Prognostic indicators of total-, cardiac- and sudden cardiac death in chest pain patients with suspected acute coronary syndrome (ACS);

with special reference to socioeconomic class, B-type natriuretic peptide (BNP), high sensitivity C-reactive protein (hsCRP), vitamin D and the omega-3 index in a northern Argentinean inland community

by

Ricardo A León de la Fuente

CONTENTS:

Acknowledgements

First, I want to express my gratitude to my main supervisor Professor Dennis W.T. Nilsen who introduced me to biomarker research in the acute coronary syndrome. He designed ARRA-RACS (ARgentinean Risk Assessment Registry in ACS) and surveilled the project as it progressed, visiting Salta on a yearly basis. His scientific experience, dedication, enthusiasm and endurance were of great importance during the last seven years of academic work, and his support is greatly appreciated.

Further, I want to thank my co-supervisor, Professor Augusto Torino, the Head of the Cardiovascular Institute at which I have been employed as a senior cardiologist during the years of research. His practical and moral support enabled me to perform the study along with my duties as a clinical cardiologist.

I greatly appreciate the continuous support by Stavanger University Hospital represented by the hospital's medical director, Professor Stein Tore Nilsen who showed great confidence in me and provided me with financial support throughout seven cumbersome years of research. Also, I appreciate the persistent support from Dr. Leik Woie who was one of the initiators of this study.

Furthermore, I thank Stavanger Health Research represented by its research director Torbjørn Aarsland who monitored the study, regularly visiting Salta twice a year. His attention to detail, guaranties the quality of the data.

Also, I acknowledge the contribution by my dedicated co-researcher, Dr. Patrycja Naesgaard who measured vitamin D and assisted in the writing process. Her dedication and moral support were of great importance during the finalization of this thesis.

The assistance of our statistician, Professor Harry Staines, and manager of the database, Associate Professor Heidi Grundt, was essential for generating and presenting the data. Their support is highly appreciated.

Thanks to my co-author, Thomas Gundersen Ph.D at AS Vitas for the measurement of fatty acids (FA) in packed red blood cells, and to my co-author, Cato

Brede Ph.D for his supervision of the vitamin D measurements. Also, I thank Tore Bolstad at AS Vitas for his technical support with the FA analysis, and I thank Audhild Aarshus and Eva May Svensson for their engagement in the laboratory work related to the BNP analysis.

A special thanks to my colleague Dr. Sven Kiserud who mediated the contact between Stavanger and Salta. Without my Argentinean coworkers, this study would not have been possible to perform. Many thanks to the participating medical doctors: Alejandro Farah (Hospital San Bernardo), Sebastian Saravia Toledo (CENESA), Sebastian Araujo (Hospital Privado Santa Clara de Asis), Pedro Kairuz (Hospital Militar), Fernando Marconetto (Sanatorio San Roque), Cesar Laspiur (Clinica San Rafael), Patricio Gallo (Sanatorio El Carmen), Fernando Rassi (Sanatorio Parque), Florencia Wayar (Clinica Guemes) and the service rendered by our devoted biochemical engineers Silvia Dib Ashur, Carolina Moreno Ten, Natalia Ruiz and Mariela Ponce. Also, I wish to extend my gratitude to Maria José Aleman and Valeria Choque for their coordinating service. The services rendered by Universidad Catolica de Salta and the affiliation to this university were of great importance for the execution of this project, so also was the support by the local government.

I am also deeply grateful to Fatima Gil, for her assistance and support for tracking the patients during the follow-up.

The hospitality of the Nilsen family during my stays in Norway, the interesting philosophical conversations with Klazien Nilsen and the walks with Lennart, the dog in the family, kept my spirits up and inspired me during my time away from home. Furthermore, thanks to Gøran Nilsen who kindly commented on my thesis.

I thank all subjects who voluntarily participated in these studies.

Many thanks to my parents for their unwavering support and encouragement throughout all times of my life.

Finally I wish to thank my beloved wife Fatima and my children Agustin, Joaquin and Sofia for their patience and their loving support during all these years of research.

List of Abbreviations

25(OH)D	25-hydroxyvitamin D
AA	Arachidonic Acid
ACS	Acute Coronary Syndrome
ARRA-RACS	ARgentinean Risk Assessment Registry in the Acute Coronary Syndrome
AUC	Area Under the Curve
BMI	Body Mass Index
BNP	B-type Natriuretic Peptide
CABG	Coronary Artery Bypass Grafting
CAD	Coronary Artery Disease
CHF	Congestive Heart Failure
CI	Confidence Interval
DHA	Docosahexaenoic Acid
DPA	Docosapentaenoic Acid
ECG	Electrocardiogram
EDTA	Ethylene Diamine Tetraacetic Acid
EF	Ejection Fraction
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
GC	Gas Chromatography
HDL	High-Density Lipoprotein

HR	Hazard Ratio
hsCRP	high sensitivity C-Reactive Protein
LDL	Low-Density Lipoprotein
LVEF	Left Ventricular Ejection Fraction
MI	Myocardial Infarction
MUFA	Monounsaturated Fatty Acid
NSTEMI	Non ST-segment Elevation Myocardial Infarction
OR	Odds Ratio
PCI	Percutaneous Coronary Intervention
PUFA	Polyunsaturated Fatty Acid
RACS	Risk in Acute Coronary Syndrome
RBC	Red Blood Cell
ROC	Receiver Operating Characteristic
SCD	Sudden Cardiac Death
STEMI	ST-segment Elevation Myocardial Infarction
TG	Triglycerides
TnT	Troponin-T
UAP	Unstable Angina Pectoris

1. GENERAL INTRODUCTION:

1.1 The Acute Coronary Syndrome (ACS)

Cardiovascular disease, one of the leading causes of death in the western society, is also becoming a major health challenge in developing countries such as India, Brazil and China (1). Implementation of a western lifestyle including cigarette smoking, physical inactivity and a diet rich in carbohydrates and unsaturated fats has resulted in increasing obesity, associated diabetes and early manifestation of atherosclerosis, all leading to a growing rate of cardiovascular disease in developing countries (1-4). Likewise, Argentina is facing a similar challenge with respect to pre-stage cardiovascular disease, with a growing prevalence of diabetes (10%), hypertension (36%) and smoking habits (28%). A similar prevalence is found in the province of Salta (5).

Chest pain is the main symptom of both chronic and acute coronary heart disease (CHD). However, chest pain is also the main symptom of other thoraco-pulmonary conditions, and to distinguish ACS patients among a population admitted with chest pain represents a diagnostic challenge.

The classification of patients with ACS is based on the electrocardiogram (ECG). As such, two categories of patients may be encountered: 1. patients with typical acute chest pain (>20 min) and persistent ST-segment elevation, termed ST-elevation ACS (STE-ACS) or ST-segment elevation myocardial infarction (STEMI), and 2. patients with acute chest pain but without persistent ST-segment elevation (NSTE-ACS). Based on the measurement of troponins, these patients are further classified into non-ST elevation MI (NSTEMI) or unstable angina pectoris (UAP) (6). In the present setting we have added troponin T (TnT) in the diagnosis of ACS.

Atherosclerotic plaque rupture or erosion, with differing degrees of superimposed thrombosis and distal embolization, results in myocardial underperfusion, and represents the main pathophysiological mechanism of ACS (7).

Risk assessment in ACS should include several prognostic indicators of future ischemic events. In the present study we have focused on various biomarkers known to be associated with CHD, such as TnT, B-type natriuretic peptide (BNP), and high sensitivity C-reactive protein (hsCRP). In addition to these we have evaluated the prognostic significance of vitamin D and omega-3 index [the sum of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)].

Risk assessment in a general setting constitutes several clinical parameters, including gender, age, smoking, hypertension, index diagnosis, diabetes mellitus (DM), congestive heart failure (CHF)(8), history of previous CHD and medication. These need to be corrected for when evaluating the prognostic utility of the individual biomarker. Women are also different from men with respect to risk assessment, and this has been focused on in a socioeconomic setting.

1.2 Population

1.2.1 City Characteristics

The studied population is from the city of Salta, the capital of the Province of Salta in Northern Argentina. The city of Salta is located in the Lerma Valley, 1152 meters (3780 feet) above sea level, at the foothills of the Andes mountains. The metropolitan area has a population of 619,000 inhabitants, which makes it the second most populated city in the northwestern part of the country. The province of Salta has 1,210,000 inhabitants (9). The climate in Salta is highland subtropical [according to the Köppen-Geiger climate classification (10)], and the city is located at 24 degrees latitude south of the Equator.

Based on its inland location and its livestock economy and traditions, beef, and not fish, forms the main component of the population's diet.

Salta's economy is diverse but relatively underdeveloped; poverty is a general feature of this society, and there are large socioeconomic inequalities. Furthermore, it is a society in which men have a higher employment rate and higher positions than women (9).

We have divided the city into four residential areas defined as: 1) slums with housing under deficit conditions, 2) suburbs closest to the city center, 3) households located downtown, and 4) residential, wealthy areas.

"Deficit conditions" (11) are defined as homes with earthen floors, and/or no piped water supply, and/or no sewage system.

1.2.2 Health System

Description of the social security model

According to official data from the 2010 National Census (9), 60% of the population of Salta have no healthcare coverage, and are in principle treated in public hospitals at no cost, 20% depend on provincial healthcare, 10% depend on a separate national retirement healthcare program, 5% depend on a national rural healthcare program, and the remaining 5% are affiliated with a public union healthcare system or private insurance program.

Until retirement, patients who are working in a provincial government agency are part of the provincial healthcare plan, which covers the cost of healthcare expenditure for the patients and their spouse and children. This social security program allows free access to public health providers and supports up to 80% of the cost at a private institution.

People working in other public agencies with union affiliation have their own healthcare coverage, and they operate in the same way as the state social security program.

The retirement healthcare program is executed only by national government appointed healthcare providers.

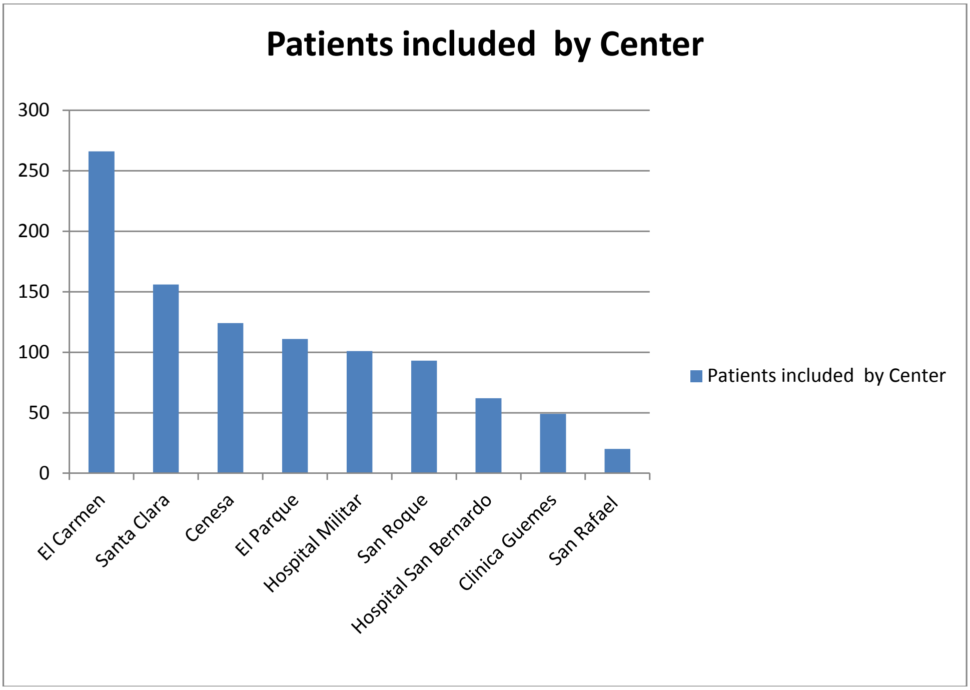
Finally, there are private insurance companies, with a high monthly cost, allowing free choice of any private health care institution and covering the total healthcare expenditure.

Public hospital and private clinics

The city of Salta has two large public hospitals, one for adults and one for obstetrics and pediatrics. In these hospitals, healthcare is at no charge to the patients. The hospitals provide comprehensive care in all medical and surgical specialties. However, aside from kidney and bone marrow transplants, patients in other transplant programs must be referred to other provinces. In Salta, there are also eleven private hospitals covering adult medicine.

For our observational study (12), we have collaborated with one public hospital (San Bernardo; responsible for adult medicine), with 450 beds, and eight private hospital institutions with a similar number of beds all together. The public hospital and five of the private clinics have cardiac catheterization facilities. Cardiac surgery is performed at the public and at two of the private hospitals.

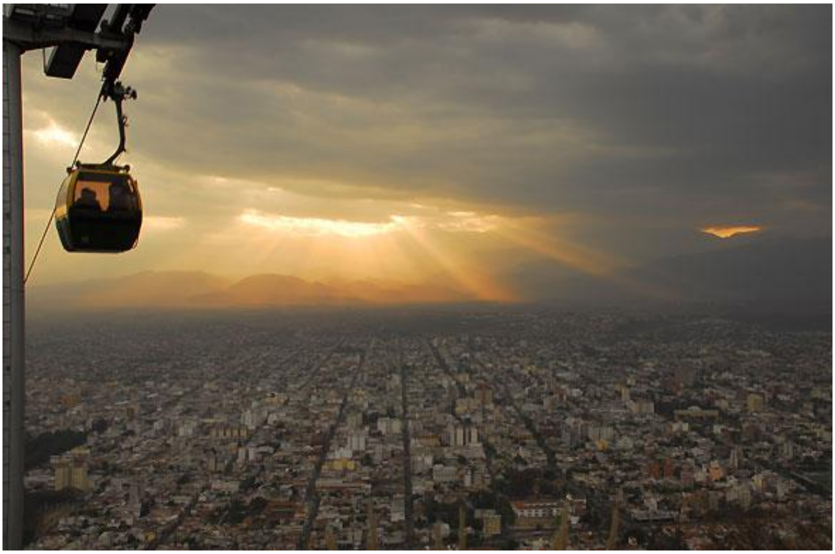
1.2.3 Inclusion by Center



1.2.4 Map of Argentina

From: http://www.visitingargentina.com/mapas/mapa-politico-argentina.jpg.
Last accessed January 04, 2012

1.2.5 Salta City Pictures



1.3 Biomarkers

1.3.1 B-type Natriuretic Peptide (BNP)

BNP is a counter-regulatory 32-amino-acid neurohormone predominantly synthesized in the ventricular myocardium, and is released into circulation in response to ventricular dilatation and pressure overload (13, 14). The actions of this peptide, like those of atrial (A-type) natriuretic peptide, include natriuresis, vasodilatation, inhibition of the renin–angiotensin–aldosterone axis, and inhibition of sympathetic nerve activity (15). The plasma level of B-type natriuretic peptide is elevated in patients with CHF and increases secondary to, and in relation with, left ventricular dysfunction. After acute myocardial infarction (MI), levels of BNP rise rapidly and peak during the first 24 hours (16-19). BNP also provides prognostic information above and beyond left ventricular ejection fraction (LVEF), as well as troponins, in patients with ACS (20-22).

BNP levels in samples obtained a few days after onset of symptoms seem to have superior predictive value as compared to measurements on admission (19-20). In particular, it is a useful marker for evaluating chest pain or dyspnea, and was shown to be helpful in differentiating cardiac and non-cardiac causes of dyspnea. On the other hand, it has limited value for initial risk stratification and hence for selecting the initial therapeutic strategy in NSTE-ACS (23-24).

1.3.2 High sensitivity C-Reactive Protein (hsCRP)

Recognizing that atherosclerosis is an inflammatory process, several plasma biomarkers of inflammation, such as hsCRP (25), have also been evaluated as potential tools for prediction of the risk of coronary events. HsCRP is an acute-phase reactant and a marker for underlying systemic inflammation, including atherosclerosis and plaque rupture with ensuing thrombus formation (26-31). Through the use of appropriate hsCRP assays, it has been possible to investigate the prognostic utility in cardiovascular disease (CVD) of plasma CRP levels previously considered to be within the normal range (32). Burke and colleagues have suggested that hsCRP in serum reflects the number of vulnerable coronary atherosclerotic plaques in sudden cardiac death (SCD) (33).

In 2009, the Canadian Cardiovascular Society recommended hsCRP assessment for patients at “intermediate risk” defined as a predicted risk of a cardiovascular event of 10 to 20% over the subsequent 10 years (34). Also in 2009, the

National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines concluded that measurement of CRP levels might be useful in the stratification of patients at intermediate risk for a cardiovascular event, although the evidence for the usefulness of measures of fibrinogen and other biomarkers of inflammation was considered to be inconclusive (32). A report from the American College of Cardiology Foundation–AHA Task Force on Practice Guidelines in 2010 stated that assessment of hsCRP levels is reasonable for patients at intermediate risk (35). It is expected that further guidelines regarding these biomarkers will emerge, such as the updated guidelines on cholesterol (Adult Treatment Panel IV) which are part of the integrated set of guidelines on cardiovascular risk reduction from the National Heart, Lung, and Blood Institute (36).

1.3.3 Vitamin D

It is well known that Vitamin D deficiency in humans is widespread and increasing (37). Vitamin D can be ingested or created in the skin on exposure to sun and is mostly derived from the latter source. Vitamin D status is commonly assessed by determination of 25-hydroxyvitamin D [25(OH)D] in serum which is a common denominator for $25(OH)D_2$ and $25(OH)D_3$ (38).

Optimal and exact cut-off levels of 25(OH)D are still under debate. The following cut-off levels have been recommended: normal, 75–250 nmol/L, insufficiency, 50–74 nmol/L and deficiency, <50 nmol/L (39-40). However, these values are based on registry data which do not fully take into account population and geographical differences, and factors such as gender and genetics (37).

Several observational studies and epidemiological data suggest that low levels of 25(OH)D may be related to mortality and CVD (41), such as MI (42) and SCD (43).

The general diet does not contain a sufficient amount of vitamin D, and without supplementation, we depend on sun exposure to obtain a satisfactory level of this vitamin. The cutaneous synthesis of vitamin D is influenced by several factors, including geographical location, latitude, altitude, season and daytime, skin colour, age and obesity (39, 44-45).

Fish, the main dietary source of vitamin D, is less preferred by the inland and highland beef-consuming population in Northern Argentina, resulting in a lower dietary intake of vitamin D. In our study population from Salta, Argentina, the dietary insufficiency of vitamin D may be outweighed by the increased sun exposure throughout the entire year at this latitude and altitude. Therefore, we have assumed that sun exposure is the essential source for vitamin D synthesis in this population.

1.3.4 Omega-3 Index

The omega-3 index is defined as the percentage in red blood cell (RBC) membranes of EPA and DHA (46), the two most important long chain n-3 fatty acids (FA) derived from fish. The index is an independent measure of the amount of n-3 FA available in the body, reflecting n-3 FA stored in the phospholipid compartment of cellular membranes. Although it correlates strongly with measurements made in whole blood, plasma and serum (47), its half-life is 4-6 times longer, therefore reflecting the average intake over several weeks (48-49). It may thus be a useful surrogate measure of the beneficial effects of the omega-3 FA. Epidemiological data suggest that n-3 FA can reduce the incidence of CVD (50-53), mainly related to fatal cardiac events. It has also been demonstrated that patients with a high tissue ratio of omega-3/omega-6 FA have a reduced risk of coronary artery events (54).

A meta-analysis of randomized trials involving patients with cardiac disease showed that supplementation with n-3 FA reduced the rate of death from CHD by 20% (55). The most significant sources for this study were the GISSI-Prevenzione (56) and JELIS trials (57). However, contrary results have been recently published by Rizos et al. (58). In this meta-analysis, the authors concluded that omega-3 polyunsaturated fatty acids (PUFA) supplementation was not associated with a lower risk of all-cause mortality, cardiac death, sudden death, myocardial infarction, or stroke, based on relative and absolute measures of association. It is difficult to conclude on the efficacy of n-3 FA supplementation based on these studies, as they differ in design, are performed in highly different patient populations with different background intake of fish, as well as reflecting the administration of a wide range of supplement doses of n-3 PUFA for varying periods of time (59).

Data from randomized and observational studies regarding the effect of the n-3 FA on the rate of cardiovascular events in populations with a very low intake of fish are currently lacking. N-3 FA display several beneficial cardiovascular properties, such as antiatherothrombogenic, antiarrhythmic, anti-inflammatory and antihypertensive effects, and they also lower the triglyceride levels and increase the high-density lipoprotein (HDL) levels (59-63).

Antilipidemic properties are present in populations with a naturally high dietary intake of marine n-3 FA (63), but clinical effects in these populations are modest (60).

2. AIMS OF THE STUDY

1. **Paper 1:** to assess total mortality, cardiac death and SCD in relation to socioeconomic class and social security in patients admitted with suspected coronary chest pain living in the city of Salta, Northern Argentina.

2. **Paper 2:** to explore the prognostic utility of BNP and hsCRP in relation to total- and cardiac mortality within 24 months in a consecutively hospitalized patient population with chest pain and suspected ACS.

3. **Paper 3:** to assess the prognostic utility of 25(OH)D in relation to total mortality, cardiac- and sudden cardiac death in an inland, high-altitude, subtropical city in Northern Argentina, characterized by a low dietary intake of fish.

4. **Paper 4:** to search for a threshold in relation to mortality (total- cardiac- and sudden cardiac death) occurring in a quartile analysis of the omega-3 index in a cohort of chest pain patients with suspected ACS and a low dietary intake of fish.

3. MATERIAL AND METHODS

3.1 ARgentinean Risk Assessment Registry in the Acute Coronary Syndrome (ARRA-RACS)

This prospective, observational study was a regional multicenter prognostic study designed to prospectively evaluate the prognostic impact of socioeconomic status and biomarkers including BNP, hsCRP, 25(OH)D and omega-3 index in a cohort of 982 patients with chest pain and suspected ACS, consecutively admitted to nine hospitals in Salta, Argentina from December 2005 to January 2009. Eight centers were private and one was public. The public hospital included sixty-two patients, representing 6.5 % of the studied population.

Patients entered into the registry were admitted for ACS as a presumptive diagnosis and had to be alive at the time of hospitalization. We used TnT levels at baseline and at six hours after admission for disease classification. Furthermore, BNP and hs-CRP were determined in all patients as quality indicators in our registry. We also measured the serum 25(OH)D content and the omega-3 index in all patients.

Clinical data were collected at each site by a trained coordinator, using a standardized nine-page case report form. Demographic characteristics, medical history, presenting symptoms, biochemical and electrocardiographic findings, treatment practices, and a variety of hospital outcome data were collected. Monthly intake of fish and fish oil supplementation was recorded.

Recorded information also included patient management data and outcome during hospitalization as well as after discharge. The patients received standard medical treatment at all centers. However, some patients needed to be referred to other more specialized hospitals with cardiac catheterization laboratory facilities when intervention was required.

To be considered eligible for the study, patients had to be at least 18 years of age at admission and have provided written, informed consent. If the patient remained unconscious until death, consent was given by a close family member. The exclusion criteria were previous inclusion in the same registry, participation in another clinical trial, or unwillingness or incapability to provide informed consent.

3.2 Socioeconomic Model

We divided the total population into three groups based on the following variables:

- The monthly income of the patient or patient's provider was scored from 1 to 4; 1 was assigned to those who earned less than 2000 Argentinean pesos a month, 2 to those with a monthly income between 2000 - 4000, 3 to those between 4000 - 10000, and 4 to those who earned more than 10000 pesos a month.
- Health insurance, also graded from 1 to 4; 1 was scored by subjects without insurance coverage, 2 by subjects on a retirement social health program, 3 by state employees and union associated employees, and 4 by patients with private health insurance.
- Residential area was graded from 1 to 4 according to the patient's housing conditions defined according to location, ranging from poor to wealthy areas, as previously defined.

We added the grading of each variable, obtaining a socioeconomic level for each subject and according to this level they were divided into three categories; low social class (3-5 points), middle class (6-8 points) and upper class (9-12 points). Accordingly, 147 out of 155 individuals without social security were found in the first category, whereas all subjects in the third belonged to a social healthcare program. Occupation/employment was not included as an individual variable in this socioeconomic model, as it is related to income in this community.

3.3 Ethics Statement

The study was approved by the Ethics Committee of the Board of the Medical School of Salta and conducted in accordance with the Helsinki Declaration of 1971, as revised in 1983. At San Bernardo Hospital and Sanatorio El Carmen, the study was also approved by the local Ethics Committee and Institutional Review Board. The Norwegian bio-bank containing Argentinean blood samples was approved by the Regional Board of Research Ethics and the Norwegian health authorities. The study was monitored by Stavanger Health Research, Stavanger, Norway. Written informed consent was obtained from all patients.

3.4 Outcome and Follow-up

The primary outcome measure of the present study was all-cause mortality from the time of inclusion up to five year follow-up. The secondary outcome was cardiac death, including sudden cardiac death. The term ACS in the present study encompasses UAP, NSTEMI and STEMI. The following classification for the index diagnosis was used: STEMI; ST-segment elevation combined with TnT values > 0.03 ng/mL. NSTEMI; Transient ST-segment elevation, ST-segment depression, or T-wave inversion in at least 2 contiguous leads combined with TnT values > 0.03 ng/mL. UAP; Transient ST-segment depression or T-wave inversion and TnT values ≤ 0.03 ng/mL, or borderline TnT values above 0.01 ng/mL up to 0.03 ng/mL without ECG changes. No-ACS: All other conditions (*i.e.* unspecific chest pain, arrhythmias, atrial fibrillation etc.) without ECG changes and with negative troponins.

The definition of cardiac death included death, preceded by a definitive MI or by chest pain > 20 minutes without a given TnT, or a history of ischemic heart disease and no other obvious cause of death (64).

SCD is defined as unexpected death due to a cardiac cause occurring within one hour of symptom onset or as a witnessed unexpected death (65).

Outcome including survival status, date and cause of death were obtained from hospital records, close family members and a link to municipal and provincial registries (Civil Registry of the Province of Salta) at regular intervals; 30 days, 6 months and annually during the 5-year follow-up period. A personal interview by a physician was performed one year from index admission. An endpoint analysis at 2 years was also recorded.

3.5 Blood and Chemical Analysis

Peripheral blood samples for determination of TnT, creatinine, glucose, lipids and hsCRP in serum and BNP in ethylene diamine tetraacetic acid (EDTA) plasma were drawn immediately following admission by direct venipuncture of an antecubital vein, applying a minimum of stasis. A repeated blood sample for the second determination of TnT was drawn six hours following the primary blood sample. Clotted whole blood and EDTA blood samples were centrifuged for 15 min with 2000 x *g* at 20°C without delay. Serum and EDTA plasma were immediately frozen in three aliquots, stored locally at -70°C and transferred in frozen condition (dry ice) to Stavanger, Norway in three different shipments, the first after collection of 100 samples, the next containing 400 samples, and remaining samples in a third shipment. These samples were stored in a Norwegian bio-bank at -70°C until measurements were performed.

TnT was quantified by a cardiac-specific second-generation TnT ELISA assay from Roche diagnostics, using a high-affinity cardiac-specific TnT isoform antibody (66). The lower detection limit of the assay used was 0.01 ng/mL. In this study a cut off level of 0.01 ng/mL was therefore used with a coefficient of variation (CV) of 10%.

BNP was analysed in EDTA plasma using the Microparticle Enzyme Immunoassay (MEIA) Abbott AxSYM® (Abbott Laboratories, Abbott Park, Illinois, USA). The dynamic range was 0-4000 pg/mL and the within-run coefficient of variation (CV) was 6.3% at 95 pg/mL and 4.7% at 1587 pg/mL, respectively.

Hs-CRP was measured with the use of an immunoturbidimetric assay (Tina-quant® C-reactive protein (latex) high sensitive assay, Roche Diagnostics, Germany) performed on a Roche automated clinical chemistry analyzer (MODULAR P). The detection limit was 0.03 mg/L and the measuring range 0.1-20.0 mg/L with an extended measuring range with automatic re-run of 0.1-300 mg/L. The between-assay CV was 3.45% at 1.19 mg/L and 2.70% at 0.43 mg/L, respectively.

3.5.1 Vitamin D

Assessment of vitamin D status was performed by determination of the metabolites $25(OH)D_3$ and $25(OH)D_2$ in serum by liquid-liquid extraction (LLE), derivatization with 4-phenyl-1,2,4-triazoline-3,5-dione reagent (PTAD, Sigma-Aldrich, St. Louis, MO, USA), and analysis by liquid chromatography coupled with tandem mass spectrometry detection (LC-MS/MS). A one-step LLE procedure was performed by mixing 50 µl serum, 50 µl internal standard solution, comprising 160 ng/ml of 6-deuterium labeled $25(OH)D_3$ (Synthetica, Oslo, Norway) in isopropanol, 350 µl of 200 mmol/L magnesium sulphate, and finally 900 µl acetone and heptane (1+1). The upper heptane layer was acquired and evaporated, followed by addition of 100 µl of 0.5 mg/ml PTAD reagent in dry acetonitrile. The LC-MS/MS analysis was performed with an Acquity UPLC coupled with a Quattro Micro (Waters, Milford Massachusetts, USA). The separation was isocratic, using a 2.1×50 mm Acquity BEH C18 UPLC column (Waters) and a 0.5 mL/min mobile phase flow consisting of 20% ammonium hydroxide (0.1%) and 80% acetonitrile. Tandem mass spectrometry detection was with positive electrospray ionization (ESI+), using 3 kV and 30 V for capillary and cone voltage, respectively. Collision energies were 15 eV for 25(OH)D_3 and 17 eV for 25(OH)D_2. The multiple reaction monitoring transitions (molecular ion > fragment ion, monoisotopic molecular weight in Da) were 558.5>298.2 for 25(OH)D_3, 564.5>298.2 for deuterium labeled 25(OH)D_3, and 570.5>298.2 for 25(OH)D_2.

Calibration of the linear relationship between peak area response ratio relative to sample concentration of 25(OH)D_3 and 25(OH)D_2 was achieved by using serum calibrator #38033 (Chromsystems, Munich, Germany). Within each microwell plate, the analytical quality was monitored by analysis of 5 different control samples: #0029 and #0030 (Chromsystems), #35080 and #35081 (Recipe, Munich, Germany), and HK10 (DEKS, Herlev, Denmark). The CV for the control samples analyzed over 25 series were in the range of 8.7–10.8% for 25(OH)D_3 and in the range of 10.7–16.5% for 25(OH)D_2. Intra-series repeatability was estimated at three different levels, and the CV's found were in the range of 2.9–8.2%. Method bias was estimated by relative difference from the quality control sample values. For 25(OH)D_3, 21–22% bias was found by analysis of control samples with reference values of 38.6 and 59 nmol/L, and 1–2% bias was found for samples with reference values of 73.4 and 136 nmol/L. For a control sample with a high reference value of 265 nmol/L, the bias was −18%. For 25(OH)D_2, the control samples with reference values of 39, 62 and 126 nmol/L, respectively, were associated with biases in the range − 3 – 8 %. Finally, a bias of – 9 % was found for a sample with a high reference value of 252 nmol/L.

3.5.2 Omega-3 Index

The RBCs were prepared as follows: After removing the plasma from the EDTA blood solution following centrifugation, the RBCs were washed once with 5 mL of 0.9% saline solution and then centrifuged at 1800 rpm for 8 min. The sediment containing the RBCs was stored in two tubes at -70°C after temporary storage at -20°C for 1 to 14 days. All RBCs samples were transferred on dry ice in one shipment to VITAS laboratories, Oslo, Norway, for measurement of FA. Frozen RBC samples were thawed overnight in a refrigerator. Approximately 40 μL of wet RBCs were transferred to a 1.8 mL GC vial using a pipette with a wide opening. After a 3 second vortex mix, 900 μL 3N methanolic HCl was added followed by another 3 second vortex mix and capping of the vial. Vials were then incubated at +80°C with mixing at 1000 rpm for 2 hours. The vials were cooled to room temperature and 500 μL hexane and 300 μL 3M KOH in water were gently added. After capping, shaking for 5 min and centrifuging for 5 min at 4000 rpm, 1 μL was injected by pulsed split less injection on a GC-FID system (Agilent G7890A, Agilent Technologies, Waldbronn, Germany). Separation was performed on a SP-2380 (30 m x 0.25 mm i.d. x 0.25 μm film thickness) column from Supelco, USA. The following temperature program was used: initial temperature of 90 °C held for 0.5 min, then increased by 50 °C/min to 150 °C, then increased by 10 °C/min to 225 °C, then increased by 120 °C/min to 245 ° C and held for 3 min. The oven was cooled before the next injection. FA were identified by comparison with known standards. An external standard containing known amounts of relevant FAME (fatty acid methyl esters) (Supelco 37 component FAME Mix, Supelco Bellafonte, USA) was included in each run to correct for differences in FA response factors. The individual FA was reported as a weight percentage of the total FAME and the omega-3 index was given by the sum of EPA and DHA. The inter-assay coefficient of variation was 4%. To adjust to the analysis previously employed in a similar study performed in the southwestern coastal region of Norway (67), C22:0, C24:0, C24:1, and unidentified peaks were removed from the denominator prior to the calculation of weight percentage. The arachidonic acid (AA)/EPA + DHA ratio was based on the unadjusted values and introduced to reflect the balance between omega-6 and omega-3 in a nutritional perspective.

We analyzed 980 out of 982 RBC samples; one sample was missing and another was coagulated. 408 samples displayed signs of oxidation, leaving 572 patients available for the present evaluation. By oxidation, we mean that the concentrations of the easily oxidizable PUFA were not normally distributed as for other FA, but showed a second distribution, severely skewed towards very low concentrations, and superimposed on the expected normal distribution for docosapentaenoic acid (DPA). This strongly suggests degradation, with PUFA concentrations approaching zero in some samples. In order to discriminate between oxidized and non-oxidized samples we applied a k-means cluster analysis (SPSS 19.0, SPSS Inc. Chicago, IL, USA), by which we divided the material into 2 groups with respect to C22:6,n-3 (DHA), C22:5,n-3 (DPA), C20:5,n-3 (EPA), C22:4,n-6, C20:4,n-6 and C20:3,n-6. By taking all these FA into consideration in combination, the cluster analysis may be regarded as more objective and powerful as compared to the evaluation of only one of these FA with respect to oxidation. The cluster analysis placed samples from 408 subjects in the oxidized group and samples from 572 patients in the non-oxidized group with a normal distribution of PUFA.

3.6 Statistics

The patients were divided into quartiles according to their BNP, hsCRP, 25(OH)D and their adjusted omega-3 index levels. Approximately normally distributed variables were given as mean and standard deviation (SD), whereas variables with skewed distributions were given as median and quartiles. The Chi-square test for association was applied between the BNP, hsCRP, 25(OH)D and omega-3 index quartiles and categorical variables at baseline. The one-way ANOVA test was used to test for equality of means of scale variables (*i.e.* age) amongst quartiles, and the two-sample t test and Mann-Whitney test were used for comparing the means and medians of two samples, respectively. The hazard ratios (HR) are presented with 95% confidence interval (CI). Stepwise Cox multivariable proportional hazards regression models with total-, cardiac- and sudden cardiac death as the dependent variables and BNP, hsCRP, 25(OH)D and omega-3 index quartiles and other variables as potential independent predictors were fitted. To examine the differences in prognosis between subjects in the upper versus the lowest quartile of BNP, hsCRP, 25(OH)D and omega-3 index we adjusted for age, sex, smoking, hypertension, index diagnosis, creatinine/estimated glomerular filtration rate (eGFR), [calculated by Modification in Diet in Renal Disease (MDRD) formula], DM, body mass index (BMI) (kg/m^2), CHF (defined by Killip-Kimball class at admission, those patients in class 2 to 4 were classified as CHF patients and class 1 as non-CHF), history of previous CHD (*i.e.* history of either angina pectoris, MI, coronary artery bypass graft (CABG), or percutaneous coronary intervention (PCI), hypercholesterolemia/use of statins, BNP, hsCRP, 25(OH)D and adjusted omega-3 index quartiles, triglycerides, HDL cholesterol, systolic and diastolic blood pressure, TnT > 0.01 ng/mL and beta-blockers prior to enrolment. Kaplan-Meier product limits were used for plotting the times to event, with the survival curves assessed by the log-rank test. In the discriminate analyses BNP, hsCRP, 25(OH)D, omega-3 index and their natural logarithm were used as individual variables. The statistical analyses were performed using the statistical package SPSS version 19.0. All tests were two-sided with a significance level of 5%.

4. LIST OF PAPERS

1. **Paper 1:** León de la Fuente R, Naesgaard PA, Nilsen ST, Woie L, Aarsland T, Staines H, Nilsen DW. *Socioeconomic assessment and impact of social security on outcome in patients admitted with suspected coronary chest-pain.* Accepted 16 April 2013; to be published in: Cardiology Research and Practice Volume 2013, Article ID 807249, 9 pages, http://dx.doi.org/10.1155/2013/807249

2. **Paper 2:** León de la Fuente R, Naesgaard PA, Nilsen ST, Woie L, Aarsland T, Gallo P, Grundt H, Staines H, Nilsen DW. *B-type natriuretic peptide and high sensitive C-reactive protein predict 2-year all-cause mortality in chest pain patients: a prospective observational study from Salta, Argentina.* BMC Cardiovasc Disord. 2011 Sep 29;11:57.

3. **Paper 3:** Naesgaard PA, León De La Fuente RA, Nilsen ST, Woie L, Aarsland T, Brede C, Staines H, Nilsen DW. *Serum 25(OH)D is a 2-year predictor of all-cause mortality, cardiac death and sudden cardiac death in chest pain patients from Northern Argentina*. PLoS One. 2012;7(9):e43228. Epub 2012 Sep 6.

4. **Paper 4:** León de la Fuente R, Naesgaard P, Nilsen S, Woie L, Aarsland T, Gundersen T, Nilsen D. *Omega-3 Index and prognosis in acute coronary chest pain patients with a low dietary intake of omega-3*. Scand Cardiovasc J. 2012 Nov 6. [Epub ahead of print].

5. SUMMARY OF RESULTS

1. Paper 1

After a follow-up period of 5 years, 173 patients (17.6%) had died. In 92 patients (9.4%) death was defined as cardiac, of which 59 patients (6.0%) were characterized as SCD. In the multivariate analysis, the HRs for all-cause mortality and cardiac death in the highest socioeconomic class as compared to the lowest was 0.42 (95% CI, 0.22 - 0.80), p = 0.008 and 0.39 (95% CI, 0.15 - 0.99), p = 0.047, respectively, whereas the results were not significant for SCD.

Comparing patients in the upper socioeconomic class, in which all individuals had a social security program, to patients without healthcare coverage, the multivariate analysis demonstrated an improved outcome with respect to total mortality, as well as a borderline difference suggesting improved survival with respect to cardiac death, in patients with health coverage. The HRs were 0.46 (95% CI, 0.23 - 0.94), p = 0.032 and 0.37 (0.14 - 1.01), p = 0.054, respectively, whereas no difference was noted for SCD. However, in the TnT positive patients a similar but significant relationship was found for both total- and cardiac death.

After extracting patients without healthcare coverage, total mortality and SCD still remained lower in the upper socioeconomic class as compared to the lowest class, with HRs of 0.42 (95% CI, 0.22 - 0.81), p = 0.009 and 0.30 (95% CI, 0.10 - 0.93), p = 0.037, respectively. No difference was found in relation to cardiac death.

2. Paper 2

The median BNP and hs-CRP concentrations in plasma were 78.1 (35.8 - 179.7) pg/mL [25 and 75% percentiles] and 3.1 (1.3 - 8.4) mg/L [25 and 75% percentiles], respectively.

In the univariate discriminate analyses, a TnT positive event at admission was correctly classified by BNP in 66.7% and by hsCRP in 64.2% of cases in their non-logarithmic form and slightly less in their logarithmic form. The specificity of non-logarithmic BNP and hsCRP for predicting all-cause mortality in the total population was 89.6% and 90.3%, respectively, with a sensitivity of 44.5% and 31.9%, respectively.

Combining the two predictors in our quartile comparisons did not increase the prognostic impact as compared to the separate analysis of BNP and hsCRP.

All-Cause Mortality

After a follow-up period of 24 months, 119 patients (12.2%) had died. The BNP and hsCRP levels were significantly higher among patients dying than in 2-year survivors; 228 (66 - 603) versus 72 (34 - 148) pq/mL [median, 25 and 75% percentiles], p = 0.000 and 7.8 (2.3 - 35.6) versus 2.9 (1.3 - 7.5) mg/L [median, 25 and 75% percentiles], p = 0.000, respectively.

In a stepwise multivariate Cox regression model, BNP was found to be a prognostic indicator of 2 year total mortality in the total patient population. The HR for BNP in the highest quartile (Q4) was 2.32 (95% CI, 1.24 - 4.35) as compared to the lowest quartile (Q1), which was statistically highly significant, p = 0.009. In the multivariate Cox regression model hsCRP levels also showed a significant relation to prognosis, with a HR of 1.97 (95% CI, 1.17 - 3.32), p = 0.011.

The area under the curve (AUC) for the receiver operator characteristics (ROC) for BNP, hsCRP and TNT was 0.711 (p = 0.000), 0.666 (p = 0.000) and 0.666 (p = 0.000), respectively.

Cardiac Death

After a follow-up period of 24 months, 66 patients (6.9%) had experienced cardiac death.

In the univariate analysis for the total population, the HRs for BNP and hsCRP were 6.97 (95% CI, 2.94 - 16.54), p = 0.000 and 2.25 (95% CI, 1.19 - 4.28), p = 0.013, respectively. In a stepwise multivariable Cox regression model, the HR for BNP was 3.34 (95% CI 1.26 - 8.85), p = 0.015, whereas the HR for hsCRP was not significant, p = 0.21.

Including patients with borderline troponin levels ≤ 0.05 ng/mL among the TnT negative patients and without adjusting for a positive troponin value, the HR of BNP for cardiac death in Q4 as compared to Q1 in the multivariate Cox regression model was 3.58 (95% CI, 1.02 - 12.60), p = 0.047, in this extended patient category. The same relation was observed for hsCRP; an HR of 2.69 (95% CI, 1.22 - 5.95), p = 0.015.

3. Paper 3

A significantly higher proportion of patients dying was found in Q1 of 25(OH)D as compared to Q4, both in the total population (25.3% vs 6.1%) and in patients with a TnT release, (43.3% vs 9.3%) each $p < 0.0001$, respectively.

The specificity of non-logarithmic 25(OH)D for predicting all-cause mortality in the total population was 62.2%, with a sensitivity of 67.2%.

When comparing 25(OH)D in Q4 to Q1 in a multivariable Cox regression model for all-cause mortality within 2 years in the total patient population, the HR was 0.37 (95% CI, 0.19 - 0.73), $p = 0.004$. For cardiac death, the HR was 0.23 (95% CI, 0.08 - 0.67), $p = 0.007$, and for SCD the HR was 0.32 (95% CI, 0.11 - 0.94), $p = 0.038$.

Patients with troponin T release

When comparing 25(OH)D in Q4 to Q1 in a multivariable Cox regression model for all-cause mortality within 2 years in patients with TnT release, the HR was 0.24 (95% CI, 0.10 - 0.54), $p = 0.001$. For cardiac death, the HR was 0.18 (95% CI, 0.05 - 0.60), $p = 0.006$, and for SCD, the HR was 0.25 (95% CI, 0.07 - 0.89), $p = 0.033$.

The area under the ROC curve for 25(OH)D was 0.276 ($p < 0.0001$).

Patients without troponin T release

After a follow-up period of 24 months, 37 patients (6.3%) of 593 with no TnT release had died. In the univariate analysis of all-cause mortality in these patients, the HR for 25(OH)D was 0.39 (95% CI, 0.15 - 1.00), $p = 0.05$, whereas 25(OH)D status did not add any prognostic information related to cardiac death and SCD.

4. Paper 4

In our main analysis, we disregarded approximately 40% of the RBC samples due to the presence of oxidation. In the 572 patients with non-oxidized samples, the mean(SD)% value of AA (C20:4,n-6) was 11.82(1.80)%, whereas the mean(SD)% of EPA, DHA, and DPA were 0.25(0.09)%, 2.57(0.74)%, and 1.65(0.37)%, respectively. The mean(SD)% of the omega-3 index was 2.81(0.79)%. After adjusting for C22:0, C24:0, C24:1, and unidentified peaks, the mean (SD)% of the omega-3 index increased to 3.58(0.99)%.

Patients were followed for a median period of 3.6 years, range 1 day to 5.5 years. At final follow-up, 100 patients (17.5%) had died. The rate of death was found to be similar in all quartiles of the AA/EPA + DHA ratio, and the prognostic utility was not improved by looking separately at the adjusted omega-3 index. In the multivariable model for the five year follow-up data, HR (95% CI) for each of the three upper adjusted omega-3 index quartiles as compared to Q1 were non-significant; Q2: 0.89 (0.48 - 1.65), Q3: 0.80 (0.43 - 1.50), Q4: 0.73 (0.38 - 1.42), respectively. Only age, TnT > 0.01, level of creatinine, hsCRP quartiles, and systolic blood pressure at admission predicted total mortality.

Cardiac death and SCD

At final follow-up, cardiac death occurred in 54 (9.4%) patients, of whom 35 (6.1%) were classified as SCD. For the endpoints of cardiac death and SCD there were no significant reductions across quartiles. In the multivariate model for cardiac death, the HR (95% CI) in each of the three upper as compared to Q1 of the adjusted omega-3 index were Q2: 1.11 (0.47 - 2.63); Q3: 0.98 (0.40 - 2.36); Q4: 0.81 (0.32 - 2.06), respectively.

6. DISCUSSION

6.1 Paper 1

A large proportion of the Argentinean population is not covered by social security programs. For example, in the city of Salta, 60% have no coverage. In this prospective observational study, 15.8% of the admitted patients were not covered by a social security program, which is mainly due to major recruitment at the private clinics. Although the public hospital is equipped with a similar number of patient beds as all the private clinics together, it only made up 6.5% of the patient population. Comparing the upper socioeconomic class to patients without a social security program in our multivariate analysis, in which age, gender, hypertension, DM type 2 and BNP were corrected for, a similar relationship was found for both total- and cardiac mortality, suggesting that the lower socioeconomic group was worse off, irrespective of a social security program. Thus, in our study we found that socioeconomic inequalities are of greater importance for survival than having social security coverage.

In our multivariate analysis, total- and cardiac mortality was elevated in the TnT positive patients belonging to the low socioeconomic class, whereas survival was unaffected by socioeconomic grouping in the TnT negative population. This would suggest that the lower socioeconomic group may have received less medical attention following the index event. In the Copenhagen Male Study, the authors suggest that potential modifiable risk factors associated with life style and working environment are strong mediators of social inequalities in risk of ischemic heart disease (68). In the Salta region there is a high rate of unemployment and a large proportion of the workers are not protected by union rights, which may have an unfavourable effect on daily life, affecting individual health conditions.

In the Scottish Heart Health Study it was shown that prevalent (69) and incident CHD (70,71) is related to housing tenure status (owner – occupiers or renters), regarded as a sensitive measure of social class, as house renting was predominantly a feature of the socially disadvantaged. In the lower socioeconomic class in our study, it is mainly the living conditions and not the possession of property that is influencing CHD mortality.

In a study based on socioeconomic inequalities in 22 European countries, access to health care was found to be one of several factors associated with inequality (72). Our findings of increased mortality in the lower socioeconomic class and among individuals without a social security program are in accordance with this statement.

An inverse relationship between education and mortality has also been reported (73). We did not include education in our socioeconomic model, as our study was not related to primary prevention, but was based on a population with suspected and documented coronary heart disease; education was not regarded as an essential mediator of health in this population. Furthermore, a primary prevention study performed in Brazil (74) concludes that there is an inverse relationship between cardiovascular mortality and income, education and poor housing conditions. However, that was a primary prevention study with univariate data, in contrast to our study, in which data have been provided in a secondary prevention setting, correcting for potential confounders.

In contrast to Argentina, nations with a social democratic healthcare system provide all inhabitants with a similar social security program, and thus the same level of medical attention irrespective of income. In these populations health will largely depend on other factors, such as education and employment. Thus, in a system with an egalitarian social security program, primary and secondary prevention is the key to better health, whereas in a system in which 60% of inhabitants have no social security, a lack of medical attention may largely explain the increase in cardiac mortality.

6.1.1 Limitations

Foremost among the limitations inherent to our study is the relatively small sample size (n = 982) compared to larger studies. Furthermore, the present study includes patients from one Argentinean city, and therefore only represents the demographics of the city of Salta, located in Northern Argentina. Our results do not necessarily represent other provinces in Argentina and should not be extrapolated to other countries. Most of our patients were included at private centers, and as only 15.8% of the expected 60% had no social security coverage, this population is highly selected.

6.2 Paper 2

The present study was carefully designed to meet the requirements for a prognostic evaluation of biomarkers. Contrary to randomized studies, patients are unselected and included on a consecutive basis, which offers a great advantage for risk identification. In this study, we have included admission samples of the two biomarkers BNP and hsCRP to investigate their impact on prognosis in patients with chest pain and suspected ACS. We have discriminated between patients with and without a release of TnT. In the stepwise multivariate model applied to the total patient material, which included both the presence and absence of TnT release, we were able to demonstrate a statistically significant prognostic impact of BNP and hsCRP on 2-year survival, both for total- and cardiac mortality. In the TnT positive subgroup we found that BNP had a statistically significant prognostic impact in the stepwise multivariate Cox regression model on all-cause mortality and cardiac mortality. On the other hand, hsCRP was found to be related only to total mortality.

In a univariate discriminant analysis we found that a TnT positive event was correctly classified by BNP or hsCRP in over 60% of cases. The specificity of BNP and hsCRP for predicting all-cause mortality in the total population was around 90% for both biomarkers, associated with sensitivities of 44.5 and 31.9%, respectively. In the present study, patients in the highest quartile for both BNP and hsCRP were older than in the remaining quartiles, and a higher proportion had TnT exceeding 0.01 ng/ml. In the highest quartile of BNP there were also more past smokers and subjects with established CHD and HF, and creatinine was elevated. These differences reflect the increased burden of risk in the upper quartiles of BNP and hsCRP, respectively. Despite some similarities in underlying risk burden, these two predictors are mechanistically different. However, their combinations were not found to strengthen the prognostic utility.

Our results indicate that both BNP and hsCRP are major predictors of outcome in a population for which invasive coronary intervention is less available as compared to wealthier communities. Indeed, only 29% of the total population and 38% of the TnT positive population underwent a revascularization procedure during the hospitalization for the index event. Thirty-one percent of the TnT positive population was classified as STEMIs, and of these patients only 42% were treated with primary PCI.

Furthermore, the use of thrombolytic therapy in this region of Argentina is uncommon and was not applied in our patient cohort. The infrequent use of reperfusion treatment in STEMI patients makes the study population unique in an epidemiological setting, optimizing the evaluation of prognostic indicators in relation to the natural course of disease.

As mentioned previously, the prognostic utilities of BNP and hsCRP remained statistically significant with respect to total mortality in both the total population and in TnT positive patients. It has been known since the mid 90's that elevated troponins are associated with a worsened prognosis in ACS patients (75), and that its prognostic utility exceeds that of all other biomarkers, including BNP (17) and hsCRP (31). Nonetheless, based on previous studies (18,19,76) addressing hsCRP, this biomarker has been considered for adoption into risk assessment algorithms (77). Recently conducted studies have, however, shown that the predictivity of hsCRP is attenuated when tested in a multivariable model in the general population (78), and together with natriuretic peptides in patients with known CAD (79-82). In our study, hsCRP appears to be a potential predictor for all-cause mortality, also when adjusted for BNP, but does not reflect cardiac mortality in the TnT positive population when introducing BNP into the model. The main prognostic impact of BNP was found in the TnT positive patients, suggesting a relation to ischemia.

In contrast to the majority of previous studies investigating the prognostic impact of various biomarkers, our study had a prospective and observational design, and blood samples were collected directly on admission. Few studies have examined the predictive value of natriuretic peptides across the spectrum of chest pain patients with suspected ACS in blood samples obtained on admission, before introduction of therapy. Therefore, as in a related study (83), we do not have to consider the potential confounding factors of late inclusions and recently introduced medical treatment in the present study. Similar considerations apply to the measurement of hsCRP. A major strength of our study is the absence of patients lost to follow-up; in fact, there were only four patients with no measurements available for hsCRP. Moreover, our study was performed in an inhomogeneous and unselected chest pain population with suspected ACS, which is representative of the one commonly dealt with in the emergency department. Our study suggests that BNP and/or hsCRP in addition to the troponins may be supplementary biomarkers in risk stratification. However, their impact in the clinical prognostic assessment of ACS patients depends on the presence of troponin release.

6.2.1 Limitations

The potential limitations of our study merit consideration. The circulating concentrations of BNP and hsCRP prior to hospitalization remain unknown and our analyses are based on a single baseline determination. Although we did not adjust for LVEF, we did adjust for known CHF and CVD, including previous MI, and other clinical risk factors.

6.3 Paper 3

This prospective observational study was designed to evaluate the prognostic utility of 25(OH)D in admission samples from consecutively included chest pain patients with suspected ACS in a beef-eating population living at a high altitude in a subtropical inland city of Argentina. We performed a comparative interquartile analysis of 25(OH)D as a prognostic biomarker in the total patient population, and in subgroups with and without TnT release, respectively. After correcting for other possible confounders including cardiovascular risk factors, we were able to demonstrate a statistically significant association between reduced levels of 25(OH)D and 2-year survival , including cardiac death and SCD, both in the total population and in patients with a TnT release.

Several other observational and epidemiological studies have also shown an inverse association between both all-cause and cardiac mortality and vitamin D. In the NHANSE III study, the lowest 25(OH)D quartile was associated with a higher risk of all-cause mortality in the general US population (84) as well as in older US adults (age >65) (85). In the general US population, the CVD mortality showed a similar trend, but did not remain statistically significant in the fully adjusted model, whereas CVD mortality was found to be statistically significant in the older US population. The Tromsø study (86) showed a significantly increased risk of all-cause death in the lowest 25(OH)D quartile as compared to the highest in the non-smoking population, but did not predict CVD outcome. Furthermore, low levels of 25(OH)D were associated with all-cause and cardiovascular mortality in the LURIC study (87), which included clinically stable patients referred for coronary angiography. In the Nurses' Health Study and the Health Professionals Follow-Up Study (88) it was suggested that a higher vitamin D intake correlated with a lower risk of CVD in men, but not in women. In the Mini-Finland Health Survey, Kilkkinen et al. (41) demonstrated that a low level of 25(OH)D may be associated with a higher risk of a fatal CVD event. Finally, two other studies suggested a higher prevalence of 25(OH)D deficiencies in patients with acute MI (42,89).

In our study, patients were recruited from a subtropical area at an altitude above 1000 m and included a predominantly Hispanic population. Despite the geographical location there were significant seasonal changes in 25(OH)D levels. The dietary contribution of vitamin D is probably negligible in this population as the intake of fatty fish, the primary source of vitamin D, is very low. Also, food fortification with vitamin D in Argentina was only introduced at the end of 2010. Despite the availability of vitamin D through sun exposure, a high proportion of the population demonstrated subnormal levels of 25(OH)D, which could be explained by a lifestyle with long working hours and a siesta in the middle of the day to avoid the heat of the sun. Furthermore, the majority of the population belonged to an urban and sheltered environment. Although the patients were living at a moderate altitude, it is insufficient to promote noteworthy additional exposure to UV radiation.

In both the univariate and multivariate analyses, we demonstrated a statistically highly significant increase in all-cause mortality, cardiac and SCD in Q1 as compared to Q4, both in the total patient population and in patients with TnT release. After adjusting for covariates, the prognostic utility of 25(OH)D was maintained for all end-points. ROC analysis supports our results related to low 25(OH)D values and high mortality in the total population (AUC 0.307, 95% CI [0.254 - 0.361], p = 0.000) and in the population with TnT release (AUC 0.276, 95% CI [0.213 - 0.339], p = 0.000).

A strength of this study is the inclusion of patients with suspected ACS, collection of blood samples at admission, a planned sub-group analysis according to TnT release and a prospective design, evaluating the prognostic value of 25(OH)D in relation to pre-specified endpoints consisting of total mortality, cardiac and sudden cardiac death.

6.3.1 Limitations

Concentrations of 25(OH)D in the healthy state prior to hospitalization remain unknown, and our analyses are based on a single baseline measurement. As patients in our ACS registry were strictly treated according to ACC guidelines, medication was not recorded specifically post-discharge. Differentiation of chest pain was usually performed prior to hospitalization and our patients were included after admission with a suspected ACS diagnosis. Although we did not adjust for LVEF, we did adjust for BNP and known CHF (Killip-Kimball class). Finally, we did not correct for parathyroid hormone.

6.4 Paper 4

In this paper, we assessed the prognostic utility of the omega-3 index in patients admitted with acute chest pain and suspected ACS, grouping the patients according to quartiles of the omega-3 index. After adjustment for potential confounders, there were no significant differences in the risk of all-cause mortality, cardiac death, or SCD between quartiles of the omega-3 index. No additional prognostic information was obtained by introducing the ratio between AA and omega-3 index. In contrast to this result, Von Schacky and Harris (47) previously demonstrated that an omega-3 index >8% is associated with 90% less risk for SCD, as compared to an omega-3 index of <4%. A retrospective case-control study from the US in ACS patients furthermore supported this proposed cut-off point (90). Our results are, however, in accordance with the results obtained in a coastal Norwegian chest-pain population (67).

An ingestion of omega-3 falling short of the aforementioned threshold may explain the results from studies demonstrating lack (63,91) or less benefit than expected (57) of omega-3 supplementation. Therefore, we designed the present study to search for a threshold related to mortality in a quartile analysis of the omega-3 index in a chest pain population with a low intake of omega-3 FA. We found no evidence of a threshold related to a worse outcome within the range of the adjusted omega-3 index. The median adjusted omega-3 index in the lowest as compared to the highest quartile was 2.5% and 4.8%, respectively, and an index level above 4% appeared to offer no cardiovascular protection. The low levels of the omega-3 index in our study population may be explained by the predominance of meat in the local diet. Indeed, the assumption of a possible threshold related to clinical benefits of omega-3 FA is mainly based on studies (92) of populations characterized by a considerably higher intake of omega-3 than in the present study. As we did not observe a decline in SCD within our omega-3 index quartiles, we presume that their anti-arrhythmic effect is not expressed in secondary prevention following ACS in a population with a very low intake of omega-3 FA.

The median of 4.8% in the upper quartile of the omega-3 index in the present study population is comparable to the median of 4.7% in the lowest quartile of the previously reported chest pain population from coastal Norway (67). As such, we had expected that the Argentineans would display omega-3 levels below the protective anti-arrhythmic threshold of the omega-3 FA. There was no gradient related to SCD risk through the quartiles of the omega-3 index, irrespective of TnT release during the index event, suggesting that levels higher than 4% of the omega-3 index are needed for anti-arrhythmic protection. There may be other factors required to yield a beneficial effect from omega-3 FA.

6.4.1 Limitations

Although oxidation was found to be present in approximately 40% of the total population, the remaining 60% were regarded as reliable, with a level of DPA (not included in the omega-3 index) approaching that of the Norwegian population in which the HS-Omega-3 index® was measured (93). Furthermore, in our Argentinean population, the mean (and median) level of unadjusted AA was 12% of total FA, far higher than our patient groups in coastal Norway, reflecting a high intake of meat. AA is also highly oxidizable, and its relative percentage supports the quality of our measurements. The loss of 40% of the total patient population may have introduced a bias in the interpretation of our results, but, fortunately, the baseline characteristics of patients with and without the presence of oxidation were found to be similar, supporting our conclusions. As the total patient sample was reduced due to the oxidation phenomenon, 42.2% of total deaths were also lost, which has decreased the statistical power of our study. We must emphasize that we have only studied the utility of the omega-3 index as a biomarker for future all-cause and cardiac death, and that our results should not be extrapolated to those of an interventional study.

6.5 General limitations

Weaknesses of all of the above studies include the relatively small sample size (n = 982, and n = 572 for Paper 4) compared with larger studies. Furthermore, our work included patients from only one Argentinean city; thus, the generalizability of the results to other cities, provinces, communities, and countries is uncertain. We could also not establish the time from symptoms onset to treatment in all patients.

This is not a randomized study, but a prospective, observational study with only few exclusion criteria limited to age, consent and prior inclusion, and all ACS patients who were asked to participate, accepted the invitation, except for two subjects. We cannot provide the exact number of patients who were not asked. However, in the private hospitals from which 93.7% of the study population was sourced, the admission rates were lower than in the public hospital, and all admittances were accounted for. Although we did not adjust for LVEF, we did adjust for BNP and known CHF (Killip-Kimball class).

7. FURTHER PERSPECTIVES

The ARRA-RACS is the first prospective, observational study of admitted chest pain patients held in the province of Salta. Consistent with available information, we showed that traditional risk factors and biomarkers measured at admission are associated with 2-5 year mortality in our cohort of patients.

Socioeconomic disparities in the study population also reflect disparities in the care of patients. This is associated with a worse prognosis in those patients without a healthcare insurance, accounting for approximately 60% of the general population in Argentina.

We also found that the levels of omega-3 index and vitamin D were very low in this almost uniquely beef eating population, suggesting that other threshold values related to cardiovascular risk should be considered in these individuals as compared to a fish eating population. Likewise, pharmacological intervention studies would be interesting to perform, both in primary and secondary prevention, in a cohort of patients with low levels of omega-3 index and vitamin D.

Based on this study, several implications and recommendations for public education, cardiovascular research, medical care, and public policy have emerged, as follows:

- Public education: the need to develop and implement an educational program, addressing not only reduction of specific cardiovascular disease risk factors, but also the societal conditions that lead to the adoption and maintenance of high-risk behaviors.
- Research: the quest for a better understanding of the links between economic policy, healthcare coverage, unemployment, and other economics phenomena and the prevention, incidence, treatment and follow-up of cardiovascular disease. Furthermore, particular attention should focus on the impact of socioeconomic status throughout a lifespan, including its influence during the prenatal and early-childhood stage.
- Medical care: the need to aggressively address preventive services, education and follow-up programs targeted toward the lower socioeconomic groups.

- Public policy: the need to establish a healthcare program for all inhabitants should be addressed by the government and raised as a political issue.

8. CONCLUSIONS

1. **Paper 1**

   The rate of total- and cardiac death was elevated in chest pain patients with suspected ACS belonging to the lowest socioeconomic class, and was not only related to the lack of a social security program.

2. **Paper 2**

   BNP and hsCRP may act as clinically useful prognostic biomarkers when obtained at hospital admission in an unselected chest pain population with potential ACS, and may improve risk stratification in troponin positive patients. However, these biomarkers failed to identify patients at risk in the troponin negative population.

3. **Paper 3**

   Vitamin D was shown to be a useful biomarker for prediction of mortality when obtained at admission in chest pain patients with suspected ACS.

4. **Paper 4**

   In a population with a very low intake of fish and fish oils, the omega-3 index did not predict future fatal events in patients with acute chest pain and suspected ACS.

5. **General Conclusion**

   Thus, the traditional risk markers BNP and hsCRP behaved as prognostic indicators in this prospectively studied cohort of chest pain patients with suspected ACS. Vitamin D levels and socioeconomic class were also found to provide prognostic information.

9. REFERENCES

1. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. Lancet 1997;349:1498-1504.

2. Yusuf S, Reddy S, Ôunpuu S and Anand S. Global Burden of Cardiovascular Diseases: Part I: General Considerations, the Epidemiologic Transition, Risk Factors, and Impact of Urbanization Circulation. 2001;104:2746-2753.

3. Yusuf S, Reddy S, Ôunpuu S and Anand S. Global Burden of Cardiovascular Diseases: Part II: Variation in Cardiovascular Disease by Specific Ethnic Groups and Geographic Regions and Prevention Strategies. Circulation. 2001;104:2855-2864.

4. Fuster V, Kelly BB and Vedanthan R. Promoting Global Cardiovascular Health: Moving Forward. Circulation. 2011;123:1671-1678.

5. Health minister of Argentina. National department of statistical and health information. http://www.deis.gov.ar/. Last accessed 14.12.12.

6. Bassand JP, Hamm CW, Ardissino D, Boersma E, Budaj A, Fernández-Avilés F, Fox KA, Hasdai D, Ohman EM, et al. Guidelines for the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes. Task Force for Diagnosis and Treatment of Non-ST-Segment Elevation Acute Coronary Syndromes of European Society of Cardiology, Eur Heart J. 2007 Jul; 28(13):1598-660.

7. Fuster V., Badimon L, Badimon JJ, Chesebro JH. The Pathogenesis of Coronary Artery Disease and the Acute Coronary Syndromes. N Engl J Med 1992;326:242-250.

8. Killip T 3rd, Kimball JT. Treatment of myocardial infarction in a coronary care unit. A two year experience with 250 patients. Am J Cardiol 1967;20:457-464.

9. National Argentinean Censo. http://www.censo2010.indec.gov.ar

10. Kottek M, Grieser J, Beck C, Rudolf B, Rubel F. World Map of the Köppen-Geiger climate classification updated. Meteorologische Zeitschrift 2006;15(3):259-263(5).

11. Indicadores básicos 2012. Ministerio de Salud. Presidencia de la Nación. Organización Panamericana de la Salud.

12. ClinicalTrials.gov Identifier: NCT01377402.

13. Maeda K, Tsutamoto T, Wada A, Hisanaga T, Kinoshita M. Plasma brain natriuretic peptide as a biochemical marker of high left ventricular end-diastolic pressure in patients with symptomatic left ventricular dysfunction. Am Heart J 1998;135(5 Pt 1):825-32.

14. Nakagawa O, Ogawa Y, Itoh H, Suga S, Komatsu Y, Kishimoto I, Nishino K, Yoshimasa T, Nakao K. Rapid transcriptional activation and early mRNA turnover of brain natriuretic peptide in cardiocyte hypertrophy. Evidence for brain natriuretic peptide as an "emergency" cardiac hormone against ventricular overload. J Clin Invest 1995;96(3):1280-7.

15. Motwani JG, McAlpine H, Kennedy N, Struthers AD. Plasma brain natriuretic peptide as an indicator for angiotensin-converting-enzyme inhibition after myocardial infarction. Lancet 1993;341:1109-1113.

16. Omland T, Persson A, Ng L, O'Brien R, Karlsson T, Herlitz J, Hartford M, Caidahl K. N-terminal pro-B-type natriuretic peptide and long-term mortality in acute coronary syndromes. Circulation 2002; 106(23):2913-18.

17. de Lemos JA, Morrow DA, Bentley JH, Omland T, Sabatine MS, McCabe CH, Hall C, Cannon CP, Braunwald E. The prognostic value of B-type natriuretic peptide in patients with acute coronary syndromes. N Engl J Med. 2001;345:1014-21.

18. Morrow DA, de Lemos JA, Sabatine MS, Murphy SA, Demopoulos LA, Dibattiste PM, McCabe CH, Gibson CM, Cannon CP, Braunwald E. Evaluation of B-type natriuretic peptide for risk assessment in unstable angina/non-ST-elevation myocardial infarction: B-type natriuretic peptide and prognosis in TACTICS-TIMI 18. J Am Coll Cardiol. 2003;41:1264-72.

19. de Lemos J, Morrow DA, Bentley JH, Omland T, Sabatine M, McCabe CH, Hall C, Cannon CP, Braunwald E. The Prognostic Value of B-Type Natriuretic Peptide in Patients with Acute Coronary Syndromes. N Engl J Med 2001; 345:1014-1021.

20. Morrow DA, de Lemos JA, Sabatine MS, Murphy SA, Demopoulos LA, Dibattiste PM, McCabe CH, Gibson CM, Cannon CP, Braunwald E. Evaluation of B-type natriuretic peptide for risk assessment in unstable angina/non-ST-elevation myocardial infarction: B-type natriuretic peptide and prognosis in TACTICS-TIMI 18. J Am Coll Cardiol. 2003;41:1264-72.

21. Richards AM, Nicholls MG, Espiner EA, Lainchbury JG, Troughton RW, Elliott J, Frampton C, Turner J, Crozier IG, Yandle T. B-type natriuretic peptides and ejection fraction for prognosis after myocardial infarction. Circ 2003;107:2786-92.

22. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115-126.

23. Steg PG, FitzGerald G, Fox KA. Risk stratification in non-ST-segment elevation acute coronary syndromes: troponin alone is not enough. Am J Med 2009;122:107-108.

24. Thygesen K, Mair J, Mueller C, Huber K, Weber M, Plebani M, Hasin Y, Biasucci LM, Giannitsis E, Lindahl B, Koenig W, Tubaro M, Collinson P, Katus H, Galvani M, Venge P, Alpert JS, Hamm C, Jaffe AS. Recommendations for the use of natriuretic peptides in acute cardiac care: a position statement from the Study Group on Biomarkers in Cardiology of the ESC Working Group on Acute Cardiac Care. Eur Heart J 2011;10:1093

25. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336:973-979.

26. Koenig W, Sund M, Frohlich M, Fischer H-G, Lowel H, Doring A, Hutchinson WL, Pepys MB. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation 1999;99:237-242.

27. Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH, Heimovitz H, Cohen HJ, Wallace R. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am J Med 1999;106:506-512.

28. Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J. Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet 1998;351:88-92.

29. Hwang S-J, Ballantyne CM, Sharrett AR, Smith LC, Davis CE, Gotto AM Jr., Boerwinkle E. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation 1997;96(12): 4219-25.

30. Berk BC, Weintraub WS, Alexander RW. Elevation of C-reactive protein in "active" coronary artery disease. Am J Cardiol. 1990; 65(3):168-72.

31. Ridker PM. Review Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation. 2003;107(3):363-9.

32. Myers GL, Christenson RH, Cushman M, Ballantyne CM, Cooper GR, Pfeffer CM, Grundy SM, Labarthe DR, Lew D, Rifai N, Wilson PW. National Academy of Clinical Biochemistry Laboratory Medicine Practice guidelines: emerging biomarkers for primary prevention of cardiovascular disease. Clin Chem 2009;55:378-384.

33. Burke AP, Tracy RP, Kolodgie F, Malcom GT, Zieske A, Kutys R, Pestaner J, Smialek J, Virmani R. Elevated C-reactive protein values and atherosclerosis in sudden coronary death: association with different pathologies. Circulation. 2002 Apr 30; 105(17):2019-23.

34. Genest J, McPherson R, Frohlich J, Anderson T, Campbell N, Carpentier R, Couture P, Dufour R, Fodor G, Francis GA, Grover S, Gupta M, Hegele RA, Lau DC, Leiter L, Lewis GF, Lonn E, Mancini GB, Nq D, Pearson GJ, Sniderman A, Stone JA, Ur E. 2009. Canadian Cardiovascular Society/Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult -- 2009 recommendations. Can J Cardiol 2009;25(10):567-579.

35. Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, Foster E, Hlatky MA, Hodgson JM, Kuschner FG, Lauer MS, Shaw LJ, Smith SC jr., Taylor AJ, Weintraub WS, Wenger NK, Jacobs AK; American college of Cardiology

Foundation/American Heart Association Task Force Practice guidelines. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation 2010; 122(25):2748-64.

36. National Heart, Lung, and Blood Institute. Detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel IV) (http://www.nhlbi.nih.gov/guidelines/cholesterol/atp4/index.htm).

37. Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, El-Hajj Fuleihan G, Josse RG, Lips P, Morales-Torres J. Review Global vitamin D status and determinants of hypovitaminosis D. IOF Committee of Scientific Advisors (CSA) Nutrition Working Group Osteoporos Int. 2009; 20(11):1807-20.

38. Judd SE, Tangpricha V. Review Vitamin D deficiency and risk for cardiovascular disease. Am J Med Sci. 2009 Jul; 338(1):40-4.

39. Holick MF. Review Vitamin D deficiency. N Engl J Med 2007; 357(3):266-81.

40. Pilz S, Tomaschitz A, März W, Drechsler C, Ritz E, Zittermann A, Cavalier E, Pieber TR, Lappe JM, Grant WB, Holick MF, Dekker JM. Vitamin D, cardiovascular disease and mortality. Clin Endocrinol 75(5): 575-84.

41. Kilkkinen A, Knekt P, Aro A, Rissanen H, Marniemi J, Heliövaara M, Impivaara O, Reunanen A. Vitamin D status and the risk of cardiovascular disease death. Am J Epidemiol. 2009; 170(8):1032-9.

42. Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. Arch Intern Med. 2008; 168(11):1174-80.

43. Pilz S, März W, Wellnitz B, Seelhorst U, Fahrleitner-Pammer A, Dimai HP, Boehm BO, Dobnig H. Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. J Clin Endocrinol Metab. 2008; 93(10):3927-35.

44. Holick MF, Garabedian M. Vitamin D: Photobiology, Metabolism, Mechanism of Action, and Clinical Applications. In: Favus MJ, editor. Primer on the metabolic bone diseases and disorders of mineral metabolism. 2006. pp 106-114.

45. Holick MF, Chen TC, Lu Z, Sauter E. Vitamin D and skin physiology: a D-lightful story. J Bone Miner Res 2007;22 Suppl 2.V28-33.

46. Harris WS, von Schacky C. The omega-3 index: a new risk factor for death from coronary heart disease? Prev. Med. 2004;39(1):212-20.

47. Von Schacky C, Harris WS. Cardiovascular benefits of omega-3 fatty acids. Cardiovasc Res. 2007;73(2):310-5.

48. Cao J, Schwichtenberg KA, Hanson NQ, Tsai MY. Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. Clin Chem. 2006;52(12):2265-72.

49. Metcalf RG, James MJ, Gibson RA, Edwards JR, Stubberfield J, Stuklis R, Roberts-Thomson K, Young GD, Cleland LG. Effects of fish-oil supplementation on myocardial fatty acids in humans. Am J Clin Nutr. 2007;85(5):1222-8.

50. Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med. 1985;312(19):1205-9.

51. Kromhout D, Feskens EJ, Bowles CH. The protective effect of a small amount of fish on coronary heart disease mortality in an elderly population. Int J Epidemiol. 1995;24(2):340-5.

52. Daviglus ML, Stamler J, Orencia AJ, Dyer AR, Liu K, Greenland P, Walsh MK, Morris D, Shekelle RB. Fish consumption and the 30-year risk of fatal myocardial infarction. N Engl J Med.1997;336(15):1046-53.

53. Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetham PM, Elwood PC, Deadman NM Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet. 1989;2(8666):757-61.

54. Harris WS, Poston WC, Haddock CK. Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. Atherosclerosis.2007;193(1):1-10.

55. Leon H, Shibata MC, Sivakumaran S, Dorgan M, Chatterley T, Tsuyuki RT. Effect of fish oil on arrhythmias and mortality: systematic review. BMJ 2008;337:a2931. doi: 10.1136/bmj.a2931

56. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction; results of GISSI-Prevenzione trial. Lancet, 1999;354(9177):447-55.

57. Yokohama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, Oikawa S, Sasaki J Hishida H, ItakuraH, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K; Japan EPA lipid intervention study (JELIS) investigators. Lancet 2007; 369(9567): 1090-8.

58. Rizos EC, Ntzani EE, Bika E, Kostapanos MS, and Elisaf MS. Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events. a systematic review and meta-analysis. JAMA 012;308(10):1024-1033.

59. Aarsetoey H, Grundt H, Nygaard O and Nilsen DWT. The Role of Long-Chained Marine N-3 Polyunsaturated Fatty Acids in Cardiovascular Disease Cardiology Research and Practice. Vol. 2012, Art. ID 303456, 15 pages.

60. Anand RG, Alkadri M, Lavie CJ, Milani RV. The role of fish oil in arrhythmia prevention. J Cardiopulm Rehabil Preven. 2008;28(2):92-8.

61. Jung UJ, Torrejon C, Tighe AP, Deckelbaum RJ. n-3 Fatty acids and cardiovascular disease: mechanisms underlying beneficial effects. Am J Clin Nutr. 2008;87(6):2003S-9S.

62. Lee JH, O'Keefe JH, Lavie CJ, Marchioli R, Harris WS. Omega-3 fatty acids for cardioprotection. Mayo Clinic Proceedings. 2008;83(3):324-32.

63. Nilsen DWT, Albrektsen G, Landmark K, Moen S, Aarsland T, Woie L. Effect of a high-dose concentrate of n-3 fatty acids or corn oil introduced early after an acute myocardial infarction on serum triacylglycerol and HDL cholesterol. Am J Clin Nutr 2001;74(1):50-6.

64. GRACE Investigators. Rationale and design of the GRACE (Global Registry of Acute Coronary Events) Project: a multinational registry of patients hospitalized with acute coronary syndromes. Am Heart J 2001; 141:190-199.

65. Kuck KH, Cappato R, Siebels J, Rüppel R. Randomized comparison of antiarrhythmic drug therapy with implantable defibrillators in patients resuscitated from cardiac arrest: the Cardiac Arrest Study Hamburg (CASH). Circulation 2000;102:748-754.

66. Müller-Bardorff M, Hallermayer K, Schröder A, Ebert C, Borgya A, Gerhardt W, Remppis A, Zehelein J, Katus HA. Improved troponin T ELISA specific for cardiac troponin T isoform: assay development and analytical and clinical validation. Clin Chem 1997; 43(3):458-66.

67. Aarsetoey H, Pönitz V, Grundt H, Staines H, Harris WS, Nilsen DW. (n-3) Fatty acid content of red blood cells does not predict risk of future cardiovascular events following an acute coronary syndrome. J Nutr. 2009;139:507-13.

68. Suadicani P, Hein AO, Gyntelberg F. Strong mediators of social inequalities in risk of ischaemic heart disease: a six yeras follow-up in the Copenhagen male study. Interventional Journal of Epidemiology 1997; 26:515-522.

69. Woodward M, Shewry MC, Smith WCS, Tunstall-Pedoe H. Social status and coronary heart disease: results from the Scottish Heart Health Study. Prev Med 1992;21:136-48.

70. Tunstall-Pedoe H, Woodward M, Tavendale R, A'Brook R, McCluskey M-K. Comparison of the prediction by 27 different factors of coronary heart disease and death in man and women of the Scottish heart health study: cohort study. BMJ 1997;315:722-9.

71. Woodward M, Oliphant J, Lowe G, Tunstall-Pedoe H. Contribution of contemporaneous risk factors to social inequality in coronary heart disease and all causes mortality. Prev Med. 2003;36(5):561-8.

72. Mackenbach JP, Stirbu I, Roskam AJ, Schaap MM, Menvielle G, Leinsalu M, Kunst AE; European Union Working Group on Socioeconomic Inequalities in Health.

Socioeconomic inequalities in health in 22 European countries. N Engl J Med 2008;358(23):2468-81.

73. Liu K, Cedres LB, Stamler J, Dyer A, Stamler R, Nanas S, Berkson DM, Paul O, Lepper M, Lindberg HA, Marquardt J, Stevens E, Schoenberger JA, Shekelle RB, Collette P, Shekelle S, Garside D. Relationship of education to major risk factors and death from coronary heart disease, cardiovascular diseases and all causes, findings of three Chicago epidemiologic studies. Circulation. 1982;66(6):1308-14.

74. Ishitani LH, Franco Gda C, Perpétuo IH, França E. Socioeconomic inequalities and premature mortality due to cardiovascular diseases in Brazil. Rev Saude Publica 2006;40(4):684-91.

75. Ohman EM, Armstrong PW, Christenson RH, Granger CB, Katus HA, Hamm CW, O'Hanesian MA, Wagner GS, Kleiman NS, Harrell FE Jr., Califf RM, Topol EJ, Lee KL for the GUSTO-IIa Investigators: Cardiac Troponin T Levels for Risk Stratification in Acute Myocardial Ischemia. N Engl J Med 1996; 335:1333-1342.

76. Ballantyne CM, Nambi V: Markers of inflammation and their clinical significance. Atheroscler Suppl 2005; 6(2):21-9.

77. Omland T, de Lemos JA, Morrow DA, Antman EM, Cannon CP, Hall C, Braunwald E. Prognostic value of N-terminal pro-atrial and pro-brain natriuretic peptide in patients with acute coronary syndromes. Am J Cardiol 2002; 89:463-5.

78. Olsen MH, Christensen MK, Hansen TW, Gustafsson F, Rasmussen S, Wachtell K, Borch-Johnsen K, Ibsen H, Jorgensen T, Hildebrandt P. High-sensitivity C-reactive protein is only weakly related to cardiovascular damage after adjustment for traditional cardiovascular risk factors. J Hypertens 2006;24:655-61.

79. de Winter RJ, Stroobants A, Koch KT, Bax M, Schotborgh CE, Mulder KJ, Sanders GT, van Straalen JP, Fischer J, Tijssen JG, Piek JJ. Plasma N-terminal pro-B-type natriuretic peptide for prediction of death or nonfatal myocardial infarction following percutaneous coronary intervention. Am J Cardiol 2004;94:1481-5.

80. Schnabel R, Rupprecht HJ, Lackner KJ, Lubos E, Bickel C, Meyer J, Munzel T, Cambien F, Tiret L, Blankenberg S: Analysis of N-terminal- pro-brain natriuretic peptide and C-reactive protein for risk stratification in stable and unstable coronary artery disease: results from the AtheroGene study. Eur Heart J 2005;26:241-9.

81. James SK, Lindahl B, Siegbahn A, Stridsberg M, Venge P, Armstrong P, Barnathan ES, Califf R, Topol EJ, Simoons ML, Wallentin L: N-terminal pro-brain natriuretic peptide and other risk markers for the separate prediction of mortality and subsequent myocardial infarction in patients with unstable coronary artery disease: a Global Utilization of Strategies To Open occluded arteries (GUSTO)-IV substudy. Circulation 2003;108:275-81.

82. James SK, Lindback J, Tilly J, Siegbahn A, Venge P, Armstrong P, Califf R, Simoons ML, Wallentin L, Lindahl B: Troponin-T and N-terminal pro-B-type natriuretic peptide predict mortality benefit from coronary revascularization in acute coronary syndromes: a GUSTO-IV substudy. J Am Coll Cardiol 2006; 48:1146-54.

83. Brugger-Andersen T, Ponitz V, Staines H, Pritchard D, Grundt H, Nilsen DWT: B-type natriuretic peptide is a long-term predictor of all-cause mortality, whereas high-sensitive C-reactive protein predicts recurrent short-term troponin T positive cardiac event in chest pain patient: a prognostic study. BMC Cardiovascular Disorders 2008; 8(1):34.

84. Melamed ML, Michos ED, Post W, Astor B. 25-Hydroxyvitamin D Levels and the Risk of Mortality in the General Population. Arch Intern Med 2008;168:1629-37.

85. Ginde AA, Scragg R, Schwartz RS, Camargo Jr. CA. Prospective Study of Serum 25-Hydroxyvitamin D Level, Cardiovascular Disease Mortality, and All- Cause Mortality in Older U.S. Adults. J Am Geriatr Soc 2009;57:1595-1603.

86. Hutchinson MS, Grimnes G, Joakimsen RM, Figenschau Y, Jorde R. Low serum 25-hydroxyvitamin D levels are associated with increased all-cause mortality risk in a general population: the Tromsø study. Eur J Endocrinol 2010;162:935-42.

87. Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, et al. Independent Association of Low Serum 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D Levels With All-Cause and Cardiovascular Mortality. Arch Intern Med 2008;168:1340-49.

88. Sun Q, Shi L, Rimm EB, Giovannucci EL, Hu FB, Manson JE, Rexrode KM. Vitamin D intake and risk of cardiovascular disease in US men and women. Am J Clin Nutr 2011; 94(2);534-542.

89. Lee JH, Gadi R, Spertus JA, Tang F, O'Keefe JH. Prevalence of Vitamin D Deficiency in Patients With Acute Myocardial Infarction. Am J Cardiol 2011;107:1636-1638.

90. Block RC, Harris WS, Reid KJ, Sand SA, Spertus JA. EPA and DHA in blood cell membranes from acute coronary syndrome patients and controls. Atherosclerosis 2008;197: 821-8.

91. Kromhout D, Giltay EJ, Geleijnse JM; Alpha Omega Trial Group. n-3 fatty acids and cardiovascular events after myocardial infarction. N Engl J Med 2010; 363: 2015-26.

92. Mozzafarian D, Rimm EB. Fish intake, contaminants and human health: evaluating the risks and benefits. JAMA 2006; 296:1885-99.

93. Aarsetoey H, Aarsetoey R, Lindner T, Staines H, Harris WS, Nilsen DW. Low levels of omega-3 index are associated with sudden cardiac arrest and remain stable in survivors in the subacute phase. Lipids 2011;46: 151-61.