Association of Calcium Channel Blocker Use and Pregnancy-Associated Plasma Protein-A Among Older Adults With Hypertension: Results From the iLSIRENTE Study

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Background. Pregnancy-associated plasma protein A (PAPP-A) is a zinc-binding matrix metalloproteinase (MMP) that was shown to increase in acute coronary syndromes. Calcium channel blockers (CCB) can influence the production of several MMPs, but no study, so far, has assessed the association between use of CCB and PAPP-A levels. The aim of the present cross-sectional study was to evaluated if, among older hypertensive adults, PAPP-A levels differ according to use of CCB.

Methods. Data are from the baseline evaluation of the iLSIRENTE study, which enrolled 364 participants 80 years old or older. For the present study, we selected 314 participants with hypertension. Analyses of covariance were performed to evaluate the differences in PAPP-A levels according to use of CCB.

Results. Mean age of participants was 85.6 years (standard deviation [SD] 4.8), 206 (66%) were women; 58 participants (19%) were using a CCB. After adjusting for potential confounders, concentration of PAPP-A was significantly lower in CCB users than in nonusers (1.58 mIU/L, 95% confidence interval [CI], 1.37–1.81 vs 1.86 mIU/L, 95% CI, 1.74–1.98; p = .03). This association was still consistent after exclusion of participants with cardiovascular disease (1.53 mIU/L, 95% CI, 1.30–1.80 vs 1.90 mIU/L, 95% CI, 1.78–2.03; p = .01).

Conclusions. Use of CCB is associated with lower levels of PAPP-A. These findings need to be confirmed in prospective studies.

Methods

The iLSIRENTE study is a prospective cohort study performed in the mountain community living in the Sirente geographic area (L’Aquila, Abruzzo) in Central Italy (13). The main aims of the iLSIRENTE study are: (i) to provide comprehensive clinical and biological information on the most important diseases in very old persons; (ii) to assess functional impairment and physical disability among very old people living in the community; (iii) to recognize multiple risk factors influencing loss of physical function among very old people in the community; (iv) to categorize the physiologic subsystems crucial for physical function and their relationship with the surrounding environment; and (v) to translate biological and clinical data into assessment tools for the early detection of physical disability.

Briefly, a preliminary list of all community-dwelling older adults born before January 1, 1924, living in the Sirente area was obtained from the 13 municipalities involved in the study. Of the initial 429 persons eligible, prevalence of refusals was 15%. As a result, the overall sample population enrolled in the iLSIRENTE study consisted of 364 persons. For the present analysis, from this sample we excluded persons lacking data on PAPP-A (n = 9) and those without hypertension (n = 41), resulting in a final sample size of 314 participants. PAPP-A levels did not differ significantly
between participants with and without hypertension (1.80 mIU/L, 95% confidence interval [CI], 1.70–1.91 vs 1.93 mIU/L, 95% CI, 1.70–2.11; p = .42).

The Minimum Data Set for Home Care (MDS-HC) form was administered to all study participants by trained assessors, following the guidelines published in the MDS-HC manual (14,15). Clinical diagnoses were recorded by study physicians based on information collected from the patient and the general practitioner, including results of a physical examination, careful review of patient clinical documentation (including laboratory tests and x-rays), and previous medical history. Hypertension was considered to be present if participants reported a history of physician-diagnosed hypertension, presented a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg, or were receiving any antihypertensive medication (CCB, angiotensin-converting enzyme inhibitors, beta blockers, thiazides, angiotensin receptor blockers, clonidine, or alpha blockers).

### Blood Measurements
Approximately 97% of participants consented to phlebotomy in the home as previously described (19). PAPP-A levels in the sera were measured by means of a commercially available high-sensitivity kit (Ultra-sensitive PAPP-A enzyme-linked immunosorbent assay [ELISA]; Diagnostic Systems Laboratories, Inc, by Pantec S.r.l, Turin, Italy). The detection limit for this method is 0.06 mIU/L. As described by Khosravi and colleagues (20), this method is suitable for studying the relationships between heart diseases and IGF-1/PAPP-A and PAPP-A concentration. C-reactive protein was measured by using a high-sensitivity ELISA kit (Bender MedSystems, Vienna, Austria).

### Data Analyses
Baseline characteristics according to CCB use were compared using analysis of variance (ANOVA) for normally distributed variables, nonparametric Kruskal-Wallis H tests for skewed variables, and chi-square analyses for dichotomous variables. Given the nonnormal distribution of PAPP-A, analyses were performed using log-transformed values. Analysis of covariance (ANCOVA) was used to compare adjusted means of log-transformed PAPP-A according to CCB use. Geometric means of PAPP-A, calculated from log-transformed values, are shown in tables and text.

Variables considered for adjustment in the ANCOVA models were those associated with CCB use at p ≤ .10 at the univariate analysis and those thought to be clinically significant.

In additional analyses, geometric means of PAPP-A were calculated among CCB users (n = 58), users of no antihypertensive drugs (n = 117), and users of other antihypertensive drugs (n = 139). Bonferroni adjustment test was used for comparisons of PAPP-A means across these three groups. All analyses were performed using SPSS software (version 10.1; SPSS Inc., Chicago, IL).

### Results
The mean age of 314 study participants was 85.6 years (standard deviation [SD] 4.8); 206 participants (66%) were women. In this study sample, PAPP-A values ranged from 0.18 to 10.30 mIU/L (mean 2.07, median 1.85, interquartile ratio 1.32–2.44); 58 participants (19%) were using a CCB. The most commonly used CCB was amiodipine (n = 19; 6% of study sample), followed by nifedipine (n = 11; 4%) and verapamil (n = 7; 2%). One participant was receiving two CCB (amiodipine and nifedipine).

The distribution of demographic, functional, clinical, and biochemical characteristics according to CCB use is presented in Table 1. Overall, CCB users were more likely to present with ADL disability, cognitive impairment, coronary artery disease, and cerebrovascular disease and were less physically active and had lower heart rate and systolic and diastolic blood pressure than nonusers, but these two groups did not significantly differ in the other variables examined (including age, gender, body mass index, and concentration of albumin, low-density [LDL] and high-density lipoprotein [HDL] cholesterol and C-reactive protein).

As shown in Table 2, according to the univariate analysis, concentration of PAPP-A was significantly lower among CCB users than among nonusers (1.57 mIU/L, 95% CI, 1.40–1.76 vs 1.86 mIU/L, 95% CI, 1.74–1.99; p = .03). This result was confirmed after adjusting for potential confounders, including age, gender, cognitive impairment, ADL disability, coronary artery disease, congestive heart failure, cerebrovascular disease, physical activity, heart rate, systolic blood pressure, and diastolic blood pressure. This association was consistent both in men (adjusted means: 1.68 mIU/L, 95% CI, 1.28–2.19 vs 2.09 mIU/L, 95% CI, 1.86–2.34; p = .16) and women (adjusted means: 1.50 mIU/L, 95% CI, 1.28–1.77 vs 1.75 mIU/L, 95% CI, 1.62–1.89; p = .10). In addition, after exclusion of participants with cardiovascular disease (including coronary artery disease, congestive heart failure, cerebrovascular disease, and peripheral artery disease; n = 64) concentration of PAPP-A was still significantly lower among CCB users than among nonusers (adjusted means: 1.53 mIU/L, 95% CI, 1.30–1.80 vs 1.90 mIU/L, 95% CI, 1.78–2.03; p = .01).

Among participants not receiving CCB, 117 were not using any antihypertensive drugs and 139 were using other antihypertensive drugs. As shown in Figure 1, CCB users had lower concentrations of PAPP-A when compared with users of other antihypertensive drugs (adjusted mean: 1.90 mIU/L, 95% CI, 1.75–2.08; p vs CCB users = .06) and with users of...
no antihypertensive drugs (adjusted mean: 1.80 mIU/L, 95% CI, 1.63–1.98; \(p = .26\)), but these associations did not reach statistical significance.

**DISCUSSION**

The present study shows that, among older adults (80 years old or older) with hypertension, use of CCB is associated with lower levels of PAPP-A. This difference was consistent after exclusion of participants with cardiovascular disease.

PAPP-A is a large, zinc-binding MMP produced by different types of cells, including fibroblasts, vascular smooth muscle cells, and male and female reproductive tissues (1). In addition, PAPP-A is produced by activated cells in unstable coronary plaques, and circulating levels of PAPP-A were significantly elevated in patients with acute coronary syndromes, chronic stable angina, and peripheral artery disease (2–8). This molecule was shown to exert a proatherosclerotic effect by degradation of IGF-1 binding proteins 4 and 5, therefore allowing active IGF-1 to bind to cell-surface receptors (1). IGF-1 induces macrophage activation, chemotaxis, LDL cholesterol uptake by macrophages, and the release of proinflammatory cytokines by these cells. However, it has been also suggested that PAPP-A may not have a causal effect in the determination of coronary syndromes, but can rather represent a sensitive signal of damage, similar to those exerted by other acute-phase proteins, being part of an endogenous compensatory pathway aimed at tissue physiological repair and replicative programs (21).

<table>
<thead>
<tr>
<th>Variables</th>
<th>CCB, N = 58 (%)</th>
<th>No CCB, N = 256 (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (mean ± SD)</td>
<td>85.6 ± 4.6</td>
<td>85.7 ± 4.8</td>
<td>.91</td>
</tr>
<tr>
<td>Female gender</td>
<td>39 (67)</td>
<td>167 (66)</td>
<td>.80</td>
</tr>
<tr>
<td>ADL disability*</td>
<td>21 (36)</td>
<td>64 (25)</td>
<td>.08</td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>15 (27)</td>
<td>40 (16)</td>
<td>.05</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>12 (21)</td>
<td>27 (11)</td>
<td>.04</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>6 (10)</td>
<td>13 (5)</td>
<td>.14</td>
</tr>
<tr>
<td>Diabetes</td>
<td>25 (43)</td>
<td>103 (40)</td>
<td>.71</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>7 (12)</td>
<td>7 (3)</td>
<td>.002</td>
</tr>
<tr>
<td>COPD</td>
<td>6 (10)</td>
<td>39 (15)</td>
<td>.33</td>
</tr>
<tr>
<td>Peripheral artery disease</td>
<td>1 (2)</td>
<td>7 (3)</td>
<td>.66</td>
</tr>
<tr>
<td>Cancer</td>
<td>2 (3)</td>
<td>11 (4)</td>
<td>.77</td>
</tr>
<tr>
<td>Body mass index, kg/m² (mean ± SD)</td>
<td>25.6 ± 4.2</td>
<td>26.0 ± 4.6</td>
<td>.58</td>
</tr>
<tr>
<td>Physical activity</td>
<td>29 (50)</td>
<td>158 (63)</td>
<td>.08</td>
</tr>
<tr>
<td>Albumin, g/dL (mean ± SD)</td>
<td>4.2 ± 0.3</td>
<td>4.2 ± 0.3</td>
<td>.29</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL (mean ± SD)</td>
<td>135.5 ± 40.6</td>
<td>130.7 ± 38.6</td>
<td>.40</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL (mean ± SD)</td>
<td>46.6 ± 12</td>
<td>45.9 ± 14</td>
<td>.75</td>
</tr>
<tr>
<td>Urea, mg/dL (mean ± SD)</td>
<td>50.3 ± 22.0</td>
<td>48.5 ± 22.1</td>
<td>.55</td>
</tr>
<tr>
<td>C-reactive protein, pg/mL (median, IQR)</td>
<td>3.5 (1.6–6.3)</td>
<td>3.0 (1.4–5.8)</td>
<td>.36</td>
</tr>
<tr>
<td>Heart rate, bpm (mean ± SD)</td>
<td>73.6 ± 9.5</td>
<td>76.5 ± 8.2</td>
<td>.02</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg (mean ± SD)</td>
<td>79.3 ± 11.4</td>
<td>84.1 ± 13.7</td>
<td>.02</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg (mean ± SD)</td>
<td>143.4 ± 21.3</td>
<td>152.2 ± 24.4</td>
<td>.01</td>
</tr>
</tbody>
</table>

*Need of assistance in one or more of the following ADLs: dressing, eating, toileting, bathing, mobility in bed, locomotion, transfer.

\(SD = \) standard deviation; COPD = chronic obstructive pulmonary disease; ADL = Activities of Daily Living; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

**Table 2. Association Between Use of Calcium Channel Blockers (CCB) and PAPP-A**

<table>
<thead>
<tr>
<th>Use of CCB</th>
<th>Unadjusted PAPP-A mIU/L</th>
<th>Adjusted PAPP-A mIU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)*</td>
</tr>
<tr>
<td>Total sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CCB, n = 256</td>
<td>1.86 (1.74–1.99)</td>
<td>1.86 (1.74–1.98)</td>
</tr>
<tr>
<td>CCB, n = 58</td>
<td>1.57 (1.40–1.76)</td>
<td>1.58 (1.37–1.81)</td>
</tr>
<tr>
<td>Sample without participants with cardiovascular disease1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CCB, n = 213</td>
<td>1.90 (1.77–2.04)</td>
<td>1.90 (1.78–2.03)</td>
</tr>
<tr>
<td>CCB, n = 37</td>
<td>1.52 (1.28–1.79)</td>
<td>1.53 (1.30–1.80)</td>
</tr>
</tbody>
</table>

*Adjusted for age, gender, cognitive impairment, Activities of Daily Living disability, coronary artery disease, congestive heart failure, cerebrovascular disease, physical activity, heart rate, systolic blood pressure, and diastolic blood pressure.

Cardiovascular disease includes coronary artery disease, congestive heart failure, cerebrovascular disease, and peripheral artery disease.

PAPP-A = pregnancy-associated plasma protein-A; CI = confidence interval.

Notes: Median and interquartile range (IQR) are shown for non-normal distributed variables.

**Table 1. Sample Characteristics According to Use of Calcium Channel Blockers (CCB)**

Notes: Geometric means are calculated from log-transformed values.

1Defined as Cognitive Performance Scale (CPS) score ≥ 2; data on CPS were missing for 3 participants.

PAPP-A = pregnancy-associated plasma protein-A; CI = confidence interval.
Previous observations have suggested an effect of CCB on the production of other MMPs. Ikeda and colleagues (12) showed that, in endothelial cells, amldipine significantly reduced the expression of MMP-1 induced by interleukin 1. This effect was not observed for nifedipine. More recently, Canavesi and colleagues (22) demonstrated that lercanidipine inhibited the release of MMP-2 and MMP-9 by macrophages. In addition, Yue and colleagues (23) showed that, in cultured rat fibroblasts, amldipine reduced and nifedipine increased the expression of MMP-2. In this latter study, neither verapamil nor diltiazem significantly altered MMP-2 expression (23). Finally, Farias and colleagues (24) demonstrated that, in murine carcinoma cells, verapamil inhibited the secretion of MMP-9, which is a critical factor for tumor invasion and metastasis. In contrast with these observations, Roth and colleagues (11) found that five different CCB can modulate metabolism of collagen within the extracellular matrix by increasing the proteolytic activity of MMP-2 secreted by human vascular smooth muscle cells. The reason for the effect of CCB on MMPs (including PAPP-A) production is not clear, but it probably relates to their antioxidant action (25). Indeed, enhanced oxidative stress is a major factor modulating MMP expression/activity, and reactive oxygen species have shown to be involved in vascular remodeling via MMP activation.

Based on our findings, it may by hypothesized that CCB may exert part of their antianginal and antiatherosclerotic effect by inhibition of PAPP-A. Indeed, it has been previously demonstrated in randomized clinical trials that CCB can significantly reduce atherosclerotic lesion formation and progression, and these finding were consistent with the results of animal studies (10). The International Nifedipine Trial on Antiatherosclerotic Therapy demonstrated that, in patients with mild coronary artery disease, nifedipine substantially suppresses disease progression as shown by the appearance of new lesions detectable by quantitative coronary arteriography (26). The Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial showed that amldipine therapy was associated with statistically significant slowing of the progression of carotid atherosclerosis, independent of blood pressure changes, and a reduced risk of cardiovascular morbidity (27). In the Coronary Angioplasty Amlodipine Restenosis Study, amldipine reduced the incidence of repeat percutaneous transluminal coronary angioplasty (PTCA) and clinical events after PTCA without a reduction in luminal loss (28). Finally, the European Lacidipine Study on Atherosclerosis showed that lacidipine (compared with atenolol) slows progression of intima-media thickness (29).

Levels of PAPP-A measured in this study differed from those observed in other studies. This difference may be related to two factors. First, we used an ultrasensitive ELISA procedure for PAPP-A determination, which has a greater sensitivity and a much lower detection limit than the conventional methods. This ultrasensitive ELISA procedure is capable of accurate determination of PAPP-A in nonpregnancy range, while providing specificity and analytical performance characteristics comparable to those found by using the conventional method. In a previous study, levels of PAPP-A determined with this method were shown to be significantly related to markers of myocardial damage (18). Second, the population of the present study includes persons 80 years old or older who have rarely been included in previous observations assessing the role of PAPP-A.

In the analysis examining PAPP-A levels according to antihypertensive drug use, use of CCB was associated with lower levels of PAPP-A compared with use of other antihypertensive drugs and no use of antihypertensive medications, but these results did not reach statistical significance. Indeed, the small sample size of the study may have limited the power of the analyses. Therefore, the association between different antihypertensive drug regimens and PAPP-A levels must be confirmed in larger studies and extended to younger populations.

In addition, the present study has other limitations. First, the ilSIRENTE sample population was composed of persons 80 years old or older, so our results may not be applicable to other age groups. Second, despite the fact that the analyses were adjusted for many health and disease-related characteristics, there could be unmeasured confounders that were not considered in this study. Finally, the data on which our analyses are based derive from a cross-sectional observational study and to truly confirm a causal association between use of CCB and PAPP-A levels; prospective studies should be performed.

Conclusion
The present study shows that, among older adults living in the community, use of CCB is associated with lower levels of PAPP-A. If confirmed in prospective studies, this finding could help to explain the antiatherosclerotic effect of CCB shown in randomized clinical trials.
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