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Research Article

Association of cardiovascular emerging risk factors with acute coronary syndrome and stroke: A case-control study

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Abstract In this study, we estimated the risk of acute coronary syndrome and stroke associated with several emerging cardiovascular risk factors. This was a case-control study, where an age - and sex-matched acute coronary syndrome group and stroke group were compared with controls. Demographic and clinical data were collected through patient interviews, and blood samples were taken for analysis. In the bivariate analysis, all cardiovascular risk factors analyzed showed as predictors of acute coronary syndrome and stroke, except total cholesterol and smoking. In the multivariate logistic regression model for acute coronary syndrome, hypertension and body mass index, N-terminal section brain natriuretic peptide and pregnancy-associated plasma protein-A were independent predictors. For stroke, the predictors were hypertension, diabetes mellitus, body mass index, and N-terminal section brain natriuretic peptide. Controlling for age, sex, and classical cardiovascular risk factors, N-terminal section brain natriuretic peptide and pregnancy-associated plasma protein-A were independent emerging cardiovascular risk factors for acute coronary syndrome, but pregnancy-associated plasma protein-A was not for stroke. High levels of cardiovascular risk factors in individuals with no episodes of cardiovascular disease requires the implementation of prevention programs, given that at least half of them are modifiable.

Key words acute coronary syndrome, cardiovascular disease, case-control study, risk factor, stroke.

INTRODUCTION

Cardiovascular disease (CVD) means any condition with a vascular origin, including acute coronary syndrome, stroke, peripheral vascular disease, aortic atherosclerosis, and aortic aneurysms. The process which causes the vast majority of vascular damage is atherosclerosis.

In the USA, this was the leading cause of death in 2011, being responsible for nearly 30% of all deaths (Hoyert & Xu, 2012). In the European Union (EU), it also represents the primary cause of death, accounting for 39% of all deaths in 2010 (European Commission, 2012), which resulted in a total cost of $216 billion (European Regional and Local Health Authorities, 2008). In Spain, CVD is also the leading cause of death; in 2012, it resulted in 30.3% of deaths (Instituto Nacional de Estadística, 2012).

According to the World Health Organization, in 2012 an estimated 17.5 million people worldwide died from CVD, representing 31% of global deaths. Of these deaths, an estimated 7.4 million were due to acute coronary syndrome and 6.7 million were due to strokes (World Health Organization, 2015). Therefore, both types of CVD are of paramount importance.

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Cardiovascular risk (CVR) is defined as the probability of suffering a cardiovascular event in a given period, which is usually within 5–10 years, and cardiovascular risk factor (CVRF), which is defined as that measurable trait that predicts an individual's likelihood of developing CVD.

Literature review

A literature search was conducted in PubMed, Cochrane Library, EMBASE, ERIC, IME and the Scielo database with a selection limit of five years from the time of the study, with the exception of landmark papers that were deemed relevant to our research. In addition, studies were selected on the basis of scientifically sound methodology and were published in any language on the condition that the summarized data was available in English. The material utilized was selected because the methodology used and the findings relating to emerging CVRF were relevant to this study, emphasizing the relationship between mentioned risk factors and both acute coronary syndrome and stroke, and the clinical implications of these findings.

Pregnancy-associated plasma protein-A (PAPP-A) has been suggested as a biomarker that produces instability and rupture of atherosclerotic plaque (Bayes-Genis *et al.*, 2001; Sangiorgi *et al.*, 2006; You *et al.*, 2010), and has also been observed in animal models (Conover *et al.,* 2010). Some studies have demonstrated the association between PAPP-A and both ischemic and hemorrhagic stroke (Fialová *et al.*, 2006). In other

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studies, PAPP-A is seen as a specific, sensitive, and early biomarker for acute coronary syndrome (Li *et al*., 2013). However, knowledge is lacking, both in relation to its method of action in different situations and with regard to other implicated co-substances, such as the insulin-like growth factor (Conover, 2012; Lawrence *et al*., 1999).

Homocysteine has been identified as an independent risk factor for acute coronary syndrome and stroke (Homocysteine Studies Collaboration, 2002), and the association between C-reactive protein (CRP) and homocysteine enables the prediction of long-term mortality in young ischemic stroke patients (Naess *et al*., 2013). Several randomized trials have attempted to decrease blood homocysteine levels with vitamin B dietary supplementation (Clarke *et al*., 2010; Martí-Carvajal *et al*., 2015).

Different applications have been assigned to N-terminal section brain natriuretic peptide (NT-proBNP) in several studies, and an acceptable diagnostic value in distinguishing ischemic stroke from other subtypes (Hajsadeghi *et al.,* 2013) also predicts presumable cardioembolic stroke independent of coronary calcification (Kara *et al.,* 2014; Yang *et al.,* 2014). Moreover, it is a significant predictor of major adverse cardiovascular events in stable coronary disease, as well as a strong predictor of death and a wide range of cardiovascular events (Linssen *et al.,* 2010; Mishra *et al.,* 2014) and some non-cardiovascular causes (Oluleye *et al.,* 2013).

The high-sensibility CRP (hsCRP) is presented in the scientific literature as a good long-term predictor of mortality in young ischemic stroke patients (Naess *et al.,* 2013), and as a strong, independent predictor of outcome in patients with acute coronary syndrome (Fiechter *et al.,* 2013). In addition, it is cited as a predictor of death, not just from CVD, but also from some non-cardiovascular causes (Oluleye *et al.,* 2013), and as an unfavorable long-term functional outcome in ischemic stroke patients (Van Gilder *et al.,* 2014).

Aim

The aim of this study was to estimate the risk of CVD, through both acute coronary syndrome and stroke, associated with various emerging CVRF (homocysteine, NT-proBNP, PAPP-A, and hsCRP) in the town of Motril (Granada, Spain).

METHODS

Design

This case-control study was composed of 201 age and sex matched patients (67 acute coronary syndrome, 67 stroke, and 67 controls). The patients were recruited from the emergency unit of the Santa Ana Hospital (Granada, Spain), and were diagnosed with acute coronary syndrome or stroke. Controls were also selected from users who attended the unit due to ophthalmic conditions or minor trauma.

Inclusion criteria for participants were acute coronary syndrome admission diagnoses (acute myocardial infarction or angina in whatever form) or stroke (hemorrhagic or ischemic), who remained as hospital inpatients and were not transferred to another referral hospital; people residing in the vicinity of the hospital; and carers of people who had disabilities. Likewise, the exclusion criteria were pregnant women; and those suffering from acute or chronic inflammatory process or cancer, acute or chronic kidney disease, acute or chronic lung disease, and acute infectious processes.

Sample

The sample size was obtained using Epidat software (version 3.0; SERGAS, Galicia, Spain) based on the prevalence contributed by previous studies on emerging CVRF included in this study. Homocysteine was the most representative variable in presenting a greater uniformity in the values in different studies. Thus, to achieve a power of 90% to detect differences in the null hypothesis, H0: $\mu 1 = \mu 2$, with a two-tailed *t*-test for two independent samples, considering the significance level was 5–15% over the number of patients for possible losses, it was necessary to include 67 experimental units in the acute coronary syndrome group, 67 in the stroke group, and 67 units in the control group, which resulted in a total of 201 enrolled participants.

The type of sample used for the selection of the acute coronary syndrome group, the stroke group, and controls was determined by systematic sampling, with a starting parameter of 320 and an interval parameter of 756. Thus, acute coronary syndrome and stroke were selected where the number of admissions most closely approached the boot parameter range and above, with a corresponding matched control starting from the first day of data collection to complete the necessary sample, which was from June 2011 to May 2013.

Ethical considerations

Approval was obtained from the Ethics Committee of the Southern Health Agency of Granada. Informed consent from the patients was also provided. This included guaranteeing the confidentiality of the obtained data. The study was conducted in accordance with the provisions of the Declaration of Helsinki.

Data collection

Data collection was conducted through interviews, during which information about age, sex, admission, diagnosis, and personal history of diagnosis of hypertension and diabetes mellitus was obtained. Height and weight were measured with a Seca 220 height–weight scale (Hamburg, Germany). Height was measured without shoes, and weight was measured with clothing on and empty pockets on admission to hospital. The smoking habits of individuals were categorized into "ex-smoker" if they had not utilized tobacco for at least one year, "smoker" if they were currently utilizing tobacco and had been doing so for at least 1 year, and "non-smoker" if they had never smoked.

Blood determinations

Blood samples for the determination of total plasma cholesterol and emerging CVRF under study were drawn using

the peripheral venous puncture system Vacutainer (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) in the first 12 h after admission. They were then analyzed in an Ethylenediaminetetraacetic acid (EDTA) tube that was kept cold until analysis by *in-vitro* quantitative determination using an enzymatic photometric test in a Roche Elecsys analyzer (Roche Diagnostics, Madrid, Spain). The measuring range was 3–750 mg/dL (0.08–19.4 mmol/L), with ≤200 mg/dL being desirable values and >240 mg/dL being high-risk values, and a stability of 20–25°C for 3 days, 7 days at 4–8°C, and 3 months at −20°C. The samples were centrifuged and analyzed within 2 h after extraction.

For the determination of NT-proBNP and PAPP-A, tubes with EDTA were utilized and immediately introduced into a cooler with ice and transported to the laboratory where an immunoassay for an *in-vitro* quantitative determination in human plasma electroquimioluminiscenia immunoassay (ECLIA) (Roche Elecsys automated analyzer; Roche Diagnostics, Spain) was used. Elecsys proBNP contains two polyclonal antibodies that recognize epitopes located in the N-terminal proBNP, (stretch), for which a sandwich technique is used with a total duration of 18 min. The measuring range of NT-proBNP was 5–35000 pg/mL, which is considered normal for values ≤100 pg/mL for men and ≤150 pg/mL for women. The samples had a stability of 3 days at 20–25°C, 6 days at 2–8°C, and 12 months at −20°C. Samples were centrifuged prior to testing, and possible effects due to the evaporation of samples, controls, and calibrators were determined within 2 h.

The same sandwich technique was used for the determination of PAPP-A, with a measuring range of 4–10,000 mIU/L. The stability of the samples was 8 h at 15–25°C, 3 days at 2–8°C, and 3 months at −20°C.

In the case of hsCRP and homocysteine, an *in-vitro* quantitative determination in human plasma by photometry using the automatic analyzer Roche/Hitachi cobas c (Roche Diagnostics, Spain), which was used in this study, was performed on a blood sample collected in the tube with EDTA. The hsCRP test is based on the principle of immune-enhanced agglutination test particles. The measuring range in this case was 0.15–20 mg/L (1.43–190 nmol/L), with a stability of 3 days at 15–25°C, 8 days at 2–8°C, and 3 years at −15 to −25°C. The result considered normal is <0.1 mg/dL. Samples were kept cold and were centrifuged within 2 h. The samples were processed by homocysteine photometry based on a novel principle that evaluates the conversion product of the homocysteine cosubstrate. The measurement range was 2.5–50 mmol/L, with a stability of 4 days at 20–25°C, 4 weeks at 0–8°C, and 4 months at −20°C. The normal range for adults is 5–100 pg/mL.

Data analysis

A descriptive analysis of the variables was performed by calculating measures of central tendency and dispersion for numeric variables, and absolute and relative frequencies for qualitative variables. With the intention of utilizing the parametric and/or non-parametric test, the normality of the variables prior to the bivariate analysis Shapiro–Wilk test was confirmed. To test whether there were differences between the three groups, for numeric variables, analysis of variance (ANOVA) test or Kruskal–Wallis test was used if the variables were not normal. The χ^2-test was used to measure the differences between categorical variables, and a level of $P < 0.05$ was considered significant. The relationship between the various traditional and emerging biomarkers with the acute coronary syndrome group and stroke group separately was analyzed using a multivariate logistic regression analysis. Furthermore, the variable selection method was backward stepwise logistic regression, where a level of $P < 0.10$ was considered significant. Analyses were performed with free software R Project for Statistical Computing version 3.0.3 (R Core Team, 2014).

RESULTS

The study population consisted of 102 men (50.75%) and 99 women (49.25%), divided into 67 acute coronary syndrome, 67 stroke, and 67 controls. No statistically-significant differences in the characteristics were found when considering the matched characteristics between the three groups. The average age of the acute coronary syndrome and stroke groups was 70.47 (standard deviation [SD]: 11.74) and 70.55 (SD: 11.73) years, respectively. Both study groups had a body mass index (BMI) classified as overweight or pre-obesity grade II (BMI = 27–29.9 kg/m^2), which was the most predominant value of BMI in acute coronary syndrome (25.37%), and the second most prevalent in stroke (23.88%). In the case of controls, the predominant group was the grade I overweight (BMI = 25–26.9 kg/m^2) at 41.79%. Only 17.91% of the controls had a BMI corresponding to normal weight (BMI = 18.5-24.9 kg/m^2) compared to 40.3% of the controls. The mean total cholesterol values of the controls was greater than the mean of the two study groups. In contrast, the levels of emerging CVRF in both study groups were higher than those of the controls (Table 1).

After controlling for age and sex, most CVRF considered in this study were significantly different between each study group and the control group. In the logistic regression analysis performed for each study group, all CVRF behaved as significant predictors of stroke or acute coronary syndrome, except smoking and total cholesterol levels for the stroke group (Table 2).

In the multivariate analysis, maintaining a significance level of $P < 0.05$, for the acute coronary syndrome group, blood pressure and BMI as adjustable CVRF, and NT-proBNP and PAPP-A as emerging CVRF, continued to be significant, independent predictors. For stroke, it was also the diabetes group, but not PAPP-A (Table 3).

The area under the Receiver Operating Characteristic (ROC) curve for the resulting model of multivariate analysis for the ACS group was 0.988, and 0.955 for the stroke group (Figs 1 and 2).

DISCUSSION

Using a matched case-control design to minimize the confounding effect, the hypertension, BMI, NT-proBNP, and PAPP-A variables remained as predictors of acute coronary syndrome. For stroke, it was also diabetes, but not PAPP-A (Table 3).

The high prevalence of hypertension in our elderly sample was consistent with national estimates for this age group (Mancia *et al.,* 2013). In the control group, despite

Table 1. Comparison of clinical and demographic characteristics between acute coronary syndrome and stroke ($n = 134$) and controls ($n = 67$)[†]

		Acute coronary syndrome group	Stroke group	Control group
Age (years)		70.4 ± 11.7	70.5 ± 11.7	70.1 ± 12.1
Sex (%)	Male	34 (50.75%)	34 (50.75%)	34 (50.75%)
	Female	33 (49.25%)	33 (49.25%)	33 (49.25%)
Diabetes mellitus (%)		23 (34.33%)	29 (43.28%)	3 (4.48%)
Hypertension (%)		47 (70.15%)	53 (79.1%)	10 (14.93)
Smoking (%)	Yes (%)	15 (22.39%)	6 (8.96%)	10 (14.93%)
	Exsmoker (%)	29 (43.28%)	23 (34.33%)	13 (19.40%)
	No (%)	23 (34.33%)	38 (56.72%)	44 (65.75%)
Body mass index (kg/m^2)		29.54 ± 4.98	28.11 ± 4.38	25.14 ± 3.04
Total colesterol (mg/dL)		175.02 ± 43.43	188 ± 35.90	192.02 ± 31.35
Homocystein (pg/mL)		14.34 ± 13.21	15.60 ± 10.09	9.74 ± 2.82
NT-proBNP (pg/mL)		2691.24 ± 705.60	2420.29 ± 448.34	46.23 ± 35.84
hsPCR (mg/dL)		2.09 ± 4.47	2.58 ± 5.39	0.31 ± 0.68
PAPP-A (mUI/L)		10.29 ± 7.64	7.38 ± 5.06	4.20 ± 1.21

[†]Means and standard deviations are shown for continuous variables; frequencies shown for categorical variables. hsPCR, high-sensibility C-reactive protein; NT-proBNP, N-terminal brain natriuretic peptide; PAPP-A, pregnancy-associated plasma protein A.

Table 2. Bivariate conditional logistic regression model of emerging and classic cardiovascular risk factor for acute coronary syndrome and stroke in Motril, Spain ($n = 201$)

		Acute coronary syndrome OR (95% CI)	*P*-value	Stroke OR (95% CI)	*P*-value
Hypertension		13.07 (5.33–34.82)	<0.001	20.87 (8.17–58.70)	<0.001
Diabetes mellitus		10.97 (3.04–60.53)	<0.001	15.96 (4.49–87.27)	<0.001
Smoking[†]	Exsmoker	4.26 (1.90–10)	<0.001	0.48 (0.21–1.08)	0.08
	Smoker	2.86 (1.12–7.58)	0.02	1.43 (0.48–4.57)	0.51
Body mass index		1.34 (1.20–1.54)	<0.001	1.26 (1.12–1.40)	<0.001
Total cholesterol		0.98 (0.97–0.99)	0.010	0.99 (0.98–1.00)	0.582
NT-proBNP		1.02 (1.01–1.03)	<0.001	1.02 (1.01–1.03)	<0.001
hsPCR		2.23 (1.22–4.07)	0.02	2.28 (1.24–4.16)	0.007
Homocystein		1.23 (1.11–1.35)	<0.001	1.24 (1.13–1.36)	<0.001
PAPP-A		2.08 (1.60–2.70)	<0.001	1.91 (1.47–2.48)	<0.001

Level of significance is $P < 0.05$. Reference group for both groups of cases is the control group. [†]Reference group is "non-smokers". hsCRP, high-sensibility C-reactive protein; NT-proBNP, N-terminal brain natriuretic peptide; OR, odds ratio; PAPP-A, pregnancy-associated plasma protein A; 95% CI, 95% confidence interval.

Table 3. Multivariate conditional logistic regression model of emerging and classic cardiovascular risk factor for acute coronary syndrome and stroke in Motril, Spain ($n = 201$)

	Acute coronary syndrome OR (95% CI)	*P*-value	Stroke OR (95% CI)	*P*-value
Diabetes mellitus	—	NS	5.45 (1.82–36.32)	0.08
Hypertension	12.40 (6.97–21.45)	0.011	3.87 (1.00–14.96)	0.05
Body mass index	1.50 (1.05–2.57)	0.003	1.27 (1.08–1.56)	0.01
NT–proBNP	1.04 (1.02–1.08)	<0.001	1.02 (1.01–1.03)	<0.001
PAPP-A	2.91 (1.62–7.29)	0.002	—	NS

Level of significance is $P < 0.10$. NT-proBNP, N-terminal brain natriuretic peptide; NS, not significant; OR, odds ratio; PAPP-A, pregnancy-associated plasma protein A; 95% CI, 95% confidence interval.

the lower prevalence of hypertension, nearly one-quarter presented a medium-high risk of CVD according to the Systematic Coronary Risk Evaluation (SCORE) risk scale adapted to the Spanish population (Sans *et al.,* 2007), which enabled us to factor in the occurrence of future cardiovascular events over the next 10 years in the population, because CVRF are modifiable through healthy lifestyles (Martínez *et al.,* 2014).

Likewise, the high prevalence of diabetes in our population is consistent with estimates related to age and sex according to the Di@bet.es Study on the prevalence of diabetes mellitus conducted in Spain in 2012 (Soriguer

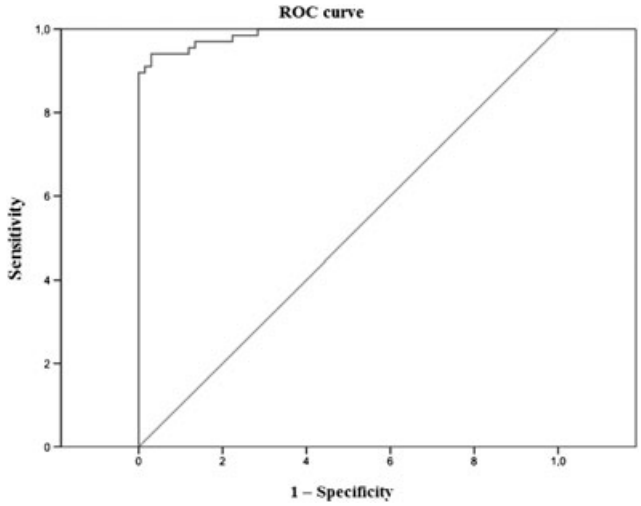

Figure 1. Receiver Operating Characteristic curve model of multivariate analysis of emerging and classic cardiovascular risk factors for acute coronary syndrome.

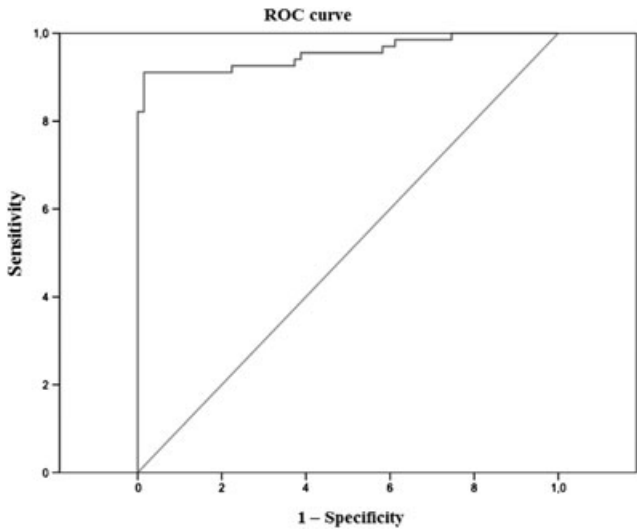

Figure 2. Receiver Operating Characteristic curve model of multivariate analysis of emerging and classic cardiovascular risk factors for stroke.

et al., 2011), where the prevalence in the control group was much lower here than in the acute coronary syndrome and stroke groups.

The means of the BMI show how the population of Motril suffering CVD can be classified as overweight or pre-obesity grade II, three-quarters of the aforementioned being above the normal weight. These numbers are higher than the mean of the Spanish population, among whom nearly half present with obesity or could be defined as being overweight (Rodríguez *et al.,* 2011). In addition, the average BMI of the control group can be classified as overweight, which enables us to anticipate future avoidable CVD events. Currently, there is no evidence to justify the replacement of BMI by any other form of measurement of overweight–obesity (European Society of Cardiology, 2012). One of the limitations of the SCORE risk scale is that it does not include the BMI in its algorithm, and, given the independent association with CVD, this is an important consideration that must be taken into account when the CVR is evaluated.

In terms of total cholesterol, this does not appear as CVRF, with statistical significance in both study groups, and the means of the two study groups and the control group, being very similar. A similar occurrence takes place with smoking, which did not appear to be statistically significant in the case of the stroke group in the bivariate analysis, but was significant in the acute coronary syndrome group. This significance disappears in the multivariate analysis, because in the logistic regression analysis, the categories of "smoker" and "ex-smoker" did not show statistical significance when compared with the "non-smoker" group.

As an emerging CVRF in the acute coronary syndrome group, NT-proBNP had a weak but statistically-significant association. Several studies have shown the relationship between NT-proBNP and CVD as well as other causes of mortality in

the general population and in the population with coronary heart disease, particularly among the elderly, including stroke (Oluleye *et al*., 2013; Linssen *et al*., 2010; Mishra *et al.,* 2014; Odden *et al*., 2014). In Niu *et al*.'s study, the plasma concentration of NT-proBNP was closely related to the size of the necrotic cardiac injury in patients with acute myocardial infarction and could be used to assess its size (Niu *et al*., 2014). However, in our study, NT-proBNP showed a weak statistical significance in the stroke group that disappeared on multivariate analysis. This could be explained by the presence of cardioembolic stroke caused by atrial fibrillation, which produces elevated NT-proBNP (Kara *et al.,* 2014; Hajsadeghi *et al*., 2013). In their meta-analysis, Yang *et al.* suggests that NT-proBNP is a good useful diagnostic marker for distinguishing cardioembolic stroke from other categories, with the consequential benefit for these patients being the initiation of preventive anticoagulant therapy (Yang *et al.,* 2014). Moreover, biochemical studies have indicated that cerebral ischemia generates NT-proBNP secretion by brain tissue (Nogami *et al.,* 2001).

Our study demonstrates a relationship of PAPP-A to the specific cardiovascular outcomes of acute coronary syndrome, but not stroke, in the general population. PAPP-A has been shown to be present in advanced stages of atherosclerosis, and was significantly associated as CVRF with both study groups in the bivariate analysis, but lost this significance to stroke in the multivariate analysis (Li *et al.,* 2013). Our results support the findings of previous studies that showed PAPP-A as a marker of unstable atherosclerotic plaques. PAPP-A is produced by many cell types, both in reproductive (testicles and endometrium) and non-reproductive tissues, including fibroblasts, vascular smooth muscle cells, and endothelial cells (Conover, 2012) Moreover, macrophages can contribute to PAPP-A overexpression due to the production of pro-inflammatory cytokines interleukin-1β and tumor necrosis factor-α. Degradation of some of these cytokines makes PAPP-A help insulin-like growth factor-1 to carry out its action (Lawrence *et al.,* 1999) Therefore, PAPP-A has a very important role in the development of atherosclerotic lesions in animal models (Conover, *et al*. 2010). In 2001, in one of the first studies conducted by Bayes-Genis *et al*., patients with coronary disease had levels of PAPP-A greater than those without this condition, which was then proposed as a new, emerging CVRF in unstable angina and acute myocardial infarction (Bayes-Genis *et al.,* 2001). A meta-analysis conducted by Li *et al*. of 14 studies and 12,830 participants concluded that high levels of PAPP-A are associated with adverse events in patients with coronary heart disease (Li *et al.,* 2013). PAPP-A has also been proposed as an early marker of atherosclerotic plaque rupture (Sangiorgi *et al.,* 2006). Moreover, in our study, no statistically-significant association between levels of PAPP-A and stroke was found; it was noted that there is minimal scientific literature available regarding this. However, in studies, such as those of Fialova *et al*., PAPP-A levels were elevated in patients with intracranial hemorrhage or brain ischemia (Fialová *et al.,* 2006). Nevertheless, in most of these studies, episodes of stroke occurred in patients with previous coronary disease that could generate thrombus. More studies are therefore needed.

In our study, the other emerging CVRF analyzed demonstrated no association with CVD. With regard to homocysteine, a large number of epidemiological studies and meta-analyses have shown that hyperhomocysteinemia is an independent risk factor of atherosclerosis and thrombosis that is relevant in both acute coronary syndrome and stroke (Homocysteine Studies Collaboration, 2002; Zylberstein *et al.,* 2004). In the same way, treatment with folic acid to reduce levels of homocysteine, and thus prevent cardiovascular events, has been unsuccessful (Martí-Carvajal *et al.,* 2015). However, other meta-analysis have given less importance to this substance as a CVRF due to the presence of confounding factors, such as metabolic nutritional aspects and lifestyle (Clarke *et al.,* 2010). Further to this, hsCRP demonstrated a statistically-significant association with both acute coronary syndrome and stroke in the univariate analysis, an effect that disappeared in the multivariate analysis. At present, the mechanism by which this substance would be involved in CVD is not well clarified. However, its origin could reside both in the presence of a complicated atherosclerotic plaque and in a myocardial or cerebral necrosis. In this case, several studies have linked hsPCR with CVD, both coronary and cerebral, and as an independent predictor of CVRF in the evolution and outcome after suffering a cardiovascular event (Fiechter *et al.,* 2013), (Naess *et al.,* 2013). However, a series of systematic reviews and meta-analyses published in recent years have highlighted the need for more studies (van Gilder *et al.,* 2014).

The strength of this study is mainly the case-control design used. This is the most appropriate for studying rare outcomes of disease processes that develop during prolonged periods (Argimón & Jiménez, 2013). It was the most suitable for studying the association between a number of emerging CVRF and CVD in its aspect of acute coronary syndrome and stroke, as CVD is a pathophysiological process that develops over many years, so transverse and longitudinal studies are inappropriate for this population. Other strengths of this study include matching of the sample by age and sex to minimize the important confounding effects of them, and the consideration of classic CVRF that could also have caused confounding effects.

Limitations

Despite its careful design and analysis, which were both tailored to our working conditions and economic constraints, our study has some limitations. The first of these is its design as a case-control study, which could limit the possibility of applying the findings more widely. However, our study could contribute to the design of future investigations with different methodologies that might serve to validate our results and lead to improvements in clinical practice.

It is known that most CVD is caused by one or more identifiable factors (CVRF) and in our study we assumed that CVD was caused by these. However, we cannot state for certain that there might not have been other unrecognized causes of CVD that were consequently not taken into account. Furthermore, while the potential confounding effects of classic CVRF were considered, we cannot rule out the possibility that the increase in blood levels of emerging CVRF included in this study might have been due to unknown reasons.

Conclusions

In summary, when taking into account the control of the confounding effects of age, sex, hypertension, diabetes mellitus, BMI, smoking, and total cholesterol, only NT-proBNP was found to be an independent predictor for acute coronary syndrome and stroke. However, PAPP-A was only found to be a good independent predictor for acute coronary syndrome, but not for stroke. The high levels of CVRF, both classical and emerging, in individuals with no CVD episodes requires the implementation of prevention programs, given that least half of them are modifiable, thereby further contributing to economic savings for our public health system and society in general.

After controlling for age, sex, and classic CVRF, PAPP-A was found to be a good predictor and biomarker of the specific cardiovascular outcome acute coronary syndrome. Its etiology and mechanism of action require further investigation. Moreover, the existence of studies and meta-analyses with conflicting results on the use of emerging cardiovascular risk factors that were included in our study makes more research necessary. It could therefore be concluded that based on sufficient evidence, PAPP-A could be used for the determination of CVR, and possibly be included in the prediction algorithms of CVR.

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CONTRIBUTIONS

Study Design: JMML, RGB, FMOM, FJSP
Data Collection and Analysis: JMML, RGB, FMOM, FJSP
Manuscript Writing: JMML, RGB, FMOM, FJSP

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