants will be selected at random to repeat measurements of all variables of the study. The sites will be monitored for all aspects by the investigators of IECS. During the monitoring the level of quality of procedures will be controlled as well as the deviations from the protocol that

take place. If it becomes necessary training will be repeated for the personal of the study that need it. Similar visits will be completed in laboratories of each site.

9.5 Quality control of the Laboratory

All laboratory exams (total cholesterol, HDL, LDL, triglycerides, glucose and plasma creatine) will be processed in the central laboratory at The Italian hospital of Buenos Aires, Argentina. This is a university hospital referent in Argentina as in Latin America. The central laboratory counts on accreditation from the American College of Pathology, in addition participates in the standardization program of lipids along with the American center of disease and control and prevention, and also as the national heart, lung and blood institute (NHLBI/NIH). Additionally counts on a long history of experience with international studies and last year successfully passed an inspection by the NIH.

The laboratory determinations will be processed at the sites and then transported to the central laboratory. Every technician of the laboratory who works on the study will complete the training program and utilize the standardized protocols of the lab to compete the analysis of the study. Between 5% and 10% of the sample will be selected at random to repeat the measurements with the purpose to quantify the variability among the samples.

10. Processing of data

10.1 Data entry system and storage

Database: For the design of the database of the study the system "OpenClinic" will be used. This is computerized system of related databases with web interface.
Data load survey forms: The electronic forms that will be used for loading the data will be designed taking into account the format of the documents and the paper source.
Data entering: The data will be entered from each site, via web. It will take place by double data entry with independent operators.
Data storage: The data will be stored on a central server, located in the IECS. Copy or backup files will be made of the data on a periodic basis, in a weekly fashion.

10.2 Data monitoring and quality control

The storage and processing of data will be centralized at the IECS. The personal in charge of entering data, will be trained in the use of the system "Openclinic" with these aims in view.
To accomplish the following quality controls:

- Double data entry with comparison of registries and control of inconsistencies.
- Rules of validation generated in accordance with the nature of the variables, programmed by means of the modules of validation of data, available on Openclinic.
- Automatic generation of queries according to rules of pre-established validation, to be responded by the investigators at the sites.
- Design manual of clarifying queries to be responded by the investigators at the sites
- Registry system, records and control of queries
- Periodic reports of the state of data entry, generation and answer of queries with monthly frequency.

11 Statistical Analysis

11.1 Calculation of sample size

This study was designed to lend precise estimates about the prevalence and measured value of the cardiovascular risk factors by gender and locality, in four age-defined categories: from 35 to 44 year, 45 to 54, 55 to 64 y 65 to 74, and the association of the development of cardiovascular events.

The calculated size of the sample is 8000 participants (2000 for each site) and abides with the recommended requirements of precision for complex data sources. This sample will be sufficient to provide precise estimates about the prevalence of major CRF and their association with the development of CVD.

The complex sample design utilized in this study becomes inapplicable to the traditional methods of statistical analysis based on the assumption of a simple random sample. The effect of the sample design on the variability of the estimates is named “design effect”, which is defined as a quotient of variables of the results of the statistical tests done over a complex sample for the results of the same statistical tests, but about a simple sample. The average design effect assumed for this study is 1.5

Table 1. Sample size for a highly complex study design for effect and design of specific proportion

Proportion	Design Effect				
	1.0	**1.5**	2.0	2.5	3.0
0.26-0.50	30	45	60	75	90
0.25	32	48	64	80	96
0.20	40	60	80	100	120
0.15	53	80	107	133	160
0.10	80	120	160	200	240
0.05	160	**240**	320	400	480

The proposed sample size is sufficient to comply with precision requirements of a complex sample that assumes that the design effect is from 1.5 and the prevalence of the risk factors of interest is 5% or greater (Table 1)
The prevalence, the levels of cardiovascular disease and the risk factors are calculated considering the factor or the sample
These coefficients will take into account the characteristics of the study sample,, the rate of non-responses, the over-sampling of some sub-groups and other demographic and geographic differences that could exist between the sample group and the total population.
The size of the sample will allow for obtaining estimates with excellent precision for the defined groups by gender (men and women) and for age (35-44, 45-54, 55-64 y 65-74 years). If necessary, the participants between 65-74 years of age will be over-sampled in order to maintain a minimum of 240 participants in each group.
The standard estimates of prevalence by age and sex will be calculated by the direct method of standardization, using most recent census data of the adult population in the areas of interest used as a reference population.
Other International population standards, such as reports from World Health Organization, can be used as additional resources to make comparisons between sites.

For analysis of the longitudinal component, the capacity to detect risk factors was calculated using a statistically significant alpha level of 0.05 and a statistical power of 85%, which will permit sufficiently detecting the moderate and large relative risks (table 2).

Table 2. Relative detectible risks

Rate of event	Proportion of risk factors CVs		
	0.10	0.25	0.50
0.01	2.29	1.86	1.76
0.03	1.68	1.46	1.40
0.05	1.50	1.34	1.30

11.2 Analysis Plan

The general characteristics of the population will be described using means of central tendency and dispersion. In the case of continuous variables, the mean and the median, range, standard deviation, and/or quartile range will be calculated according to the distribution of each variable. In the case of categorical variables, the absolute and relative frequency of each will be calculated.

The distribution of each risk factor will be examined by means proportions and the construction of bar graphs, box plots and normal probability charts.

In order to determine the prevalence and incidence of risk factors and cardiovascular events the corresponding design effect of the unit of the first stage of sampling will be considered and apply the corresponding weights beginning with the relation between the number of individuals finally included in the study and the population data of every locality according to most recent census data.

Likewise, the analysis will be carried out by stratified means according to socioeconomic strata defined in sampling stage for the different categories of age and sex by location. (35–44, 45–54, 55–64, and 65–74 years) and by gender and locale.

For the associational cross-sectional analysis, linear models of linear regression and simple and multiple logistical regression will be used according to the nature of the variables of the responses.

In the case of continuous variables that do not match with those hypothesized by a linear model they will be evaluated by the application of transformations and categorizations that apply.

There will be an analysis of subgroups of the variables described according to sex, age, and the variable of interest.

On the longitudinal component, the relationship between each risk factor and the development of cardiovascular disease will be examined in a longitudinal manner.

The secular tendency of the risk factors in time will be evaluated with methods of statistical analysis of correlation between repeated measures. To evaluate the changes in risk factors over time by sub-groups of interest, generalized estimation equations will be used. To estimate the rate of accumulated

cardiovascular incidents, the Kaplan-Meir method will be used. The log rank test will be used to compare the differences between the curves of accumulated incidence events. The log rank test of tendencies will be applied to analyze the relationship between the accumulated risk by quartile group or by created groups of interest. In order to quantify the relationship between risk factors and the incidence of cardiovascular events, the Cox method will be used for proportional hazards, both in the univariate analysis as well as in multivariable analysis. These methods assume differences in the time of follow up and do not require that the distribution of time is exponential.

The models will be initially generated only with variables of interest Later, how the association of interest behaves in relation to potential confounders of measurement that is incorporated to the model will be explored.

The statistical significance will be evaluated using the log likelihood test with a level of significance of 0.05. The proportion of risks will be controlled using interaction terms of time dependent on the same criteria.

The age of the participants will be used to construct a time scale for all analysis of time to the event. The election of the scale, (time of origin) of the analysis of time to event will be the scale with the most impact on risk. In a longitudinal study of the general population, it is recommendable to use age as a scale of time (birth as a time of origin) rather than time of the study, since risk of cardiovascular disease is more appreciable as changes as functions of age. Then the adjustment of the model due to confounding variables is controlled adequately of its itself using the appropriate diagnostics.

In the case of predictive categorical variables, the relationship of interest will be tested for no linearity using tests of tendency. Such variables will be entered by categories (dummy variable) according to correspondence.

In all cases, fulfillment of assumptions in the model by means of exploration of residual behavior will be verified and the necessity of applying transformations when needed will be also be evaluated.

Likewise atypical observations, outliers, and potential influences will be investigated by means of standardized residual calculation and indicators D-Beta Cooks Distance according to appropriate results.

The statistical analysis of the data will be completed by a team of investigators from Tulane University, whom possess vast experience with data analysis on cross sectional studies and prospective cohorts done in cardiovascular disease

Prevalence of COPD by age and gender will be estimated for each city. The age-gender-standardized prevalence of COPD will be compared among the 4 cities by socio-economic status, lifestyle risk factors, exposure to air pollution, and other risk factors. In addition to descriptive analyses, logistic regression models will be used to examine the association between risk factors and development of

COPD. Incidence of COPD as well as changes in pulmonary function over time will be estimated. The association between baseline risk factors and incidence of COPD and decline in pulmonary function will be analyzed
Statistical analysis programs STATA 10.0 and SAS 9.0 will be used.

12. Ethical Aspects

Recruitment and Informed Consent (See Appendix B)

The study will carried out strictly following the guidelines for the protection of the rights of human volunteers. All investigators and personnel in the study have attended a training session regarding the theme, certified by NIH. All participants will sign the informed consent during the initial visit. This form will clearly indicate the purpose of the study, the selection criteria, characteristics of the study, and the benefits and potential risks of participating in the study. The right to refuse to participate or withdraw from the study at any time will also be clearly explained. Before administering the questionnaires, the interviewer will explain the details of consent orally to the participant, and will answer any questions that pertain to the study. Concretely, during the process of informed consent the following actions will be followed out:

- The participant will be informed that participation in the study is voluntary and that his/her refusal to participate will not result in any sanction or loss of benefits.
- The participant will be informed about the ends of the study and the development of the investigation.
- The participant will be informed about any reasonable perceived risks.
- The participant will be informed about the benefits of the study.
- The participant will be informed about his/her right to confidentiality and how to Project his/herself.
- The participant will be informed about the right to withdraw from the study at any time without suffering any penalty for doing so.
- The participant will be informed about the right to receive answers to their questions about the study and who to contact to obtain them, or how consequences of lesions related to the study.
- The participant will be informed about the existence of indemnity related to their participation in the study.

To protect the confidentiality of data, the forms and questionnaires of the study will be maintained in locked file cabinets. These also will be located in rooms locked with a key to which only personnel authorized by the study will have access. The forms and questionnaires that contain personal identification will

not be transmitted to the central coordinator. The information included in database will not contain the personal information of the participant. Each participant in the study will be assigned a unique identification code (ID) which will not be a mathematical derivation of the medical registration of the patient or any other personal identifier.
All transmission of data obtained in the field, along with the central lab results and the coordination of the data will take place via a secure website with password. Blood samples will be stickered with the ID only, to be transmitted and processed in the central lab. No personal information of any type will be in any type of presentation or publication. In addition, all personnel of the study will sign a confidentiality agreement to not divulge information or data related to the theme of the study.

Protection against risks: The risks of physical, psychological, social or foreseeable judicial harm are minimal for this study
There is a minimal risk that the participants might feel uncomfortable with certain questions in the interviews, but they can refuse to answer them or stop the interview at any time with no negative consequences. There is also a slight risk of fainting or hematomas during the blood draws. During blood draws, the participant will be seated on a chair with their legs elevated to reduce the risk of fainting.
In addition, juice and a snack will be available to the participants soon after the phlebotomy procedure. The venipuncture will be carried out by nurses trained at the site of the clinic where certified doctors will be available in case of medical emergencies. Trained laboratory technicians, will use standard techniques.
The participants may experience minor discomfort during the blood pressure measurement due to pressure on the artery caused by the blood pressure cuff.
Participants will receive contact information for the coordinators of the study and of the principal investigators in order to ask questions or report complaints.

The potential benefits: The participants of the study will receive the results of the blood tests, record of the arterial tension, as well as the results of the electrocardiogram and spirometry. All participants identified with hyperlipidemia, glucose intolerance, signs of diabetes, hypertension, pre-hypertension or COPD will be directed to see their primary physician; if they do not have a primary physician, one will be assigned to them under the auspices of this study.

Importance of the information gathered: This study will have important clinical

implications, as well as for the good of public health. It is the first population based, longitudinal study in this region, which will have estimate the impact on the risk factors of CVD in the incidence of cardiovascular events. The data collected in this study will fill voids of information and will allow for future political decisions.

13. Timeline of the study

The data will be collected during the first half of the second year of the study and will continue until the third year with a total of 2 years (24 months) from the beginning of the data Collection. The analysis and publication of the baseline data will take place towards the end of the second year of the data collection (month 31- 54). The second phase of follow up will begin during the 3rd year (after the 30th month) and will consist of a minimum of 2 years follow up continuing up to the 5th year (month 54). The final 6 months of the year will be dedicated to the analysis an publication of the data collected (see Appendix C)

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