Original Article

# Plasma renin activity to plasma aldosterone concentration ratio correlates with night-time and pulse pressures in essential hypertensive patients treated with angiotensin-converting enzyme inhibitors/AT1 blockers

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**Objectives:** Angiotensin-converting enzyme inhibitors (ACE-I) and AT1 blockers (ARB) are commonly used antihypertensive drugs, but several factors may affect their effectiveness. We evaluated the associations between ambulatory blood pressure (BP) monitoring (ABPM) parameters and plasma renin activity (PRA)-to-plasma aldosterone concentration (PAC) ratio (RAR) to test renin–angiotensin–aldosterone system inhibition in essential hypertensive patients treated with ACE-I or ARB for at least 12 months.

**Methods:** We evaluated 194 consecutive patients referred to our Hypertension Centre. ABPM, PRA and PAC tests were performed without any changes in drug therapy. RAR, PRA and PAC tertiles were considered for the analyses.

**Results:** Mean age: $57.4 \pm 12.0$ years; male prevalence: 63.9%. No differences between RAR tertiles regarding the use of ACE-I or ARB ($P = 0.385$), as well as the other antihypertensive drug classes, were found. A reduction of all ABPM values considered (24-h BP, daytime BP and night-time BP and 24-h pulse pressure (PP), daytime PP and night-time PP) and a better BP control were observed at increasing RAR tertiles, with an odds ratio = 0.12 to be not controlled during night-time period for patients in the third tertile compared with patients in the first tertile ($P < 0.001$). This association remained significant even after adjusting for 24-h BP control. All the associations were also confirmed for PRA tertiles, but not for PAC tertiles.

**Conclusion:** Higher RAR values indicate effective renin–angiotensin–aldosterone system inhibition and lower night-time and pulse pressures in real-life clinical practice. It could be a useful biomarker in the management of essential hypertensive patients treated with ACE-I or ARB.

**Keywords:** ambulatory blood pressure measurement, blood pressure control, hypertension, night-time blood pressure, plasma aldosterone concentration, plasma renin activity, pulse pressure

**Abbreviations:** ABPM, ambulatory blood pressure monitoring; ACE-I, angiotensin-converting enzyme inhibitors; ARB, AT1 blockers; ARR, aldosterone-to-renin ratio; BP, blood pressure; eGFR, estimated glomerular filtration rate; PAC, plasma aldosterone concentration; PP, pulse pressure; PRA, plasma renin activity; RAAS, renin–angiotensin–aldosterone system; RAR, plasma renin activity-to-plasma aldosterone concentration ratio; TIS, treatment intensity score

## INTRODUCTION

High blood pressure (BP) is a major risk factor for cardiovascular and renal diseases and a leading worldwide risk factor for morbidity and mortality [1]. Treatment of hypertension has been one of the greatest advances in medicine of the last century. The recent findings of the landmark SPRINT trial, which reported that intensive BP control in high-risk hypertensive patients reduces mortality and adverse outcomes, indicates that optimizing BP measurement and control is a high priority [2]. Despite the introduction of more effective and well tolerated medications during the past 30 years, the control of hypertension continues to be inadequate. Approximately 65% of Americans with hypertension do not have their BP

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controlled to levels below 140/90 mmHg [3]. Data from general practitioners showed a prevalence of uncontrolled hypertensive patients next to 40% in Italy, with a sharp improvement compared with the survey realized 8 years earlier (56.8%) [4]. However, individuals with uncontrolled hypertension increased from 605 to 978 million worldwide, because of population growth and aging. It is estimated that 20% of all disability adjusted life years in individuals more than 70 years and 15% in individuals aged 50–69 years are lost due to uncontrolled BP [5].

Angiotensin-converting enzyme inhibitors (ACE-I) and AT1 blockers (ARB), antagonizing the renin–angiotensin–aldosterone system (RAAS), are the most commonly used BP-lowering agents [6,7]. Pharmacokinetic and pharmacodynamic factors, as well as the adherence to the prescribed therapy, may influence the effectiveness of these drugs in reducing BP. Therefore, continuous therapy with ACE-I or ARB may not necessarily produce the expected decrease of plasma aldosterone concentration (PAC), together with the expected increase in plasma renin activity (PRA) [8,9].

The aim of our study was to evaluate the effectiveness of a stable therapy based on ACE-I or ARB, using PRA-to-PAC ratio (RAR) as a biomarker to test RAAS inhibition in real-life clinical practice. Given that the expected response to ACE-I or ARB treatment is indeed an increase in PRA and a decrease in PAC [10,11], our hypothesis was that the RAR might be the best way to fully evaluate RAAS inhibition during ACE-I or ARB treatment: the higher the value of RAR, due to larger increase in PRA and decrease in PAC, the more effective should be RAAS inhibition by the prescribed (and likely taken) ACE-I or ARB in each patient. In particular, we analyzed the associations between this ratio and ambulatory BP monitoring (ABPM) parameters, especially night-time BP and pulse pressures (PPs) to assess its feasibility as a biomarker of BP control.

## METHODS

We evaluated 194 consecutive outpatients referred to our Hypertension Centre from January 2015 to December 2015. All participants have given their informed written consent and clinical investigations have been conducted according to the principles expressed in the Declaration of Helsinki. This study was approved by the local institutional ethics committee. Inclusion criteria: age at least 18 years, diagnosis of essential hypertension, stable treatment for at least 12 months with an ACE-I or ARB as part of the treatment (excluding patients in therapy with mineralocorticoid receptor antagonists, direct renin inhibitors, amiloride or oral contraceptives), a 24-h ABPM. Patients with a probable low adherence to prescribed therapy were also excluded after testing with the modified Morisky Medical Adherence Scale [12]. A treatment intensity score (TIS) was also calculated to compare the different drug associations. As previously reported [13], the recorded daily dose taken by patients was divided by the maximum recommended daily dose to obtain a proportional dose (intensity) for that medication. The maximum recommended daily doses established by the Italian National Drug Agency were used for these calculations. Dual-class drugs were separated into their components and intensity was calculated separately for each compound. The sum of all the different values was recorded as the TIS. In the statistical analyses, we also considered ACE-I/ARB TIS. The following clinical parameters were also evaluated: patients' history, laboratory measurements, anthropometric measurements and ABPM parameters.

### Laboratory measurements

We considered the following laboratory parameters: PRA, PAC, creatinine, estimated glomerular filtration rate (eGFR), serum sodium and potassium. The eGFR was estimated using the Modification of Diet in Renal Disease study equation [14] taking into account the measurement of creatinine by Jaffe's reaction. Blood samples for PAC and PRA were obtained in the morning after at least 2 h in the upright position. PRA (ng/ml per h) was determined by radioimmunoassay using commercial kits (Sorin Biomedical Diagnostic, Vercelli, Italy), whereas PAC (pg/ml) was determined using an ELISA kit (DRG Instruments, Marburg, Germany).

As reported in the introduction, the expected response to ACE-I or ARB treatment is a decrease in PAC levels together with an increase in PRA levels [10]. Therefore, RAR was used to evaluate RAAS inhibition during ACE-I or ARB treatment. RAR was multiplied by 100 to facilitate reading. Subsequently, PRA and PAC were also analyzed separately.

### Anthropometric measurements

Body weight and height were measured on a standard beam balance scale with an attached ruler. Body weight was measured to the nearest 0.1 kg, and height was measured to the nearest 1 cm. BMI was calculated as weight in kilogram divided by the square of height in meters. Waist circumference was measured with the patient standing relaxed, arms freely by each side and feet close together.

### Blood pressure measurements

A 24-h ABPM and tests for PRA and PAC were performed within the same week, without any changes in drug therapy. Most of the times, tests for PRA and PAC were performed on the same day the ABPM was placed or removed.

ABPM was performed as part of the usual clinical practice to evaluate BP control in treated hypertensive patients, in patients who reported recently uncontrolled home BP values or in patients who had clinical features associated with higher risk of poor nocturnal BP control, according to the latest ESH Guidelines [7]. ABPM was performed using Spacelabs 90207 and 90217 (SpaceLabs Healthcare, Snoqualmie, Washington, USA), with the appropriate cuff and bladder dimensions for the arms circumferences. Minimum quality criteria considered for a satisfactory ABPM recording were: at least a 21-h period of valid BP recording, at least 70% of expected readings (sample frequency was set at one measurement every 15 min during the daytime and 30 min during the night-time), and at least 20 valid measurements during the daytime and seven during the night-time [15]. Twenty-four-hour BP, daytime BP (defined as the BP values from 0600 to 2200 h), night-time BP (defined as the BP values from 2200 to 0600 h) and PP (defined as the difference between SBP and DBP) were evaluated for each

patient. The definitions of 'day' and 'night' periods in our center were based on the most common answers to a questionnaire in which patients were asked about their sleeping behavior. The night-to-day ratio represents the ratio between mean night-time and daytime ABPM values. Night-to-day ratios were multiplied by 100, therefore expressing night-time BP as a percentage of a daytime level. A ratio of 100% or higher signified the absence of a BP fall at night. Patients with mean 24-h BP less than 130/80 mmHg, mean daytime BP less than 135/85 mmHg and mean night-time BP less than 120/70 mmHg were defined as well controlled by therapy [7].

## Statistical analysis

Data were analyzed with the Statistical Package for Social Science version 13 (SPSS Inc. Chicago, Illinois, USA). A value of $P$ less than 0.05 was defined as statistically significant. Normal continuous variables were expressed as mean ± SD. Skewed variables were expressed as median and interquartile range. Categorical variables were expressed as absolute number and percentage. Twenty-four-hour PP, daytime PP and night-time PP were natural logarithmically transformed to normalize their distributions. RAR tertiles (first tertile 0.08–0.82, second tertile 0.88–3.53 and third tertile 3.75–50.98), PRA tertiles (first tertile 0.2–1.0 ng/ml per h, second tertile 1.2–4.2 ng/ml per h and third tertile 4.4–52 ng/ml per h) and PAC tertiles (first tertile 16–101 pg/ml, second tertile 102–148 pg/ml and third tertile 150–675 pg/ml) were considered for the analyses. The $\chi^2$ test was used to analyze the differences between categorical variables. The unpaired $t$ test, analysis of variance and Kruskal–Wallis test (for multiple comparisons) were used to compare quantitative variables. Logistic and linear regression analyses and analysis of covariance were used to create adjusted models.

# RESULTS

General characteristics of the studied population are shown in Table 1, stratified by RAR tertiles. Mean age was 57.4 ± 12.0 years, with male prevalence (63.9%). No clear associations emerged between RAR tertiles and age, sex or BMI. Mean 24-h SBP and DBP were: 130.4 ± 15.3 and 78.5 ± 10.4 mmHg, respectively. Mean daytime SBP and DBP were: 133.7 ± 15.2 and 81.4 ± 10.8 mmHg, respectively. Mean night-time SBP and DBP were: 122.0 ± 17.3 and 71.2 ± 10.9 mmHg, respectively. Renal function, sodium and potassium maintained similar mean values between RAR tertiles. As expected, there was a clear increase in median values of PRA with increasing RAR tertiles, whereas no clear trend emerged for median values of PAC.

As antihypertensive therapy, an ARB was taken by 67% of the studied population, whereas an ACE-I was taken by the rest of the population (33%). We found no statistical differences among RAR, PRA and PAC tertiles regarding the use of ACE-I or ARB ($P = 0.385$, 0.926 and 0.781, respectively) as well as the other antihypertensive drug classes. Moreover, no differences were found among RAR, PRA and PAC tertiles in intensity of antihypertensive treatment, expressed by TIS (Tables 1–3 in Supplemental file, http://links.lww.com/HJH/A795), as well as no differences were found in ACE-I/ARB TIS (Tables 4–6 in Supplemental file, http://links.lww.com/HJH/A795).

## Effectiveness of renin–angiotensin–aldosterone system blockade and ambulatory blood pressure monitoring parameters

The increase of RAR tertiles was associated with a reduction of 24-h, daytime and night-time BP values. These associations remained significant even after adjusting for cofactors (Fig. 1, panel a–c). The same results were found with PRA tertiles, as shown in Table 2. On the contrary, no association was found between PAC tertiles and ambulatory BP values (Table 7 in Supplemental file, http://links.lww.com/HJH/A795). There was a decrease of night-to-day ratio at increasing PRA tertiles (first PRA tertile: 92.6% ± 6.4%; second PRA tertile: 91.5% ± 7.3%; third PRA tertile: 89.5% ± 7.5%; $P = 0.034$ after adjusting for age, sex, BMI, eGFR and TIS), whereas RAR and PAC showed no significant associations with night-to-day ratio.

**TABLE 1. General characteristics**

| Clinical characteristics | All patients (no. 194) | 1st RAR tertile (no. 64) | 2nd RAR tertile (no. 65) | 3rd RAR tertile (no. 65) | P |
|---|---|---|---|---|---|
| Age (years) | 57.4 ± 12.0 | 60.3 ± 10.8 | 54.1 ± 10.4 | 57.9 ± 14.0 | 0.012 |
| Sex (male) | 124 (63.9%) | 39 (60.9%) | 43 (66.2%) | 42 (64.6%) | 0.818 |
| BMI (kg/m$^2$) | 28.6 ± 5.1 | 27.7 ± 5.0 | 29.3 ± 5.4 | 28.9 ± 4.8 | 0.181 |
| Waist (cm) | 100.8 ± 12.3 | 98.4 ± 12.1 | 103.3 ± 14.2 | 100.5 ± 10.0 | 0.108 |
| Smoking habit | 58 (29.9%) | 16 (25%) | 22 (33.8%) | 20 (30.8%) | 0.538 |
| Diabetes | 21 (10.8%) | 8 (12.5%) | 8 (12.3%) | 5 (7.7%) | 0.608 |
| Lab parameters | | | | | |
| eGFR (ml/min per 1.73 m$^2$) | 95.4 ± 23.1 | 96.4 ± 22.4 | 96.1 ± 22.8 | 93.6 ± 24.3 | 0.766 |
| Serum sodium (mEq/l) | 140.5 ± 2.5 | 141.2 ± 2.3 | 140.1 ± 2.6 | 140.1 ± 2.6 | 0.026 |
| Serum potassium (mEq/l) | 4.3 ± 0.4 | 4.2 ± 0.4 | 4.3 ± 0.4 | 4.4 ± 0.4 | 0.079 |
| PRA (ng/ml per h) | 2.2 (25–75° pcs: 0.6–6.6) | 0.4 (25–75° pcs: 0.2–0.8) | 2.2 (25–75° pcs: 1.6–3.6) | 9.6 (25–75° pcs: 5.0–18.0) | <0.001 |
| PAC (pg/ml) | 123.0 (25–75° pcs: 89.8–170.0) | 124.5 (25–75° pcs: 99.8–154.0) | 139.0 (25–75° pcs: 94.5–235.0) | 105.0 (25–75° pcs: 64.5–152.5) | 0.015 |
| Antihypertensive therapy | | | | | |
| Beta blockers | 37 (19.1%) | 15 (23.4%) | 12 (18.5%) | 10 (15.4%) | 0.502 |
| Diuretics | 88 (45.4%) | 29 (45.3%) | 27 (41.5%) | 32 (49.2%) | 0.678 |
| Alpha blockers | 8 (4.1%) | 2 (3.1%) | 2 (3.1%) | 4 (6.2%) | 0.387 |
| Calcium channel blockers | 84 (43.3%) | 27 (42.2%) | 34 (52.3%) | 23 (35.4%) | 0.147 |

eGFR, estimated glomerular filtration rate; PAC, plasma aldosterone concentration; PRA, plasma renin activity; RAR, plasma renin activity to plasma aldosterone concentration ratio.

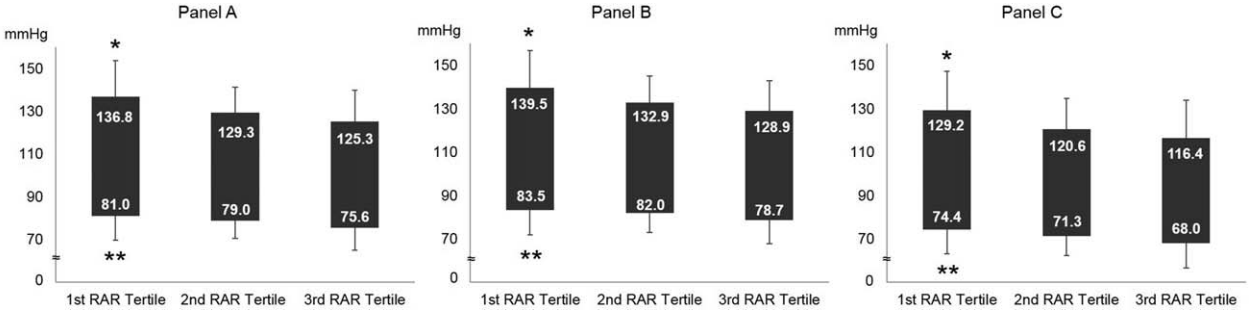


**FIGURE 1** Ambulatory blood pressure values and plasma renin activity to plasma aldosterone concentration ratio tertiles, panel (a) 24-h blood pressure values and plasma renin activity to plasma aldosterone concentration ratio tertiles, panel (b) daytime blood pressure values and plasma renin activity to plasma aldosterone concentration ratio tertiles, panel (c) night-time blood pressure values and plasma renin activity to plasma aldosterone concentration ratio tertiles. ANCOVA, adjusted for age, sex, BMI, estimated glomerular filtration rate and treatment intensity score. $^{*}P < 0.001$ for SBPs. $^{**}P < 0.001$ for DBPs. eGFR, estimated glomerular filtration rate; RAR, plasma renin activity to plasma aldosterone concentration ratio; TIS, treatment intensity score.

## Effectiveness of renin–angiotensin–aldosterone system blockade and blood pressure control

A well controlled 24-h BP (<130/80 mmHg) was found in 70 patients (36.1%). Men were more not controlled compared with women (odds ratio 1.9; $P = 0.036$), whereas no associations were found between 24-h BP control and age, BMI, eGFR and different antihypertensive drug classes. An increasing in RAR tertiles was associated with better BP control (24 h, daytime and night-time BP control), as shown in Table 3. Patients in the second and third RAR tertiles had, respectively, a 75 and an 88% lower risk to be not controlled in night-time period, compared with patients in the first tertile. PRA tertiles showed the same significant associations (Table 3), whereas PAC tertiles did not. The associations

**TABLE 2. Ambulatory blood pressure values and plasma renin activity tertiles**

| | 1st PRA tertile | 2nd PRA tertile | 3rd PRA tertile | *P* |
|---|---|---|---|---|
| 24-h SBP (mmHg) | 136.6 ± 15.9 | 129.8 ± 14.0 | 124.4 ± 13.4 | <0.001 |
| 24-h DBP (mmHg) | 80.7 ± 10.6 | 80.0 ± 9.6 | 74.8 ± 10.1 | <0.001 |
| Daytime SBP (mmHg) | 139.8 ± 16.0 | 132.6 ± 13.8 | 128.3 ± 13.5 | <0.001 |
| Daytime DBP (mmHg) | 83.6 ± 10.9 | 82.3 ± 10.0 | 78.2 ± 10.8 | 0.001 |
| Night-time SBP (mmHg) | 129.4 ± 17.2 | 121.3 ± 16.2 | 114.8 ± 15.4 | <0.001 |
| Night-time DBP (mmHg) | 74.3 ± 10.5 | 72.3 ± 10.9 | 66.8 ± 10.0 | <0.001 |

ANCOVA, adjusted for age, sex, BMI, eGFR and TIS. BP, blood pressure; PRA, plasma renin activity.

**TABLE 3. Logistic regression for lack of blood pressure control**

| | Model 1 | | | Model 2 | | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | | | OR (95% CI) | | |
| Variables[a] | 24-h BP control | Daytime BP control | Night-time BP control | 24-h BP control | Daytime BP control | Night-time BP control |
| Age | 0.98 (0.95–1.01) | 0.98 (0.95–1.00) | 0.98 (0.95–1.01) | 0.98 (0.95–1.01) | 0.97 (0.95–1–00) | 0.98 (0.95–1.01) |
| Sex (ref: male) | **2.21 (1.11–4.37)**$^{*}$ | 1.21 (0.63–2.31) | **2.92 (1.42–6.00)**$^{*}$ | **2.10 (1.06–4.18)**$^{*}$ | 1.16 (0.60–2.24) | **2.98 (1.43–6.23)**$^{*}$ |
| BMI | 0.96 (0.90–1.03) | 0.97 (0.91–1.03) | 0.97 (0.91–1.04) | 0.97 (0.91–1.03) | 0.97 (0.91–1.03) | 0.98 (0.91–1.04) |
| eGFR | 1.00 (0.99–1.02) | 1.00 (0.99–1.02) | 1.00 (0.98–1.01) | 1.00 (0.99–1.02) | 1.00 (0.99–1.02) | 1.00 (0.98–1.01) |
| TIS | 1.27 (0.85–1.89) | 1.15 (0.79–1.66) | 1.12 (0.74–1.71) | 1.31 (0.88–2.00) | 1.17 (0.80–1.70) | 1.13 (0.74–1.73) |
| 2nd RAR tertile (ref: 1st RAR tertile) | 0.63 (0.26–1.50) | 0.68 (0.31–1.50) | **0.25 (0.09–0.66)**$^{*}$ | / | / | / |
| 3rd RAR tertile (ref: 1st RAR tertile) | **0.24 (0.10–0.54)**$^{*}$ | **0.32 (0.15–0.69)**$^{*}$ | **0.12 (0.05–0.31)**$^{**}$ | / | / | / |
| 2nd PRA tertile (ref: 1st PRA tertile) | / | / | / | 0.49 (0.20–1.17) | 0.56 (0.26–1.28) | **0.20 (0.07–0.55)**$^{*}$ |
| 3rd PRA tertile (ref: 1st PRA tertile) | / | / | / | **0.20 (0.09–0.45)**$^{**}$ | **0.24 (0.11–0.53)**$^{**}$ | **0.09 (0.04–0.25)**$^{**}$ |

CI, confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity; RAR, plasma renin activity to plasma aldosterone concentration ratio; TIS, treatment intensity score. Bold indicates statistical significance.
[a]For continuous variables OR was for a one unit increase. Model 1: age, sex (reference = male), BMI, eGFR, TIS and RAR tertiles (reference = first tertile). Model 2: age, sex (reference = male), BMI, eGFR, TIS and PRA tertiles (reference = first tertile).
$^{*}P < 0.05$.
$^{**}P < 0.001$.

among RAR tertiles, PRA tertiles and night-time BP control remained significant even after further adjusting for 24-h BP control.

### Effectiveness of renin–angiotensin–aldosterone system blockade and pulse pressures

At univariate analysis, patients with higher PPs (24-h PP, daytime PP and night-time PP) were older ($r = 0.400$, 0.392, 0.390, all $P < 0.001$, respectively), had higher 24-h SBP ($r = 0.742$, 0.724, 0.730, all $P < 0.001$, respectively) and higher TIS ($r = 0.259$, 0.251, 0.255, all $P < 0.001$, respectively). People with diabetes and uncontrolled hypertensive patients showed higher PP values. Moreover, all PPs were associated with RAR tertiles and PRA tertiles at univariate analysis, whereas no association was found between PPs and PAC tertiles. In multiple linear regression models, age and 24-h BP control showed the strongest association with PPs. Nevertheless, associations remained significant also among 24 h, night-time PP and RAR tertiles and between night-time PP and PRA tertiles (Table 4).

## DISCUSSION

Our study shows that a higher RAR, used as an index of a more effective RAAS blockade during treatment with ACE-I or ARB, is associated with lower ambulatory BP values and better BP control especially during night-time period, and lower PP values. Similar results were found for PRA, but not for PAC. To the best of our knowledge, this is the first study that investigated the relationship between RAAS activity, evaluated by PRA and PAC levels, and ABPM parameters in a population of essential hypertensive patients treated with ACE-I or ARB.

The RAAS plays a central role in acute and chronic regulation of BP, affecting both arterial vasoconstriction and plasma volume. Therefore, hypertensive disorders are often the expression of a primary or a reactive dysregulation of this system. The understanding of the crucial role of the RAAS in the pathophysiology of hypertension has laid the basis for the development of current antihypertensive drugs [16,17].

In our study on treated patients, RAR was not affected by age, BMI or renal function or by antihypertensive therapy. In particular, there was no difference between ACE-I or ARB in RAAS inhibition and there was only a trend for beta blockers that were more taken in the first RAR tertile, as expected. Even the intensity of treatment, expressed by TIS and particularly ACE-I/ARB TIS, did not affect RAR in our population, although these results could have been biased by the high prevalence of high doses of antihypertensive drugs taken by most of our patients. RAR may be affected also by the pretreatment degree of RAAS activity and/or interindividual differences in response to antihypertensive drugs.

### Effectiveness of renin–angiotensin–aldosterone system blockade and ambulatory blood pressure monitoring parameters

At increasing RAR tertiles, there was a progressive reduction in all ambulatory BP values with a $\Delta$ of 13 mmHg for night-time SBP values between first and third tertile, even after adjusting for main confounding factors. These associations were further confirmed by a better BP control reported in the highest tertile, regardless of age, sex, BMI, renal function and treatment intensity. In particular, the main finding of our study is the strong relationship between RAR and night-time BP control, reaching an 88% lower risk to be not controlled during night-time period of patients in third tertile compared with patients in first tertile. Similar results were observed for PRA tertiles. These findings were independent of 24-h BP control. Analyzing nocturnal BP fall, patients in higher RAR tertiles showed a trend of better night-to-day ratio profile that became statistically significant if considering PRA tertiles.

Several studies reported that an altered circadian BP pattern is associated both with cardiac damage (left ventricular hypertrophy, diastolic dysfunction and left atrial enlargement) [18,19] and higher risk of major cardiovascular events compared with a normal BP pattern. The importance of the nocturnal BP fall was stressed in a recent meta-analysis of 17 312 hypertensive patients from three continents. Nocturnal BP fall, analyzed both as continuous variable (night-to-day ratio) and as categorical variable (dipping status), provided substantial prognostic information, independent of 24-h SBP. Night-to-day ratio

**TABLE 4. Multiple linear regression for pulse pressures**

| | Model 1 | | | Model 2 | | |
|---|---|---|---|---|---|---|
| | β | | | β | | |
| Variables | 24-h PP | Daytime PP | Night-time PP | 24-h PP | Daytime PP | Night-time PP |
| Age | **0.352*** | **0.343*** | **0.337*** | **0.353*** | **0.343*** | **0.334*** |
| Sex | −0.012 | −0.036 | −0.038 | −0.016 | −0.039 | −0.041 |
| BMI | **0.128*** | 0.097 | **0.178*** | **0.126*** | 0.096 | **0.178*** |
| Smoking habit | 0.072 | 0.060 | 0.047 | 0.066 | 0.055 | 0.041 |
| Diabetes | **0.190*** | **0.162*** | **0.182*** | **0.188*** | **0.160*** | **0.176*** |
| eGFR | −0.014 | −0.011 | −0.020 | −0.010 | −0.009 | −0.020 |
| 24-h BP control | **0.313*** | **0.315*** | **0.338*** | **0.316*** | **0.315*** | **0.332*** |
| TIS | 0.078 | 0.079 | 0.089 | 0.082 | 0.082 | 0.093 |
| RAR tertiles | **−0.137*** | −0.117 | **−0.141*** | / | / | / |
| PRA tertiles | / | / | / | −0.109 | −0.103 | **−0.146*** |

Model 1: age, sex, BMI, smoking habit, diabetes, eGFR, 24-h BP control, TIS and RAR tertiles. Model 2: age, sex, BMI, smoking habit, diabetes, eGFR, 24-h BP control, TIS and PRA tertiles. eGFR, estimated glomerular filtration rate; PAC, plasma aldosterone concentration; PP, pulse pressure; PRA, plasma renin activity; RAR, plasma renin activity to plasma aldosterone concentration ratio; TIS, treatment intensity score. Bold indicates statistical significance.
*$P < 0.05$.

predicted all end-points analyzed (strokes, coronary heart disease, total cardiovascular events, cardiovascular mortality and all-cause mortality) [20].

Previous studies have analyzed the relationship between PRA levels and office BP values in treated hypertensive patients. Sim *et al.* showed the highest SBP and office DBP in the lowest PRA quartile with a BP fall across the PRA quartiles (from 146/81 mmHg in first PRA quartile to 134/76 mmHg in fourth PRA quartile) in a population of 7887 treated hypertensive patients. Similarly to our study, the lowest PRA quartile had the poorest BP control, whereas the highest PRA quartile had the best BP control. Median PRA increased 40-fold across the PRA quartiles, whereas there was no such trend in PAC [21]. Similar trends of PRA and PAC emerged across the RAR tertiles in our study: median PRA increased about 20-fold across RAR tertiles, whereas no clear linear trend was found for PAC.

## Role of plasma renin activity and plasma aldosterone concentration in the assessment of renin–angiotensin–aldosterone system inhibition and cardiovascular risk

The expected pathophysiological response to ACE-I/ARB is a decrease in PAC levels together with an increase in PRA levels [10]. Therefore, as reported above, our hypothesis was that the RAR might be a powerful tool to evaluate RAAS inhibition during ACE-I or ARB treatment. Analyzing separately the two components, we observed that the relationship between RAR and ABPM parameters was guided by PRA and not by PAC. In fact, all the associations emerged for RAR tertiles were also confirmed for PRA tertiles. PAC can be affected by slight increases of serum potassium, one of its major secretagogue, that could over-ride the expected reduction because of angiotensin-II-reduced synthesis (by ACE-I) or AT1 antagonism (by ARB). Another possible mechanistic explanation for this finding may be related to the phenomenon of the 'aldosterone breakthrough', in which the cleavage of angiotensin I to angiotensin II, through enzymes different from ACE, can increase aldosterone levels despite therapy [9]. Moreover, severe adiposity can stimulate aldosterone secretion by several mechanisms, leading to inappropriately normal or even higher PAC levels in obese essential hypertensive patients, despite treatment [22,23].

On the other side, the increase in PRA is probably the best 'downstream' measurement of the RAAS inhibition, because the more effective the blockade, the greater the loss of negative feedback by angiotensin II through AT1 receptors on renin secretion from the juxtaglomerular apparatus in the kidney [24]. ACE-I and ARB lead to several-fold increases in plasma renin, through the inhibition of this negative feedback [25].

Although no association emerged in our study between PAC and ABPM parameters, previous studies reported strong association between aldosterone and cardiovascular, renal and metabolic disease in general community, even with aldosterone in the normal range and after adjusting for antihypertensive therapy [26]. In a general population, PAC is also a predictor of new hypertension, central obesity and type 2 diabetes in a 4-year follow-up study. [27] Other studies showed that renin was associated with cardiovascular events [28], all-cause and cardiovascular mortality in untreated hypertensive patients [29].

In daily clinical practice, PRA and PAC tests are performed mainly to exclude secondary hypertension (primary aldosteronism), using PAC-to-PRA ratio (ARR), even without antihypertensive treatment cessation [30]. Some authors have also proposed renin measurement to guide antihypertensive therapy [31–33]. Previous studies reported a possible predictive power by pretreatment renin levels on BP response to antihypertensive therapies [21,33] even if parameters that precisely predict the response to RAAS blockade are still lacking [34].

As well as ACE-I and ARB, all other antihypertensive drugs can affect plasmatic renin and aldosterone levels. For example, diuretics could cause a volume depletion with a reactive increase in renin levels. On the contrary, beta blockers reduce renin levels by inhibiting its secretion [35]. In our study, we could not analyze the volume status of patients; however, no significant differences in antihypertensive therapy were found between RAR tertiles.

## Effectiveness of renin–angiotensin–aldosterone system blockade and pulse pressures

A weak association among RAR, PRA and PP values was found in our study, even after adjusting for known predictors of PP, such as age, diabetes and BP control. In our previous studies, we found that higher PP values were associated with more severe cardiac organ damage [18,19]. Moreover, PP is a well known predictor of cardiovascular events and mortality, especially in patients over 60 years old [36,37]. Behold in poor cardiovascular outcome, RAAS is involved in many molecular pathways that contribute to vascular remodeling, inflammation, fibrosis and vascular smooth muscle cell hypertrophy and proliferation. These may lead to the development of large artery stiffness [34]. Although the relationship is not straightforward and better markers of arterial stiffness are currently used, higher PP can be also considered an indirect index of reduced arterial elasticity and an expression of structural alterations of the arterial wall. Previously, it has even assumed that PP may represent a marker of 'preclinical vascular disease' [38,39]. To confirm the relationship between RAAS and ambulatory PP, a recent study showed a linear correlation between PP and aldosterone levels in a group of untreated essential hypertensive patients [40]. Moreover, previous studies reported a larger reduction in PP with ACE-I and ARB compared with other antihypertensive drug classes [41,42]. Therefore, these antihypertensive drugs are effective not only on BP lowering, but may also have beneficial effects on arterial compliance, by reducing oxidative stress and inflammation, leading to vasodilation and prevention of the arterial wall remodeling [43].

## Study limits

The strengths of our study include a precise PRA and PAC measurement and a systematic evaluation of BP profile with ABPM. PRA and PAC were measured in the same laboratory throughout the study, using a method with sufficient sensitivity and accuracy and ABPM was performed within the same week.

Our study has also some limitations that require consideration. First of all, our investigation is a cross-sectional study that did not allow us to establish causality for the associations observed, but we can only speculate regarding the possible biological mechanisms that might have caused them. Moreover, pretreatment values of PRA and PAC were not available. We could not evaluate the variations of such hormones after the introduction of therapy. Therefore, it was not possible to determine whether pretreatment renin or aldosterone levels could have been predictive for BP response in the first place and/or whether our reported levels reflected pharmacodynamic mechanisms. However, the aim of our study was not to evaluate the predictive power of PRA and PAC, but to analyze the associations between long-term on-treatment PRA and PAC levels and ABPM parameters in a real-life practice.

Second, we included patients in stable treatment for at least 12 months with an ACE-I or ARB, but we did not know the precise duration of antihypertensive therapy or other informations about their hypertensive history, so we could not investigate the effects of RAAS inhibition on main target organ damages, which generally takes years to establish. Finally, we were unable to obtain data of known regulators of RAAS, such as dietary sodium and volume depletion.

In conclusion, our study shows that a better RAAS inhibition, as revealed by RAR, is associated with better control of the most important ABPM parameters, suggesting a likely reduction of cardiovascular events. RAR and PRA resulted to be strongly associated with night-time BP control, one of the most important predictors of cardiovascular events and mortality, and also with a marker of arterial stiffness, such as PP.

RAR may be a useful biomarker of effective RAAS inhibition in real-life clinical practice. PRA and PAC tests, routinely performed mostly as a screening for primary aldosteronism, may also be useful in the management of essential hypertensive patients treated with ACE-I or ARB. For instance, it could be useful to identify patients that are poorly adherent to prescribed therapy. Further studies are needed to better define clinical usefulness of PRA and PAC levels and RAR in essential treated hypertensive patients, to help physicians in assessing real-life efficacy of ACE-I-based or ARB-based therapies.

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### Conflicts of interest

There are no conflicts of interest.

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## Reviewers' Summary Evaluations

### Reviewer 1

ACE inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are front-line therapy to treat, hypertension. Treatment with ACEIs and ARBs causes an increase in plasma renin activity (PRA), and a decrease in plasma aldosterone concentration (PAC). This study evaluated the relationship, between ambulatory blood pressure monitoring and PRA to PAC ratio (RAR) in essential, hypertensives treated with ACEIs and ARBs for 12 months, whereby a higher RAR reflected more, effective blood pressure control. Higher RAR is associated with lower ambulatory blood pressure, and better blood pressure control. RAR could be a useful to manage essential hypertensives, treated with ACEIs and ARBs.