ROLE OF PLASMA RENIN ACTIVITY IN PERSONALIZING ANTIHYPERTENSIVE THERAPY IN EUROPEAN AND AFRICAN AMERICANS WITH UNCOMPLICATED HYPERTENSION

By

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To my lovely family
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<td>African American</td>
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<td>ACE</td>
<td>Angiotensin Converting Enzyme</td>
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<td>ADRB1</td>
<td>Adrenergic Beta1 Receptor</td>
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<td>AHA</td>
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<td>DBP</td>
<td>Diastolic Blood Pressure</td>
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<td>EA</td>
<td>European American</td>
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<td>EBF1</td>
<td>Early B-cell Factor 1</td>
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<td>ENaC</td>
<td>β-chain of Epithelial Sodium Channel</td>
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<td>FDA</td>
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<td>HCTZ</td>
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<td>HOT</td>
<td>Hypertension Optimal Treatment</td>
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<td>HTN</td>
<td>Hypertension</td>
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<td>LD</td>
<td>Linkage Disequilibrium</td>
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<td>MET</td>
<td>Metoprolol</td>
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<td>MOV10</td>
<td>Putative Helicase</td>
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<td>NEDD4L</td>
<td>Neural Precursor Cell Expressed Developmentally Down-regulated Protein 4</td>
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<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
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<td>NPV</td>
<td>Negative Predictive Value</td>
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<td>PC</td>
<td>Principal Component</td>
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<td>PCA</td>
<td>Principal Component Analysis</td>
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<td>PEAR</td>
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<td>PPV</td>
<td>Positive Predictive Value</td>
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<td>PRA</td>
<td>Plasma Renin Activity</td>
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<td>Protein Kinase C Alpha</td>
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<td>QALY</td>
<td>Quality-adjusted Life Years</td>
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<td>RAAS</td>
<td>Renin-angiotensin-aldosterone System</td>
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<td>RAS</td>
<td>Renin Angiotensin System</td>
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<td>ROC</td>
<td>Receiver Operating Characteristic</td>
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<td>SBP</td>
<td>Systolic Blood Pressure</td>
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<td>SD</td>
<td>Standard Deviation</td>
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<td>SH2B</td>
<td>3SH2B Adaptor Protein 3</td>
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<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
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<tr>
<td>TPR</td>
<td>Total Peripheral Resistance</td>
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Abstract of Thesis Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacy

ROLE OF PLASMA RENIN ACTIVITY IN PERSONALIZING ANTIHYPERTENSIVE THERAPY IN EUROPEAN AND AFRICAN AMERICANS WITH UNCOMPLICATED HYPERTENSION

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Major: Pharmaceutical Sciences

Only half of hypertensive patients have controlled BP. PRA is a potential biomarker for personalizing antihypertensive therapy, and is suggested to improve BP control. After categorizing by PRA, DBP responses to MET and CTD were compared in EA and AA subjects who were sequentially treated with MET, followed by CTD in the PEAR-2 trial. To determine PRA cut point in AAs, data from PEAR-2 were used to estimate the cut point associated with BP responses using multivariable linear regression models. The derived cut point was then tested in a meta-analysis of two independent AA cohorts, by estimating pooled adjusted mean differences in SBP response to RAS blockers and HCTZ. EA subjects with lower-PRA (<0.65 ng/mL/hr) responded better to CTD than to MET (-11.9±8.1 vs. -7.5±7.7 mmHg, adjusted P=0.0024), whereas those with higher-PRA (≥0.65 ng/mL/hr) responded better to MET (-12.9±8.3 vs. -7.0±7.6 mmHg, adjusted P<0.0001). No strong relationship was found between PRA and DBP responses in PEAR-2 AAs. After determining PRA cut point in AAs from PEAR-2, those with PRA≥1.3 ng/mL/hr (PEAR2-derived cut point) given RAS blockers had a greater reduction in mean SBP of -7.6 mmHg than those given HCTZ.
(95% CI, -13.3 to -1.8 mmHg; meta-analysis $P=0.01$). However, the derived cut point had only 10% sensitivity to identify the subset of AAs who had good response to RAS blockers and an ROC analysis showed that PRA did not discriminate SBP response. These findings suggest that PRA is a useful predictive biomarker of BP response in EA, not AA population.
CHAPTER 1
INTRODUCTION AND BACKGROUND

HTN and Uncontrolled BP

HTN is one of the most common chronic diseases, with a global prevalence in 2014 of 22% among adults aged 18 years old and over.\textsuperscript{1} According to the NHANES 2011-2014 data, the age-adjusted prevalence of HTN in the U.S is estimated to be 34%, which is equivalent to 85.7 million adults.\textsuperscript{2} High BP is a primary risk factor for multiple adverse cardiovascular outcomes. A prospective observational study where 1.25 million individuals had been followed up for a median of 5 years demonstrated that hypertensive patients at 30 years of age have an overall life-time cardiovascular risk of 63.3%, whereas normotensive individuals at the same age have an overall lifetime risk of 46.1%.\textsuperscript{3} This study also reported that the lifetime risk of angina, myocardial infarction, heart failure, and cardiac arrest is almost twice as high in hypertensive patients as in normotensive individuals.\textsuperscript{3} Furthermore, HTN is also associated with a high global mortality rate that reached 9.4 million deaths in 2010.\textsuperscript{1}

It has been demonstrated that controlling BP using antihypertensive drugs reduces the risk of stroke by 30%, coronary heart disease by 10-20%, congestive heart failure by 40-50%, and overall mortality by 10%.\textsuperscript{4} Despite the availability of multiple antihypertensive drug classes with numerous options within each class, data collected by NHANES in 2011-2014 showed that only 54.4% of patients with a HTN diagnosis have controlled BP.\textsuperscript{2} A study combining data from the NHANES III, the HOT trial, and the Dallas-based AHA estimated that uncontrolled BP results in 39,702 additional cardiovascular events and 8,374 additional deaths every year in the U.S.\textsuperscript{5} Another epidemiological study showed that uncontrolled BP is estimated to be associated with
an increase of 58,000 major cardiovascular events annually in the U.K. In line with these statistics, a recently published prospective observational study found that hypertensive patients with uncontrolled BP were more likely to have reduced left ventricular myocardial function - a leading risk factor for heart failure - compared with those with controlled BP. In addition to the potential increased cardiovascular risks, uncontrolled BP often leads to the prescription of additional antihypertensive drugs, which may lead to poor compliance, increase risk for adverse events, and result in additional treatment cost that has been estimated to be US $467 million per year among American patients receiving antihypertensive drugs. Thus, adequate BP control is of great public health importance.

Several studies postulate that restricted access to healthcare, along with medication non-adherence, are major contributors to poor BP control; yet, there is evidence that even patients who have health insurance and those who adhere to their medications have uncontrolled BP. This uncontrolled BP likely reflects the high inter-individual variability in BP response to various antihypertensive drugs, which is primarily caused by the heterogeneity in the pathophysiologic pathways underlying HTN among hypertensive patients.

**RAAS: an Important BP Regulator**

The RAAS is one of the physiologic mechanisms which plays a significant role in regulating BP. Figure 1-1 outlines how this system works. In normotensive individuals, BP is primarily maintained by body sodium, which determines the extracellular and plasma volumes, and in turn determines the amount of blood pumped by the heart per minute (CO). At the same time, kidneys monitor the plasma volume and BP, and accordingly, adjust renin secretion. Renin cleaves plasma angiotensinogen into
angiotensin I (inactive) which is rapidly converted by the widely-distributed ACE into angiotensin II, that raises BP by constricting the arterioles (vasoconstriction) and stimulating the release of aldosterone hormone from the adrenal glands.\textsuperscript{11, 13} The aldosterone release increases sodium reabsorption from the kidneys, leading to sodium retention.\textsuperscript{13}

When the kidneys retain more sodium than usual, arterial sodium-volume is increased, resulting in increased BP, which exerts a negative feedback on the kidneys, causing renin secretion to fall to prevent BP from rising over the long term, and to keep BP at a normal level.\textsuperscript{11, 13} Suppression of RAAS activity buffers the effect of any increase in the arterial sodium-volume.\textsuperscript{13} In contrast, in cases of decreased sodium intake, diuresis, hemorrhage, infection, Addison’s disease (decreased aldosterone secretion from the adrenals), or hypotension, the RAAS is activated as a compensatory mechanism, stimulating the release of the vasoconstrictor renin from the kidney nephrons, thus preventing a fall in BP.\textsuperscript{11-13} Although there are many vasoconstrictors other than renin (prostaglandins, bradykinin, nitric oxide, and endothelin), when compared to renin, they only have briefer or more localized effects on certain tissues.\textsuperscript{14} Maintaining BP at its normal level ensures an adequate blood flow to the tissues, thus allowing the delivery of nutrients and removal of waste products.\textsuperscript{11-13}

Hence, BP is predominantly regulated by two factors: the volume factor (dietary sodium), and the vasoconstrictor factor (renin). This is also supported by the Poiseuille’s equation (BP=CO x TPR) which was first described in the 1840s, and states that BP is equal to CO multiplied by the TPR. These two components are
primarily determined by the arterial sodium-volume status (V) (preload) and renin-induced arterial vasoconstriction (R) (afterload), respectively.\textsuperscript{14}

**Measurement of PRA to Assess the RAAS Activity in Normotensive Individuals**

Measuring the circulating renin in plasma provides an assessment of RAAS activity, which normally fluctuates in relation to the state of sodium balance and BP. Several decades ago, immunoassays were developed to directly measure the plasma renin concentrations (reported as pg/mL or ng/L) using monoclonal antibodies.\textsuperscript{15-18} However, renin exists in relatively minute amounts in adult plasma: its concentration is less than $10^{-12}$ mol/L (five times and two times lower than that of cortisol and insulin respectively), thus Sealey et al. demonstrated that these assays have low sensitivity, and might fail to detect the very low renin concentrations.\textsuperscript{12, 19} Instead, a more sensitive assay has been developed to alternatively measure the amount of angiotensin I produced by renin (plasma renin “activity” or PRA) by incubating renin in plasma samples and generating thousands of copies of angiotensin I, while also inhibiting the ACE to prevent the conversion of angiotensin I into angiotensin II. This PRA enzyme-kinetic assay is reported as the hourly rate at which angiotensin I is generated by plasma renin (in nanograms per milliliter per hour [ng/mL/hr]).\textsuperscript{12} To determine the normal PRA range, Laragh and colleagues have constructed nomograms showing the relation between the PRA measurements from normotensive young subjects of primarily European ancestry and the 24-hour urinary sodium excretion (a reflection of the daily sodium intake) using average ranges of sodium intake (renin profiling). Using the enzyme-kinetic assay, PRA levels in normotensive young European subjects have been found to range from 0.65 to 4.50 ng/mL/hr.\textsuperscript{20-22}
Disturbances in the RAAS Activity among Hypertensive Patients with Different BP Responses to Various Antihypertensive Agents

Nomograms of normotensive individuals have been compared with those of hypertensive patients. It has been found that approximately 30% of hypertensives had PRA-sodium values below the lower limit of the PRA range observed in the normotensives (PRA < 0.65 ng/mL/hr), while 14% exhibited levels above the upper limit of the PRA range in normotensives (PRA > 4.50 ng/mL/hr).\textsuperscript{20, 22}

It has been hypothesized that the elevated BP in patients with lower PRA (< 0.65 ng/mL/hr) is maintained by inappropriate renal retention of sodium.\textsuperscript{23} Sodium retention occurs when the nephrons either do not filter enough salt (inability to excrete dietary salt) or reabsorb too much, in which case the resulting rise in BP does not exert a negative feedback on the kidneys since renin secretion is already maximally suppressed (sodium volume-dependent HTN).\textsuperscript{13, 23} In contrast, in patients with normal and higher PRA (≥ 0.65 ng/mL/hr), some nephrons become ischemic and try to correct this under-perfusion by increasing the rate of renin secretion, causing vasoconstriction and aldosterone-induced sodium retention.\textsuperscript{12, 13} The ischemic nephrons do not suppress the renin secretion appropriately in response to rise in BP, therefore, a form of HTN is formed which has both vasoconstriction and volume components (vasoconstriction-dependent HTN).\textsuperscript{12, 13} Although some hypertensives in this category have PRA levels within the normal range for normotensive individuals, these levels are still considered inappropriately high since PRA is not adequately suppressed in response to elevated BP.\textsuperscript{23, 24} Those with this two-component HTN are more susceptible to cardiovascular sequelae compared with those with sodium volume-dependent HTN. A study published in 2011, where 3,791 hypertensive patients were followed for an average of 16 years,
showed that patients with higher PRA had a three-fold higher risk of developing myocardial infarction and a 70% higher risk of cardiovascular mortality than patients with lower PRA.\textsuperscript{25} Additionally, patients with elevated PRA had a greater risk for congestive heart failure than those with lower PRA.\textsuperscript{26}

In the 1970s, Laragh and other investigators began to observe that patients with lower PRA and those with normal or higher PRA responded differently to various antihypertensive drug classes.\textsuperscript{27-31} The first observation was made by Bühler et al., who showed that a strong positive relationship existed between the BP response to propranolol and baseline PRA levels in a study conducted on 96 hypertensive patients. This study found that propranolol resulted in a significantly greater drop in the mean DBP of 32.2 mmHg in patients with higher PRA versus 4.7 mmHg in those with lower PRA.\textsuperscript{28} Also, investigators demonstrated that propranolol treatment was associated with decreased PRA levels, and that the BP lowering effect of this drug was directly correlated with the degree of the induced-PRA decline.\textsuperscript{27} Since propranolol is a non-selective β-blocker, and its antihypertensive efficacy may be related to the several effects it exerts on heart and blood vessels rather than its inhibitory effect of renin release, researchers have sought to further investigate the association between PRA and BP responses to antihypertensive agents which act directly on the RAAS. As a validation of the previous results, subsequent studies showed that the BP responses to the drugs which directly inhibit the activation of the renin-produced angiotensin I (ACE inhibitors saralasin and SQ20881) were positively correlated with the baseline PRA levels. The studies also demonstrated that these drugs were only effective in reducing the BP of patients with normal and higher, but not lower PRA.\textsuperscript{29-31}
Moreover, several studies showed that treatment with the diuretics HCTZ, spironolactone, and CTD were significantly more effective in patients with lower PRA, compared with those having normal or higher PRA levels. A study conducted on 31 patients who were treated with either spironolactone or HCTZ found that those with lower PRA had a significantly greater BP response to both diuretics than those with higher PRA.\textsuperscript{32} In a double-blinded study where 24 subjects were randomized to either spironolactone or placebo, showed that patients with lower PRA had a significantly mean reduction in their SBP of 20 mmHg in response to spironolactone, whereas those with higher PRA had no significant reduction in their BP.\textsuperscript{33} Additionally, diuretics have been shown to induce a reactive rise in PRA levels in response to loss of body sodium (volume depletion) during treatment. Therefore, investigators suggested that the elevated BP of patients who did not respond well to diuretics was maintained by renin-induced vasoconstriction rather than by sodium volume retention.\textsuperscript{32-34} Moreover, when compared with propranolol, CTD resulted in a significantly greater BP reduction among patients with lower PRA.\textsuperscript{35} In a study conducted in 534 patients who were treated with either HCTZ or propranolol, those with lower PRA had a better response to HCTZ, whereas those with higher PRA had a better response to propranolol.\textsuperscript{36} Other studies demonstrated that either the α-blocker prazocin or calcium channel blockers resulted in a greater BP reduction and even more significant orthostatic hypotension in patients with lower PRA, compared to patients with higher PRA.\textsuperscript{37-39} In each of the previously mentioned studies, patients were categorized into low, normal, and high PRA groups based on nomograms constructed in both normotensive and hypertensive subjects.
Compared with the nomograms constructed by Laragh, similar hyperbolic relationships with similar PRA ranges were observed.

**Differences in the RAAS Activity among Hypertensive Patients of Different Races and Different Age Groups**

Numerous studies demonstrated differences in RAAS activity and BP response comparing EAs and AAs, and younger and older patients.\(^{20, 26, 40, 41}\) Overall, AA patients have lower PRA values and a higher prevalence of salt sensitivity (a rise in BP in response to sodium or salt intake) than EA patients, and this usually explains the observed differences in BP responses to various antihypertensive drugs between the two races.\(^{20, 26, 42}\) AAs are likely to have a better antihypertensive response to HCTZ (thiazide diuretic), whereas EAs are likely to have a better response to atenolol, labetolol, propranolol (β-blockers), captopril (ACE inhibitor), and candesartan (ARB).\(^{43-49}\)

Earlier studies proposed that the lower PRA levels found in AAs than in EAs might be explained by their higher sodium intake; however, studies demonstrated no difference in sodium intake comparing the two races.\(^{49-51}\) Moreover, lower PRA levels have been observed in AAs with both low and high sodium intakes.\(^{48}\) Given the same sodium load (2 liter-infusion of normal saline), Luft et al. observed that AAs retain more sodium than EAs.\(^{52}\) A hypothesis was made by Wilson and colleagues, who proposed that the slave trade from Africa to the Americas resulted in severe sodium depletion due to diarrhea, infections, and limited access to water, which, in turn, led to selection for genes responsible for retaining more sodium as an evolutionary adaptation.\(^{53}\) Rayner et al. showed that a genetic mutation, the p.Arg563Gln (or R563Q) in the ENaC gene, was associated with low-renin HTN in South Africans.\(^{54}\) Additionally, the GRK-4 p.Arg65Leu and p.Ala142Val genetic variants were associated with impaired sodium excretion in the
African population. According to Kailasam’s study, another explanation of the lower PRA levels found in AA compared with EA hypertensive patients is that the former have lower levels of renal kallikrein -which converts the inactive renin into active renin- than the later.57

Lastly, older hypertensives tend to have lower PRA levels than the younger hypertensives, and this is at least partially explained by the fact that as age increases, BP increases, which leads to reduced release of renin from the kidneys.12, 26, 58-61 This can explain the differences in BP response comparing age subgroups: previous reports showed that younger patients were less responsive to HCTZ and were more responsive to propranolol (β-blocker) than older patients.62-64

The Proposed PRA-Guided Treatment Approach

Based on the observations of different BP responses to various antihypertensive drugs between patients with lower PRA and those with higher PRA, Laragh and colleagues were the first to advocate that PRA testing can determine the relative contribution of the concurrent volume and vasoconstrictor factors, and thus can guide the initial selection of antihypertensive drugs. Laragh’s proposed PRA-guided treatment approach has the goal of controlling BP with monotherapy or with no more than 2 antihypertensive agents. In the PRA-guided treatment approach, it has been suggested that BP of the hypertensive patients with PRA < 0.65 ng/mL/hr can be controlled by sodium restricted diet and anti-sodium volume (anti-V) drugs which increase the renal sodium excretion either by inhibiting the reabsorption of salt and water from the renal tubules (diuretics) or by causing renal vasodilatation (α-blockers and calcium channel blockers) (Figure 1-2a). In contrast, hypertensive patients with PRA ≥ 0.65 ng/mL/hr can be successfully treated by anti-renin (anti-R) drugs (RAS blockers) that block one of the
three different sites in the overactive RAAS hormonal cascade: either by blocking the formation of angiotensin II (ACE inhibitors), blocking the action of angiotensin II (ARBs), or directly blocking the β-sympathetic stimulation of the renal renin release (β-blockers) (Figure 1-2b). Table 1-1 shows the anti-V and anti-R drug classes and the drugs within each class. Therefore, identifying the disturbances/ abnormalities in the RAAS activity in hypertensive patients can determine which antihypertensive drug class would reduce BP most effectively and improve BP control.13, 34, 65, 66

Additionally, based on the observed differences in the RAAS activity and BP response between different races and different age groups, Preston and colleagues proposed that race and age could be used as surrogates for PRA to personalize antihypertensive therapy; whereby AA race and older age could be used as markers for lower PRA, while EA race and younger age could be used as markers for higher PRA.67 However, a retrospective analysis from the GERA study, where hypertensive subjects were treated with either HCTZ or candesartan, indicated that the PRA treatment strategy was associated with a BP control rate of 69.4% versus 61.3% with the age-race based strategy (P<0.0001).46 Additionally, another retrospective analysis from the PEAR study, where subjects were randomized to either HCTZ monotherapy or to atenolol monotherapy, showed that the PRA-guided approach was associated with a 10% higher BP control rate compared with the age-race strategy (P=0.0004).47

**Why PRA-Guided Treatment Approach Has Been Obsolete for Many Years**

Although the PRA-guided treatment strategy appears compelling and promising, many factors rendered it obsolete for many years and prevented it from being clinically applied. First, the fact that renin exists in very low concentrations has resulted in the development of several time-consuming protocols which attempted to stimulate the
endogenous renin levels through collecting blood samples after ambulation for at least four hours, maintaining the patient on a reduced sodium intake, or through diuretic administration. These protocols were being used for several years, even after the development of the alternative method of measuring PRA instead of plasma renin. Also, there was a misconception that sodium intake should be monitored through measuring the urinary sodium and potassium in urine samples collected over 24 hours. Subsequently, investigators realized that these conditions are unnecessary and that PRA is best measured under the usual clinic settings, normal sodium intake, without diuretic administration, and without the need of 24-hour urinary collection.\textsuperscript{12}

As mentioned above, antihypertensive drugs can cause changes in the PRA levels and this has been one of the challenges that hindered PRA testing for years. The anti-V drugs can cause a reactive rise in renin because the reduced sodium content caused by diuresis stimulates renal renin release.\textsuperscript{68, 69} Through a different mechanism, ACE inhibitors and ARBs (anti-R drugs, RAS blockers) can also increase renin secretion by blocking angiotensin II formation or its action, respectively, thus inhibiting the negative feedback effect on renin release.\textsuperscript{31} This reactive rise in renin, especially as associated with the anti-R drugs, can be very large and can even cause a paradoxical pressor response.\textsuperscript{70} Conversely, β-blockers decrease renin levels by directly blocking the β-sympathetic stimulation of the renal renin release.\textsuperscript{26, 27, 71} Based on that, it was recommended that all antihypertensive medications be discontinued three weeks before testing. Scientists later realized that PRA could, and should be measured without any change in the antihypertensives administered, because this truly reflects the PRA level in response to these medications, especially in the setting of uncontrolled BP.
Furthermore, Laragh and associates developed guidelines for using PRA to guide the treatment of uncontrolled hypertensive patients. These guidelines are based on adding or subtracting antihypertensive medications as a result of the PRA changes induced by the drugs being administered which might cause a pressor response.\textsuperscript{65}

Additionally, PRA assay encountered some methodologic problems. These methodologic problems, which were widely adopted in 1970s included measuring PRA at high pH, that was found to destroy renin leading to falsely low levels, and chilling of plasma samples, that was found to convert the inactive prorenin into the active renin, making the levels falsely elevated. Moreover, although Laragh and colleagues recommended incubating the plasma samples which exhibit PRA levels < 0.65 ng/mL/hr for an additional 18 hours, the shorter incubation time used in some laboratories has led to an inability to detect low PRA levels, and the inability to differentiate between patients with low versus high PRA.\textsuperscript{12, 66, 72} Years later, these technical problems were resolved by measuring PRA at a pH of 5.7, avoiding chilling of plasma samples to temperature below 6 degree Celsius, and incubating samples with PRA < 0.65 ng/mL/hr for 18 hours.\textsuperscript{12} However, the resulting confusion greatly discouraged clinicians from measuring PRA and using it to guide therapy of hypertensive patients over several years.\textsuperscript{23, 65}

Recently, the questionable reproducibility of the PRA enzyme-kinetic assay among different laboratories, and its required long incubation time, especially for the low-PRA plasma samples, have further prevented its extensive use in guiding the antihypertensive medication selection in clinical practice. Also, investigators suggested that this assay might produce inaccurate results since it is not only dependent on renin, but also on its substrate (angiotensinogen) concentration.\textsuperscript{73, 74} Therefore, a new direct
immunochemiluminometric direct renin assay has been introduced, in which monoclonal antibodies specific to renin are labelled with a chemiluminescent reagent. Studies have shown that this assay has good sensitivity, short turnaround time, and good correlation with the PRA measurements, but it has been withdrawn from the market before further investigation of its reproducibility. Subsequently, the DiaSorin company has recently developed another immunochemiluminometric assay which directly measures the plasma renin concentrations in µIU/mL and involves the incubation of the plasma samples for only 30 minutes. In a multicenter international study published in 2010 in which twelve centers have participated, Morganti and associates compared the new DiaSorin direct renin assay with the PRA assay. This study showed a significantly good correlation between PRA and plasma renin measurements. Moreover, the new assay has been shown to have substantially better intra and inter-laboratory reproducibility than the PRA assay. Unlike the old direct renin assays, the new assay has been demonstrated to have very good sensitivity since it can detect low plasma renin concentrations ranging from 1.60 to 1.96 µIU/mL (approximately equivalent to 0.07 to 0.09 ng/mL/hr for PRA measurements). Morganti et al. concluded that the new renin assay is simpler and quicker than the PRA assay, thus it is more practical for application in clinical settings. This assay was approved by the US FDA in 2013, however, the PRA assay is still more widely used.

Interest Is Regained in PRA-Guided Treatment Approach

Only recently have researchers regained interest in the PRA’s promising role in guiding antihypertensive therapy, and a number of PRA studies have been conducted in the last few years. In a recent study, the 24-hour, daytime, and nighttime SBP and DBP responses to valsartan (ARB, anti-R drug) have been found to be significantly correlated
with the pretreatment log-transformed PRA values in Japanese patients, indicating a better response when baseline PRA values were higher. In line with these results, in a study conducted on 439 participants from GERA, the log-transformed PRA was a significant predictor of both SBP and DBP responses to candesartan, which also increased the predictability of SBP and DBP responses by approximately 14% and 53% respectively. Additionally, candesartan-induced PRA rise was significantly correlated with BP response, and its incorporation in the models increased the percent of the explained variability in SBP and DBP responses by 3% and 4% respectively. In another study conducted on 1096 subjects from GERA, Schwartz and colleagues, compared the BP responses of subjects who were treated with HCTZ with those treated with candesartan based on PRA category, and found that AA subjects with PRA < 0.60 ng/mL/hr who were treated with HCTZ had a significantly greater drop in BP of 11/4 mmHg than those treated with candesartan. In contrast, AAs with PRA ≥ 0.60 ng/mL/hr treated with candesartan had a greater decrease in DBP of 3 mmHg compared with those treated with HCTZ (P<0.01). It has been also shown that EAs with PRA < 0.60 ng/mL/hr given HCTZ had a greater reduction in DBP of 2.7 mmHg compared with those given candesartan, while those with PRA ≥ 0.60 ng/mL/hr had a better response to candesartan. In a recently published retrospective analysis conducted on 733 participants from PEAR, where the participants were randomized to either HCTZ monotherapy or atenolol monotherapy, consistent results were also obtained in the lower-PRA AA and the higher-PRA EA subgroups. However, AAs with PRA ≥ 0.60 ng/mL/hr and EAs with PRA < 0.60 ng/mL/hr responded similarly to both HCTZ and atenolol. The promising role for PRA in improving BP control has not only been
observed in untreated hypertensive patients, but also in unsuccessfully treated and resistant hypertensive patients. In a randomized, open-label clinical trial conducted on 77 unsuccessfully treated patients, Egan et al. demonstrated that patients who were randomized to a PRA-guided treatment approach had a significantly greater reduction of 10 mmHg in SBP, and were treated with fewer anti-R medications compared with those randomized to the usual clinical HTN specialists’ care. The ASPIRANT study was a multicenter, randomized, placebo-controlled, double-blinded clinical trial, where 117 patients with resistant HTN were randomized to either spironolactone or placebo, as an add-on therapy to their current antihypertensive drugs. This study showed that baseline PRA was a significant predictor of 24-hour SBP response. When the trial participants were divided into three tertiles based on their PRA levels, the mean reductions in 24-hour SBP in response to spironolactone were 19.0 mmHg for the low-renin (≤ 0.12 ng/mL/hr), 12.0 mmHg for the middle-renin (0.13-1.34 ng/mL/hr), and 4.0 mmHg for the high-renin (≥ 1.34 ng/mL/hr) tertiles. Furthermore, a pilot study was recently published, where 44 resistant hypertensive patients of primarily African ancestry patients were randomized to either spironolactone or to PRA-guided approach. This study showed that although spironolactone reduced the SBP and DBP by 17.6/4.0 versus 20.4/9.7 mmHg in patients treated according to PRA-guided approach ($P=0.7/0.1$), the PRA-guided algorithm was associated with the addition of fewer medications. The authors also advocated that the non-significant findings obtained can be attributed to the small sample size used in this study.

**Conclusion**

In summary, hypertensive patients respond differently to various antihypertensive drugs. Thus, the empiric, routine use of a single class of antihypertensives, for example,
thiazide or thiazide-like diuretics in all patients with uncomplicated essential HTN as recommended by the current treatment guidelines, is unlikely to achieve optimal BP control. Alternatively, a more personalized treatment approach which matches the antihypertensive drug’s mechanism of action with the individual patient’s pathophysiologic type of HTN is needed. The PRA-guided approach has the potential to individualize therapy based on RAAS activity and type of HTN (lower-PRA or volume-dependent HTN versus higher-PRA or vasoconstriction-dependent HTN). It was hypothesized that using PRA testing in HTN treatment could improve BP control and decrease the number of drugs needed to successfully treat the patient, which would in turn lead to better compliance, fewer adverse events, and less treatment costs.

The concept of PRA measurement to guide the initial selection of antihypertensive treatment is old. However, earlier PRA studies had the limitations of including small sample sizes, measuring PRA using different methods, and using different cut points for PRA categorization. Only recently, researchers started to regain interest in PRA’s potential role and a few studies have been conducted which included larger sample sizes and used the 0.60-0.65 ng/mL/hr cut point derived from the earlier Laragh’s studies. In all recently published studies evaluating the use of PRA, the only tested anti-V drug was HCTZ. Since there is an evidence that CTD is more potent BP lowering drug than HCTZ, our first aim is to study the potential role of PRA in guiding therapy in EA and AA patients who were sequentially treated with MET (β-blocker, anti-R drug) and then CTD (thiazide-like diuretic, anti-V drug). The paired study design will also have the advantage of yielding more accurate and reliable results since there is reduced inter-patient variability. This is especially important in view of the fact
that BP response is a complex trait which is affected by many environmental and genetic factors that are highly variable among hypertensive individuals.\textsuperscript{90} As the PRA cut point of 0.60-0.65 ng/mL/hr was determined based on a EA population,\textsuperscript{20-22} we also aim to identify the optimal cut point in AA patients, in order to better identify the subset of AA who could be successfully treated with anti-R drugs. The results from our present studies will shed light on the potential role of PRA in guiding therapy, and if proven to be clinically useful, could provide the basis for a more personalized treatment approach to HTN.
Figure 1-1. Diagram showing how the RAAS works

*Poiseuille’s equation which states that $BP = CO \times TPR$, which are mainly determined by the sodium-volume status (V) and renin-induced vasoconstriction (R).
Figure 1-2. Diagrams showing the mechanisms of action of anti-V and anti-R drugs in treating different forms of HTN. A) sodium volume-dependent or lower-renin HTN (PRA < 0.65 ng/mL/hr) can be treated with anti-V drugs shown in blue boxes. Diuretics act by inhibiting the sodium reabsorption from the renal
tubules. α-blockers block the α-receptors in the kidney resulting in renal vasodilatation and increased sodium excretion. Calcium channel blockers (CCBs) cause renal vasodilation, thus natriuresis by reducing the intracellular calcium. B) vasoconstriction-dependent or higher-renin HTN (PRA ≥ 0.65 ng/mL/hr) can be treated with anti-R drugs shown in yellow boxes. β-blockers inhibit the renal renin secretion by blocking the β-adrenergic receptors. ACE inhibitors block the conversion of angiotensin I into the vasoconstrictor angiotensin II. ARBs block the angiotensin II (AT II) receptors and its vasoconstrictor action.
## Table 1-1. Anti-V and anti-R antihypertensive drugs

<table>
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<tr>
<th>Anti-V drugs</th>
<th>Anti-R drugs</th>
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<tbody>
<tr>
<td><strong>Diuretics</strong></td>
<td><strong>ACE inhibitors</strong></td>
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<tr>
<td>Thiazides (hydrochlorothiazide, chlorthalidone, chlorthiazide, indapamide, metolazone)</td>
<td>Benazepril</td>
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<td>Captopril</td>
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<td>Ramipril</td>
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<td></td>
<td>Trandolapril</td>
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<td></td>
<td>Moexipril</td>
</tr>
<tr>
<td>Loop (furosemide, torsemide, bumetanide, ethacrylic acid)</td>
<td></td>
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<tr>
<td>SARA (spironolactone, eplerenone)</td>
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### Abbreviations:
- SARA, selective aldosterone receptor antagonists
- CCBs, calcium channel blockers

### α-blockers
- Doxazosin
- Prazosin
- Terazosin

### CCBs
- Dihydropyridine (amlodipine, felodipine, isradipine, nicardipine, nifedipine, nisoldipine)

### Non-dihyrdopyridine
- Diltiazem, verapamil

### β-blockers
- Non-selective agents
- β₁-selective agents (acebutolol, atenolol, betaxolol, bisoprolol, esmolol, nebivolol, metoprolol, and celiprolol)
CHAPTER 2
BP RESPONSE TO CTD AND MET AMONG A DIVERSE COHORT OF CONTEMPORARY HYPERTENSIVES

Introduction

Despite the availability of multiple, effective antihypertensive drug classes, only half of patients with HTN have BP that is controlled.\textsuperscript{91} The World Health Organization postulates that medication non-adherence is a major contributor to poor BP control,\textsuperscript{92} however, there is evidence that even adherent patients have uncontrolled BP.\textsuperscript{9} This likely reflects the high inter-individual variability in BP response to various antihypertensive drugs, which often leads to prescription of additional antihypertensive drugs, which may or may not provide additional BP reduction, but will increase treatment costs and risk for adverse events.\textsuperscript{93}

Pathophysiologic heterogeneity in pathways underlying elevated BP contributes to the variability in antihypertensive drug response,\textsuperscript{94, 95} and therefore, empiric, routine use of a single class of antihypertensives, for example, thiazide or thiazide-like diuretics in all hypertensive patients, is unlikely to achieve optimal BP control for all hypertensives. Accordingly, we sought to compare BP response to optimized doses of contemporary antihypertensive drugs (CTD and MET tartrate) and identify patient characteristics associated with BP response, amongst a diverse population of well characterized essential HTN patients who were sequentially treated with both drugs as monotherapy.

Methods

Study Population

The PEAR-2 study was a prospective, multicenter, sequential, open-label clinical trial (clinicaltrials.gov identifier: NCT01203852) and has been described in detail
previously. Briefly, participants who had mild to moderate uncomplicated, essential HTN, between the ages of 17-65, of any race or ethnicity were included. Recruitment took place at 3 centers: The University of Florida (Gainesville, FL), Emory University (Atlanta, GA), and Mayo Clinic (Rochester, MN). Participants were excluded if they had secondary HTN, isolated systolic HTN, clinic or home measured SBP or DBP > 180 mmHg or 110 mmHg respectively, cardiovascular disease, diabetes mellitus, renal insufficiency, or liver dysfunction. Pregnant or lactating women were also excluded from the trial. All participants provided written, voluntary informed consent to participate in the study, which was approved by the institution review board at each study site and was conducted in accordance with the Declaration of Helsinki.

**Study Procedures**

Following a systematic withdrawal of current antihypertensive medications, baseline BP and blood samples were obtained for analysis of multiple biomarkers. Participants were initially treated with MET tartrate 50 mg twice daily for two weeks, followed by a dose titration to 100 mg twice daily for an additional six weeks if BP remained > 120/70 mmHg. Participants then underwent a 4-week washout period and once clinic DBP > 90 mmHg, baseline BP and blood samples were again obtained. Participants were then treated with CTD 15 mg daily for two weeks, followed by a titration to 25 mg daily over an additional six weeks if BP remained > 120/70 mmHg.

Clinic BP was measured using Microlife monitors, model #s BP3AC1-PC and BP3MC1-PC (Dunedin, FL). The monitors were set to obtain three sequential BP measurements separated by two minutes each, and the average of the three measurements was recorded.
Laboratory Analysis

Baseline levels of PRA were determined by the core laboratory at the Mayo Clinic, from plasma samples obtained after washout but prior to treatment with MET and CTD. PRA was determined using a radioimmunoassay of the generated angiotensin I based on Sealey’s method. The reagents used were purchased from DiaSorin (Stillwater, MN) and the incubation period for angiotensin I generation was 3 hours except for samples with PRA < 1 ng/mL/hr, which were incubated for 18 hours. All samples were assayed in duplicate or triplicate, and the mean PRA value for each participant was determined prior to treatment with MET and again before treatment with CTD.

Statistical Analyses

To identify the characteristics associated with BP response to MET and CTD, we compared the BP response to both drugs within each racial group by age and by PRA category since the contribution of these characteristics to BP response variability has been inconsistently reported. All participants included in this analysis were treated sequentially with MET monotherapy followed by CTD monotherapy. Among these, participants were excluded if their PRA level obtained during the post-MET washout period did not return to the pre-MET level based on PRA category (<0.65 or ≥0.65 ng/mL/hr). All data for quantitative variables are summarized as means with SD, except for PRA, which was not normally distributed, thus summarized as medians with interquartile ranges. Categorical variables are summarized as numbers and percentages.
Primary outcome

DBP responses to MET monotherapy and CTD monotherapy for each individual patient were defined by subtracting the pretreatment clinic DBP from the posttreatment clinic DBP, such that negative values indicate declines in DBP in response to drug treatment. We focused on DBP response since participants were enrolled based on DBP measurements. DBP responses to CTD versus MET were compared within each racial group in all participants, by age subgroups (< 50 and ≥ 50 years) and by PRA subgroups (< 0.65 and ≥ 0.65 ng/mL/hr). The age threshold was chosen based on the mean age (50 years) of the study participants, and the 0.65 PRA cut point was used since it has been determined based on nomograms constructed in Laragh’s earlier studies and used in previous clinical trials of PRA-guided treatment approach.20, 23, 84, 86

Paired comparison of DBP response to MET and CTD

After confirming the data normality using normal probability plots, paired t-test was used to compare the DBP responses to MET and CTD within each racial group in all participants, by age and by PRA subgroups.

Differences in DBP response to CTD versus MET

To estimate the adjusted mean differences in DBP response to CTD versus MET, multivariable mixed effects linear regression models were used. The analyses were conducted within each racial group in all participants, by age and by PRA subgroups and all the models were adjusted for baseline DBP and gender. A negative value in DBP response difference means a participant had a better response to CTD than to MET. All statistical tests were 2-tailed and \( P<0.05 \) was considered significant. SAS version 9.4 (SAS institute, Cary, NC) was used in all statistical analyses.
ROC curves in EA

Based on our findings which were suggestive of a strong relationship between PRA and DBP response in EA participants, we further evaluated PRA’s predictive value using ROC analysis. The binary outcome was whether or not the difference in DBP response to CTD compared with that to MET was at least 3 mmHg. Since SBP is more difficult to control, we additionally assessed the test performance of PRA to predict SBP response using the binary outcome of whether or not the difference in SBP response to CTD compared with that to MET was at least 5 mmHg. These BP thresholds were chosen based on the evidence that reduction in DBP of 3 mmHg or SBP of 5 mmHg was associated with 27% decline in risk of stroke. The optimal PRA cut point value was also determined using the Youden index which assesses the performance of a diagnostic test based on the false positives and false negatives, giving an equal weight to sensitivity and specificity. MedCalc statistical software, version 16.8.4 (MedCalc Software, Mariakerke, Belgium, http://www.medcalc.org) was used for the ROC analysis.

Results

Study Population

The final study population included 237 participants, 134 (56.5%) EA and 103 (43.5%) AA (Figure 2-1). The baseline characteristics of the population are summarized in Table 2-1. Overall, the population was 47.3% female, and on average, participants were overweight or obese (body mass index = 30.7 ± 5 kg/m²). EA participants had higher PRA levels than AA participants ($P<0.0001$) at the pre-MET and pre-CTD time points. PRA categorization according to age and race is shown in Table 2-2.
Comparison of DBP Response to MET and CTD

BP response is summarized in Table 2-3 according to race. At baseline, clinic BP was similar among EA and AA participants before treatment with MET and CTD. Among EA, DBP response was greater to MET than to CTD (-11.1±8.5 vs. -8.6±8.1 mmHg, \(P=0.02\)) (Figure 2-2a). This finding was consistent amongst those < 50 years (-10.0±8.7 vs. -5.9±6.8 mmHg, \(P=0.01\)), while in those ≥ 50 years, DBP responses were similar to MET and CTD (\(P=0.3\)). Among EA with PRA < 0.65 ng/mL/hr, DBP response was greater to CTD than to MET (-11.9±8.1 vs. -7.5±7.7 mmHg, \(P=0.004\)), whereas those with PRA ≥ 0.65 ng/mL/hr had a better DBP response to MET than to CTD (-12.9±8.3 vs. -7±7.6 mmHg, \(P<0.0001\)).

Among AA, DBP response to MET and CTD was similar (-7.0±8.5 vs. -8.9±8.0 mmHg, \(P=0.1\)) (Figure 2-2b), and there was no significant difference in response to MET and CTD among those < 50 years old (\(P=0.7\)) or ≥ 50 years old (\(P=0.07\)). While AA with PRA < 0.65 ng/mL/hr had a greater DBP reduction to CTD than to MET (-10.0±8.0 vs. -6.2 ± 7.7 mmHg, \(P=0.004\)), DBP response among AA with PRA ≥ 0.65 ng/mL/hr to MET and CTD was similar (\(P=0.2\)).

Following adjustment for baseline DBP and gender, consistent DBP responses to MET and CTD were obtained in all subgroups with the exception of the AA cohort overall and AA ≥ 50 years old cohort, where in both cases there was a 3 mm Hg greater DBP decrease to CTD (adjusted \(P \text{ value} = 0.0045\), and 0.01 respectively) (Figure 2-3).

**ROC Analyses in EA**

Because PRA was significantly associated with DBP response to both drugs among EA, ROC analyses were performed to further confirm the role of PRA as a predictive biomarker of BP response to MET and CTD. The areas under the ROC
curves for the DBP and SBP responses were 0.74 and 0.69 respectively \((P<0.001)\) (Figure 2-4). The optimal PRA cut point value with the highest Youden index was 0.6 ng/mL/hr, with a sensitivity of 54.2% and a specificity of 85% to predict the DBP response and with a sensitivity of 48.3% and a specificity of 85.1% to predict the SBP response to MET and CTD.

**Discussion**

To our knowledge, this is the first study to evaluate the contribution of specific patient characteristics to BP response variability among a cohort of diverse, uncomplicated hypertensive patients exposed to sequential treatment of two contemporary antihypertensive monotherapies. Our data suggest that within the EA and AA race groups, both age and PRA are associated with DBP response to MET and CTD. Additionally, we demonstrated that a PRA cut point of 0.65 ng/mL/hr is a useful biomarker with good sensitivity and specificity and could be used to guide the initial antihypertensive drug selection in EA patients with uncomplicated HTN.

Overall in EA, the average DBP response was greater to MET, a β-blocker than to CTD, a thiazide-like diuretic, while overall in AA, the average DBP response was greater to CTD than to MET. Our results are consistent with those of several previous studies\(^99, 104-106\) and are likely explained by the higher PRA levels among EA than AA.\(^46, 49, 67\) The RAAS is an important pathophysiologic regulator of BP. Renin produces angiotensin I, which is rapidly converted by the ACE into angiotensin II, which causes vasoconstriction and stimulates the release of aldosterone, leading to sodium retention and elevated BP.\(^13\) Laragh and colleagues suggested that hypertensive patients with PRA < 0.65 ng/mL/hr likely have sodium volume-dependent HTN and would respond better to diuretics, whereas those with PRA ≥ 0.65 ng/mL/hr likely have angiotensin-
mediated vasoconstriction-dependent HTN and would respond better to RAS blockers that either block the formation of angiotensin II (ACE inhibitors), block its action (ARBs), decrease renin release from the kidneys (β-blockers), or block enzymatic activity of renin.65

In the present study, younger EA participants had a greater DBP response to MET, whereas older EA participants had a similar response to both drugs. On the other hand, younger AA participants had a similar DBP response to both drugs, while older AA participants had a better response to CTD. Consistent with previous studies,59-61 we observed that both EA and AA participants with higher-PRA were significantly younger than those with lower-PRA (data not shown). This difference in PRA by age group may explain the BP response differences between the age subgroups.62 Accordingly, it has been suggested that age could be used as a surrogate for PRA in personalizing antihypertensive therapy whereby younger-age could be used as a marker for higher-PRA, while older-age could be used as a marker for lower-PRA.67 However, our results demonstrate that there are subsets of patients whose PRA levels were inconsistent with their age subgroup: 25% of younger-EA and 60% of younger-AA had lower-PRA, whereas 61% of older-EA and 21% of older-AA had higher-PRA. Similar findings were reported by a large cohort which included over 4000 hypertensive patients and suggested that although age was negatively correlated with PRA levels, age is not an accurate estimation of the RAS activity.107 Lastly, we have previously observed that BP response to HCTZ and candesartan or atenolol was significantly greater based on PRA category than age category, and regardless of race, significantly greater BP control rates were observed when a PRA-guided strategy was used to model BP response.46, 47
Interestingly, we observed that EA with lower-PRA had a greater DBP response to CTD than to MET. In a previous study among participants treated with either atenolol or HCTZ monotherapy, we demonstrated that lower-PRA EA participants had similar BP responses to both drugs,\textsuperscript{47} while another study showed that the lower-PRA EA subjects treated with candesartan monotherapy had even a greater DBP response compared with those treated with HCTZ monotherapy.\textsuperscript{46} This inconsistency could be due to the higher potency of CTD, compared with that of HCTZ. This is supported by a recent study which suggested that CTD 25 mg was associated with 5/4 mm Hg significantly greater SBP and DBP reduction, compared with either HCTZ 25 mg or 50 mg.\textsuperscript{88}

Moreover, among patients treated sequentially with HCTZ and then CTD, additional BP lowering was observed.\textsuperscript{89}

In contrast, among our EA population with higher-PRA, we observed a greater average DBP response following treatment with MET than to CTD, which is consistent with other studies that demonstrated a greater response to atenolol vs HCTZ and candesartan vs HCTZ among EA with higher-PRA.\textsuperscript{46, 47} Additionally, Egan et al. demonstrated that EA subjects with uncontrolled BP who were prospectively randomized to a PRA-guided antihypertensive medication selection approach had a significantly greater reduction in SBP compared with those randomized to the usual medication selection approach.\textsuperscript{84} Based on these findings, we further tested the predictive performance of PRA in this population and demonstrated, for the first time, that PRA at a cut point of 0.60-0.65 ng/mL/hr, derived among a cohort of Caucasians decades ago, is an informative predictive biomarker of both SBP and DBP responses with good sensitivity and specificity.\textsuperscript{65}
Among AA participants, we observed that those with lower-PRA responded
to CTD better than to MET, whereas those with higher-PRA had similar response to
both drugs and this is consistent with previous studies.\textsuperscript{46, 47} Some have suggested that
PRA is not a useful biomarker in AA,\textsuperscript{86, 99, 100} while others suggest personalizing
antihypertensive therapy in younger-AA based on PRA is possible.\textsuperscript{46} Our results
indicate that even the younger-AA with higher-PRA had similar response to both CTD
and MET (data not shown). While the higher potency of CTD may explain this
inconsistency, the difference in potency has been documented primarily in a EA
population.\textsuperscript{88, 89} Therefore, it remains uncertain whether or not PRA is a reliable
predictive biomarker of BP response in AA hypertensives, or whether the 0.65 ng/mL/hr
cut point, originally derived in EA subjects,\textsuperscript{20, 23, 99} is not the optimal cut point for AA
hypertensives.

Our study has a number of strengths. First, to the best of our knowledge, this is
the first study to identify clinical factors associated with the BP response using
participants who were systematically exposed to the sequential treatment with MET and
CTD monotherapies, therefore minimizing interpatient variability. Among studies
investigating the role of PRA in personalizing antihypertensive therapy, this study is the
first to utilize the more potent thiazide-like diuretic, CTD rather than HCTZ.\textsuperscript{36, 46, 47, 99} To
our knowledge, one of the largest randomized clinical trials which utilized CTD, the
ALLHAT did not test PRA.\textsuperscript{108} It is also the first study of its kind to test and confirm the
predictive value of a 0.65 ng/mL/hr PRA cut point among EA. On the other hand, the
results of the present study should be also viewed in light of its limitations. This was a
retrospective analysis of data from a multicenter clinical trial with a relatively short
period of observation for each drug treatment. Also, the small sample size, especially in some subgroups limits our power, however, we overcame this issue by conducting an additional analysis using all PEAR-2 subjects (n=681) and obtained similar results (data not shown). Lastly, it is uncertain whether or not our results can be applied to RAS blockers other than the β-blocker, MET.

In conclusion, our data suggest that race, age and PRA are important characteristics associated with DBP response to MET and CTD among a population of uncomplicated hypertensives. A PRA cut point of 0.60-0.65 ng/mL/hr is reasonable for use in EA for a more personalized approach to antihypertensive medication selection, especially when CTD is the diuretic of choice. Using a PRA-guided treatment approach has been suggested to reduce the number of the antihypertensive drugs needed to control BP, thus improving medication adherence, and decreasing treatment costs and the potential for adverse events. Additional investigation is necessary to determine the optimal PRA cut point in AAs.
Table 2-1. Baseline characteristics overall and by race group

<table>
<thead>
<tr>
<th></th>
<th>Overall (n = 237)</th>
<th>EA (n = 134)</th>
<th>AA (n=103)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>50.9 (9.0)</td>
<td>51.4 (9.1)</td>
<td>50.2 (9.0)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.7 (5.0)</td>
<td>30.3 (4.9)</td>
<td>31.1 (5.2)</td>
</tr>
<tr>
<td>Female no. (%)</td>
<td>112 (47.3%)</td>
<td>59 (44.0%)</td>
<td>53 (51.5%)</td>
</tr>
<tr>
<td>Pre-MET PRA, ng/mL/hr</td>
<td>0.7 (0.3-1.3)</td>
<td>1.0 (0.4-1.8)</td>
<td>0.4 (0.2-0.7)</td>
</tr>
<tr>
<td>Pre-CTD PRA, ng/mL/hr</td>
<td>0.7 (0.3-1.3)</td>
<td>0.9 (0.5-1.5)</td>
<td>0.4 (0.3-0.7)</td>
</tr>
</tbody>
</table>

All continuous variables are summarized as mean (SD) except PRA, which is expressed as median (upper and lower quartiles). Discrete variables are summarized as frequency (group percentage).
Table 2-2. PRA categorization according to age and race

<table>
<thead>
<tr>
<th>PRA categorization</th>
<th>Age &lt; 50 years</th>
<th>Age ≥ 50 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA (n = 45)</td>
<td>EA (n = 49)</td>
</tr>
<tr>
<td>PRA &lt; 0.65 ng/mL/hr, no. (%)</td>
<td>27 (60.0)</td>
<td>12 (24.5)</td>
</tr>
<tr>
<td>PRA ≥ 0.65 ng/mL/hr, no. (%)</td>
<td>18 (40.0)</td>
<td>37 (75.5)</td>
</tr>
</tbody>
</table>
Table 2-3. Baseline and post-treatment BP according to race

<table>
<thead>
<tr>
<th></th>
<th>MET</th>
<th></th>
<th></th>
<th></th>
<th>CTD</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-BP mmHg (SD)</td>
<td>Post-BP mmHg (SD)</td>
<td>Delta-BP mmHg (SD)</td>
<td>% change</td>
<td>Pre-BP mmHg (SD)</td>
<td>Post-BP mmHg (SD)</td>
<td>Delta-BP mmHg (SD)</td>
<td>% change</td>
<td></td>
</tr>
<tr>
<td>EA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>149.9 (12.5)</td>
<td>137.1 (15.6)</td>
<td>-12.8 (14.7)</td>
<td>8.4</td>
<td>150.9 (13.0)</td>
<td>134.9 (12.3)</td>
<td>-16.0 (14.1)</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>97.6 (4.7)</td>
<td>86.5 (8.0)</td>
<td>-11.1 (8.5)</td>
<td>11.2</td>
<td>98.6 (5.4)</td>
<td>90.0 (7.9)</td>
<td>-8.6 (8.1)</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>150.9 (12.1)</td>
<td>143.6 (16.5)</td>
<td>-7.3 (16.3)</td>
<td>4.6</td>
<td>148.8 (12.0)</td>
<td>133.3 (11.5)</td>
<td>-15.5 (14.1)</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>99.0 (6.1)</td>
<td>91.9 (8.5)</td>
<td>-7.0 (8.5)</td>
<td>7.0</td>
<td>97.8 (5.1)</td>
<td>88.9 (8.0)</td>
<td>-8.9 (8.0)</td>
<td>9.0</td>
<td></td>
</tr>
</tbody>
</table>

Data are summarized as mean (SD). Percent change in BP in response to each drug was calculated using this equation:

\[
\frac{\text{Post-treatment BP} - \text{Pretreatment BP}}{\text{Pretreatment BP}} \times 100
\]
Figure 2-1. Flow diagram showing the study subjects who were included in the final analysis.
Figure 2-2. Clinic DBP in response to MET and to CTD in (A) EAs and in (B) AAs overall, by age and by PRA subgroups.
Figure 2-3. Adjusted mean differences in clinic DBP response (mean, 95% confidence interval (CI)) to CTD vs. MET for each racial group overall, by age and by PRA subgroups. All the values are adjusted for baseline DBP and gender.
Figure 2-4. The ROC curves in EA subjects (n=134) of PRA as a predictive biomarker of (A) DBP response where the vertical axis shows whether or not the difference in DBP response to CTD compared with that to MET was at least 3 mmHg. The area under the curve (AUC) was 0.74 (95% confidence interval [95% CI], 0.65-0.81, \( P < 0.0001 \)); cut point with the highest Youden index (0.39) was 0.6 ng/mL/hr with a sensitivity of 54.2% and specificity of 85%; and of (B) SBP response where the vertical axis shows whether or not the difference in SBP response to CTD compared with that to MET was at least 5 mmHg. The AUC was 0.69 (95% CI, 0.60-0.76, \( P = 0.0001 \)); cut point value with the highest Youden index (0.33) was 0.6 ng/mL/hr with a sensitivity of 48.3% and a specificity of 85.1%.
CHAPTER 3
PRA IS NOT A PREDICTIVE BIOMARKER OF BP RESPONSE TO RAS BLOCKERS AND THIAZIDE DIURETICS IN AA

Introduction

AAs develop HTN at an earlier age, have higher BP on average, and are at a greater risk for developing cardiovascular disease and target organ damage, compared with EA. Use of antihypertensive drugs to lower BP, particularly SBP, has been proven to reduce cardiovascular risks and prevent target organ damage. Although AAs are likely to have a better antihypertensive response to thiazide diuretics than to RAS blockers, including β-blockers, ACE inhibitors and ARBs, there is still a high inter-individual variability in BP response.

Published literature suggests that PRA can be a useful, predictive biomarker of BP response that can differentiate between the volume-dependent (PRA < 0.65 ng/mL/hr) and the vasoconstriction-dependent (PRA ≥ 0.65 ng/mL/hr) mechanisms underpinning HTN, and thus inform antihypertensive treatment selection to optimize BP response and improve BP control rates. While in EA individuals, PRA does have the potential to improve the precision of antihypertensive therapy, in AA individuals, investigations of PRA’s potential role as a predictive tool for BP response has yielded inconsistent results.

Because the 0.65 ng/mL/hr cut point for differentiating volume vs vasoconstriction-dependent mechanisms of HTN was derived in EAs and since there is a paucity of data regarding PRA level and the associated BP response in AA population, we sought to determine and validate a PRA cut point that could predict antihypertensive drug response amongst AAs.
Methods

Study Population

The PEAR-2 and PEAR-1 studies

The PEAR-2 was a prospective, multicenter, open-label, sequential trial (clinicaltrials.gov identifier: NCT01203852). The detailed description of this study has been previously published.96 Patients who were diagnosed with mild to moderate, uncomplicated essential HTN, between the ages of 17-65 were enrolled. Exclusion criteria were secondary HTN, clinic or home measured SBP or DBP > 180 or 110 mmHg respectively, isolated systolic HTN, cardiovascular disease, diabetes mellitus, heart rate < 55 beats/min, renal or hepatic dysfunction. Also, pregnant or lactating women were excluded. The study has been conducted at 3 centers: The University of Florida (Gainesville, FL), Emory University (Atlanta, GA), and the Mayo Clinic (Rochester, MN). Participants underwent 4-6 weeks washout period to allow the re-establishment of DBP > 90 mmHg after withdrawal of their current antihypertensive medications. Then, at baseline, BP was measured and blood samples were collected for analysis of multiple biomarkers. Participants were initially given MET tartrate 50 mg twice daily for two weeks, then the dose was titrated to 100 mg twice daily over a duration of six weeks if BP remained above 120/70 mmHg. After a second washout period, baseline BP was recorded and blood samples were again collected. Participants were then given CTD 15 mg daily for two weeks followed by a titration to 25 mg daily over a duration of six weeks if BP remained above 120/70 mmHg. Clinic BP was measured using monitors manufactured by Microlife, model #s BP3AC1-PC and BP3MC1-PC (Dunedin, FL). Three sequential BP measurements, separated by two minutes each, were recorded and the mean of these measurements was used in all
analyses. This analysis focuses on the AA subjects who were treated with MET or CTD monotherapies in the PEAR-2 study.

The PEAR-1 study was a prospective, multicenter, randomized, open-label, crossover clinical trial (clinicaltrials.gov identifier: NCT00246519). The detailed description of the PEAR aims, design and procedures has been previously reported. The inclusion and exclusion criteria as well as the study recruitment sites of the PEAR-1 were the same as that of the PEAR-2. Participants underwent 4-6 weeks washout period following the withdrawal of their current antihypertensive medications. Then, baseline BP was measured and blood samples were obtained for analysis of multiple clinical biomarkers. Participants were then randomized to either atenolol 50 mg (titrated to 100 mg if BP remained above 120/70 mmHg), followed by HCTZ 12.5 mg (titrated to 25 mg if BP remained above 120/70 mmHg), or HCTZ 12.5 mg (titrated in a similar way), followed by atenolol 50 mg (titrated in a similar way). Clinic BP at baseline, post monotherapy and post the combination therapy were measured using an automated oscillometric sphygmomanometer (Microlife 3AC1-PC; Microlife, Minneapolis, MN). The monitors were set to take three BP measurements and the average of the three was recorded and used in all analyses. This analysis focuses on the AA subjects who were randomized to either atenolol monotherapy or HCTZ monotherapy in the PEAR-1 study.

All the study participants provided written voluntary informed consents prior to participation in each study. The protocols of the two studies were approved by the institutional review board at each study site and they were conducted in accordance with the Declaration of Helsinki.
The GERA

Subjects were treated with HCTZ in GERA-1 and they were treated with candesartan (ARB) in GERA-2 (clinicaltrials.gov identifier: NCT00005520). The detailed description of GERA design and procedures has been previously described. AA and EA subjects with primary HTN, between the ages of 30-59 were recruited at Emory University in Atlanta, Georgia and at the Mayo Clinic in Rochester, Minnesota respectively. Subjects were excluded if they had secondary HTN, SBP or DBP >180 mmHg or 110 mmHg respectively, kidney or hepatic dysfunction, serious cardiovascular disease, diabetes mellitus, gout, or sulfa allergy. Females receiving oral contraceptives were also excluded. Subjects were maintained on a standard sodium intake of 2 mmol/kg/day throughout the study duration. Following withdrawal of the subjects’ current antihypertensive medications, they underwent a 4-6 week washout period to allow the achievement of DBP > 90 mmHg. Baseline BP and blood samples were obtained for analysis of multiple biomarkers. Subjects were then given HCTZ 25 mg once daily for four weeks (GERA-1) or candesartan 16 mg once daily for two weeks, followed by a titration to 32 mg once daily for an additional four weeks (GERA-2). BP was measured in triplicate in the morning using an automated oscillometric sphygmomanometer (Omron HEM-907; Omron, Bannockburn, IL) and the average of the second and third readings were used in all analyses. All the study participants provided written, voluntary informed consent to participate. The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board at each study site. This analysis focuses on the AA subjects who were treated with either HCTZ (GERA-1) or candesartan (GERA-2).
Laboratory Analysis

Baseline levels of PRA were determined from plasma samples obtained before treatment with each drug monotherapy in both PEAR studies and GERA studies. PRA was measured by radioimmunoassay of the generated angiotensin I using reagents purchased form DiaSorin company (Stillwater, MN) in PEAR-2, PEAR-1 and GERA-2 (candesartan), and from Dupont company (Boston, MA) in GERA-1 (HCTZ). PRA measurements by both assays were performed in accordance with the manufacturer’s recommendations. The standard incubation time for the DiaSorin assay was 3 hours, whereas the incubation time for the Dupont assay was 1 hour. All PRA samples were assayed in duplicate or triplicate, and the mean value for each participant was used in all analyses.

Statistical Analyses

AA participants enrolled in the PEAR and GERA studies were included in this study. Data for all continuous variables are summarized as means with SD, except for PRA, which was not normally distributed, and thus summarized as medians with interquartile ranges. Categorical variables are summarized as numbers and percentages. BP response to each drug for each individual was determined by subtracting the pretreatment clinic BP from the post-treatment BP, such that negative values indicate reductions in BP in response to drug treatment.

The flow chart in Figure 3-1 outlines the analytic steps in this study. Because AA patients are less likely to have controlled BP, are more likely to have cardiovascular complications compared with other populations, and previous studies have shown that elevated SBP is associated with more cardiovascular adverse events, we focused this investigation on SBP. Data from the PEAR-2 study (discovery cohort) were
used to estimate the PRA cut point associated with BP response. The PEAR-2 derived PRA cut point was then tested in two independent validation cohorts of AAs enrolled in PEAR-1 and GERA. In all analyses, all statistical tests were 2-tailed and $P<0.05$ was considered significant. SAS version 9.4 (SAS institute, Cary, NC) was used for all analyses.

**Assessment of the correlation between log-transformed PRA and SBP responses in PEAR-2**

After excluding outliers from the distribution of the log-transformed PRA and SBP responses ($\pm 4$ SD from the mean), the partial correlations between baseline log-transformed PRA and SBP responses to MET monotherapy and to CTD monotherapy were assessed using Pearson’s correlation coefficients, adjusted for baseline SBP.

**Determination of the PRA cut point in PEAR-2 AA participants**

It has been suggested that EA patients with PRA $< 0.65$ ng/mL/hr are more effectively treated with thiazides, whereas those with PRA $\geq 0.65$ ng/mL/hr are more effectively treated with RAS blockers. Accordingly, we sought to determine the PRA level (cut point) at which both MET ($\beta$-blocker, RAS blocker) and CTD (thiazide-like diuretic) in PEAR-2 were associated with the same SBP response. We hypothesized that patients who have PRA values below this cut point would have a better response to thiazides, whereas those who have PRA values above this cut point would have a better response to RAS blockers.

Two multivariable linear regression models were constructed using SAS software where the outcome variables were SBP responses to MET monotherapy and to CTD monotherapy. The log-transformed PRA along with the age, gender, baseline SBP, smoking status, alcohol drinking status, height and waist were initially forced into the
two models. These characteristics have been previously implicated as predictors of BP responses to β-blockers and diuretics.\textsuperscript{112} Next, only the predictors (other than the log-transformed PRA) which were statistically significant in at least one model and had the same direction of effect (the same sign) in both models were retained. Then, the (solve) function in the R statistical computing and graphics software, version 3.3.1 (R Development Core Team, \url{http://www.r-project.org}) was used to equate and solve the two regression equations to estimate the PRA cut point.

**Ancestry of higher-PRA versus lower-PRA AA subjects**

A considerable body of evidence supports that EA have higher PRA values than AA.\textsuperscript{46-49} Thus, we aimed for comparing the ancestry of the higher versus lower-PRA AA subjects based on the new determined cut point using the PCA analysis in PEAR-1 and PEAR-2, which was used to assess the ancestry and also to confirm the race of the study participants.\textsuperscript{115, 116} PCA analysis was performed using EIGENSTRAT method on a LD set of SNPs.\textsuperscript{117} Participants were clustered into different ancestry groups based on the PCA data. Herein, we plotted the PC1 versus PC2 of the AA subjects in PEAR-1 and in PEAR-2 by the different PRA subgroups. We chose the PCs 1 and 2 because they explain a large proportion of the variance, thus provide the best separation of ancestry clusters.

**Testing the PEAR2-derived PRA cut point in two independent cohorts**

To test the ability of the derived PRA cut point to differentiate SBP response among independent cohorts of AA subjects, SBP responses to RAS blocker and thiazide diuretic were compared within each PRA subgroup in each validation cohort. This was done by estimating the adjusted mean differences in SBP response between atenolol and HCTZ monotherapies in PEAR-1 and between candesartan and HCTZ
monotherapies in GERA. Multivariable linear regression models, adjusted for baseline SBP were used in SAS.

A meta-analysis of PEAR-1 and GERA was conducted using the metafor R package (https://cran.rproject.org/web/packages/metafor/index.html). The measure of effect was the pooled adjusted mean differences in SBP response between atenolol/candesartan and HCTZ with their corresponding 95% confidence intervals within each PRA subgroup based on the determined cut point. Between-study heterogeneity was assessed using the Cochrane Q statistic\textsuperscript{118} and the random-effects models, which incorporates both within- and between-study variability, was used regardless of heterogeneity.\textsuperscript{119}

**Determination of the sensitivity and specificity of the PEAR2-derived cut point**

Since assessing the clinical utility of a biomarker using a certain cut point requires the evaluation of its predictive performance,\textsuperscript{120} the predictive performance of the derived PRA cut point was evaluated by determining the sensitivity, specificity, PPV and NPV. Since AAs overall have a better antihypertensive response to thiazides than to RAS blockers,\textsuperscript{111} and since PRA biomarker has been suggested to identify the subset of AA patients who can be successfully treated with RAS blockers,\textsuperscript{65} we focused on SBP response to RAS blockers. Combined data of RAS blockers’ observations (atenolol and candesartan) from PEAR-1 and GERA respectively were used in this analysis. Table 3-1 shows the definitions of the true positives, true negatives, false positives and false negatives used to conduct this analysis. The cutoff of a 5 mmHg reduction in SBP from baseline (good response) was chosen based on the evidence that a reduction in SBP of this magnitude was associated with 27% and 15% declines in risk of coronary heart disease and stroke respectively.\textsuperscript{102} The epiR R package
was used to conduct this analysis.

Assessing the performance of PRA biomarker in AA

Based on our findings which showed that the derived cut point had low sensitivity, PRA’s predictive value was assessed using an ROC analysis where PRA was used as a continuous variable. To evaluate how well PRA can discriminate between β-blockers’ good responders and poor responders, an ROC analysis was conducted using data of atenolol observations from PEAR-1, where the binary outcome was whether or not atenolol was associated with a fall in the SBP ≥ 5 mmHg from baseline.

To further validate our results, the predictive performance of PRA biomarker to identify the subset of AA patients who had a better response to a RAS blocker compared with a thiazide diuretic was assessed. The ROC curve was plotted using data from subjects who were sequentially treated with both MET and CTD monotherapies in PEAR-2 (paired data). The binary outcome was whether or not the difference in SBP response to MET compared with that to CTD was at least 5 mmHg. These analyses were conducted using MedCalc statistical software, version 16.8.4 (MedCalc Software, Mariakerke, Belgium, http://www.medcalc.org).

Results

Study Population

AA participants included in the final analysis from PEAR-2, PEAR-1 and GERA cohorts are shown in Figures 3-2, 3-3 and 3-4. The baseline characteristics and BP responses in each cohort are summarized in Tables 3-2 and 3-3 respectively. In PEAR-1 and GERA, the drug groups did not differ significantly in the characteristics measured
at study enrollment. However, in GERA, the median PRA of subjects given candesartan was significantly lower than that of subjects treated with HCTZ, which is likely due to the different measurement assays used as described in the methods. Clinic SBP was similar in AA participants before treatment with each drug in PEAR-1 and GERA. Overall, AA subjects had a significantly better SBP response to HCTZ than to atenolol or candesartan ($P<0.001$ for all comparisons).

**Assessment of the Correlations between Log-Transformed PRA and SBP Responses in PEAR-2**

Only one subject with outlying post MET SBP value was excluded. The log-transformed baseline PRA was significantly correlated with the SBP responses to MET and to CTD in PEAR-2 (Figure 3-5). The negative correlation ($r = -0.22$, $P=0.0048$) indicates a greater SBP response to MET when baseline PRA values were higher, whereas positive correlation ($r = 0.3$, $P=0.0003$) indicates a greater SBP response to CTD when baseline PRA values were lower.

**Determination of the PRA Cut Point in PEAR-2**

Based on our specified criteria for selecting the covariates to be retained in the final two models, only the baseline SBP was retained in both models along with the log-transformed PRA (Table 3-4). The directionally opposite predictive effects (coefficients) of log-transformed PRA for the two drugs indicate the opposite relationships of PRA with the SBP response to each drug (as also indicated by the correlation coefficients presented above). After solving the regression equations, the estimated PRA cut point was 1.3 ng/mL/hr. Figure 3-6 shows the PRA cut point (the intersecting point) between the two regression lines which represent the SBP response to MET and the SBP response to CTD.
Ancestry of Higher-PRA Versus Lower-PRA AA Subjects

When the PC1 vs. PC2 were plotted in PEAR-1 and in PEAR-2, no distinct separation was observed between the AA subjects with PRA < 1.3 and those with PRA ≥ 1.3 ng/mL/hr. This indicates that all the AA subjects in both study cohorts had the same ancestry regardless of their PRA status (Figure 3-7).

Testing the PEAR2-Derived (1.3 ng/mL/hr) Cut Point in PEAR-1 and GERA

Figures 3-8 and 3-9 shows the adjusted mean differences in SBP responses (mean, 95% confidence intervals (CI)) and the adjusted mean SBP responses to HCTZ vs. atenolol monotherapies in PEAR-1 and to HCTZ vs. candesartan in GERA by PRA subgroups (< 1.3 and ≥ 1.3 ng/mL/hr). In PEAR-1, AA subjects with higher-PRA (≥ 1.3 ng/mL/hr) had similar SBP response to HCTZ monotherapy and atenolol monotherapy (P= 0.3). However, in GERA, AA subjects with PRA ≥ 1.3 ng/mL/hr given candesartan had a greater reduction in SBP of -7.5 mmHg (95% CI= -14.2 to -0.8 mmHg, P=0.028) than those given HCTZ. To check whether our non-significant results in the PRA ≥ 1.3 ng/mL/hr subgroup in PEAR-1 cohort were due to a lack of statistical power, a post-hoc power analysis was performed using GPower version 3.1.9.2 program with α set at 0.05, two-tailed. The power to detect a statistical significance was 0.22, thus PEAR-1 and GERA were meta-analyzed to improve our power.

In the meta-analysis of PEAR-1 and GERA, AA subjects with PRA < 1.3 ng/mL/hr had a significantly better SBP response to HCTZ than to atenolol or candesartan (meta-analysis P<0.0001). In contrast, AA subjects with PRA ≥ 1.3 ng/mL/hr who received a RAS blocker had a greater reduction in mean SBP of -7.6 mmHg (95% CI= -13.3 to -1.8, meta-analysis P=0.01) than those who received HCTZ (Figure 3-10).
Determination of the Sensitivity and Specificity of the PEAR2-Derived (1.3 ng/mL/hr) Cut Point

The total number of AA subjects included in this analysis was 417. As shown in Table 3-5, the 1.3 ng/mL/hr PRA cut point had a specificity of 97%, yet, a sensitivity of 10%. The high specificity indicates few false positives. In contrast, the low sensitivity indicates many false negatives, reflecting a high proportion of AA subjects with PRA < 1.3 ng/mL/hr (239 out of 385 subjects or 62%) who had a good response (a fall in SBP ≥ 5 mmHg from baseline) to RAS blockers (Table 3-6).

Assessing the Performance of PRA Biomarker in AA

Based on the finding which showed that the derived 1.3 ng/mL/hr cut point had low sensitivity to identify the subset of AA patients who had a good response to RAS blockers, ROC analyses were conducted to assess the predictive performance of PRA biomarker in this population. The area under the ROC curve shown in Figure 3-11a (0.59 (95% CI=0.51-0.67, P=0.06)) indicates that PRA biomarker cannot discriminate between the AA patients who had a good SBP response to atenolol from those who had a poor response.

There was no significant difference between SBP measured before treatment with MET and that measured before treatment with CTD in AA participants who were sequentially treated with both drug monotherapies. Also, PRA values were similar before treatment with MET and CTD. The area under the ROC curve shown in Figure 3-11b (0.60 (95% CI=0.51-0.69, P=0.07)) indicates that PRA did not also discriminate between the AA patients who had a better response to MET than to CTD from those who did not.
Discussion

To our knowledge, this is the first study undertaken to determine whether an optimal PRA cut point could be identified among a cohort of AA patients with uncomplicated HTN. We estimated the PRA cut point from PEAR-2 cohort, tested the derived cut point in two independent cohorts and assessed its predictive performance. We were unable to identify a PRA cut point that was predictive of BP response with high sensitivity in this population. Moreover, we demonstrated that PRA did not discriminate among AAs based on their SBP response, thus PRA is not a predictive biomarker in these patients.

AA hypertensive patients have less adequate BP control and more cardiovascular complications than the other populations. They are likely to have a better antihypertensive response to thiazide diuretics than to RAS blockers, which is also confirmed by our data. However, high inter-individual variability in BP response to different antihypertensive drug classes, especially to RAS blockers has been previously reported. A recent study suggested that 20-30% of AA could be effectively treated with β-blockers. Therefore, identifying biomarkers which can predict BP response is the key to personalize antihypertensive therapy in AA, better control their BP and reduce the associated cardiovascular risks.

PRA is considered one of the most promising biomarkers studied to date, especially in hypertensive EA. Laragh and associates were the first to advocate that the lower-PRA status (PRA < 0.65 ng/mL/hr) reflects volume-dependent HTN, which is caused by a higher tendency of sodium retention, thus can be corrected by diuretic therapy. In contrast, the higher-PRA status (PRA ≥ 0.65 ng/mL/hr) indicates vasoconstriction-dependent HTN, which is caused by an over-activity of the RAS, thus
can be treated by RAS blockers.\textsuperscript{65, 109} The 0.65 cut point was determined based on nomograms which showed the relationship between PRA measurements and the 24-hour urinary sodium excretion. These nomograms were constructed using normotensive and hypertensive subjects of primarily EA ancestry who have higher PRA levels than AA subjects.\textsuperscript{20} Moreover, the few earlier studies which did not find an association between PRA and BP response in AA population enrolled small number of subjects, measured PRA using different methods, used different thresholds for PRA categorization and focused exclusively on DBP response.\textsuperscript{36, 43, 99, 100} Using data from PEAR-1 and GERA, two recently published studies suggested that AA patients with PRA < 0.6 ng/mL/hr had a significantly greater BP reduction to HCTZ, compared with candesartan or atenolol (RAS blockers) respectively, whereas those with PRA ≥ 0.6 ng/mL/hr had a trend towards a better response to the RAS blockers, however, this did not reach a statistical significance.\textsuperscript{46, 47} Consistent results were also obtained when CTD was compared with MET within each PRA subgroup in PEAR-2 subjects who were sequentially treated with both drug monotherapies.\textsuperscript{124} Accordingly, we speculated that PRA might be a useful predictive biomarker in AA; yet, a more optimal cut point is needed.

Based on a systematic approach, we estimated the PRA level (cut point) at which MET and CTD monotherapies were associated with the same SBP response in PEAR-2 cohort. The cut point was estimated to be 1.3 ng/mL/hr, which is very close to the PRA cut point (1.2 ng/mL/hr) estimated by a retrospective analysis from PEAR-1. This analysis suggested that AA subjects with poorly controlled BP who have PRA ≥ 1.2 ng/mL/hr can be successfully treated with atenolol monotherapy.\textsuperscript{125} The meta-analysis
of the two independent validation cohorts showed that PRA of the 1.3 ng/mL/hr cut point was associated with SBP responses: AA subjects with PRA < 1.3 ng/mL/hr had -on average- a better response to HCTZ than to atenolol or candesartan (RAS blockers), whereas those with PRA ≥ 1.3 ng/mL/hr had -on average- a better response to the RAS blockers.

However, when we assessed the performance of the derived cut point to identify the subset of AA patients who could be effectively treated with RAS blockers, we found that the 1.3 ng/mL/hr cut point had very low sensitivity, indicating the presence of many false negatives. This likely reflects the high percentage (about 62%) of the AA subjects with PRA < 1.3 ng/mL/hr who had a good response (a fall in SBP ≥ 5 mmHg from baseline) to RAS blockers. The high variability in the SBP response to RAS blockers within the lower-PRA (< 1.3 ng/mL/hr) subgroup was not reflected by the average responses obtained from the meta-analysis. Our data also indicate that although AA subjects with PRA < 1.3 ng/mL/hr who were sequentially treated with MET and CTD in PEAR-2 had -on average- a significantly better response to CTD, about 27% of these subjects had a better response to MET than to CTD (a difference in SBP response to MET compared with that to CTD of at least 5 mmHg) (data not shown). In line with these observations, Minami et al. demonstrated that most large clinical trials reported a high variability (large SDs) in BP responses to RAS blockers. Therefore, it is difficult to predict the response for an individual patient based on the average effects if there is a high variability in treatment response. Additionally, when we assessed the predictive performance of PRA biomarker, we found that PRA did not discriminate between the AA subjects based on their response.
Our study has several strengths. To the best of our knowledge, it is the first study undertaken to determine whether an optimal PRA cut point could be identified among a cohort of hypertensive AA. It is also the first of its kind to assess the predictive performance of PRA biomarker in this population utilizing data from two studies which used two different RAS blockers. Additionally, our study is the first to report such a high percentage of AA patients who were successfully treated with RAS blockers. On the other hand, the results of this study should be considered in light of its limitations. First, this study was a retrospective analysis from three independent cohorts with a relatively short period of observation. However, we assume that the inferences from this analysis is unlikely to be biased since the populations used, inclusion and exclusion criteria were very similar among these studies. A second limitation is that we estimated the 1.3 ng/mL/hr PRA cut point based on the average baseline SBP of the PEAR-2 AA subjects. Although this might have introduced some potential error, using data from PEAR-2 study, where one third of the subjects were sequentially exposed to the same antihypertensive agents is expected to be associated with a minimal inter-patient variability. Additionally, the same cut point was obtained using unadjusted regression analyses. Another limitation is that PRA was measured using two different assays: DiaSorin assay used in PEAR-1, PEAR-2 and GERA-2 was associated with lower PRA values than the Dupont assay used in GERA-1 due to the longer incubation time of the former. An analysis using the GERA samples from which PRA was measured by both assays suggests that about 7% of the subjects would have been categorized differently by the two assays using the 1.3 cut point (data not shown). It is unclear how these differences in PRA categorization might affect our results when we tested the cut point
in GERA. However, we expect that these differences might have only a little effect on the results of the meta-analysis since both PEAR-1 (atenolol and HCTZ) and GERA-2 (candesartan) used the same assay. Also, these differences have no effect on the sensitivity and ROC results since we only included the RAS blockers’ observations.

In conclusion, the mechanisms for BP response variation in AA still remain poorly understood. We were unable to identify a PRA cut point with good sensitivity to predict BP response in AA. Our findings also suggest that PRA provides only little information for drug selection, which do not warrant the expense of its determination in this population. Our observations emphasize that the empiric use of thiazides in all AA with uncomplicated HTN -as recommended by the Joint National Committee guidelines - is not the optimal treatment approach since we found that a considerable proportion of these patients could be successfully treated with RAS blockers. Previous studies suggested that the genetic variants which have been associated with BP responses in AA only account for < 5% of the variability. Therefore, the future utility of metabolomics, transcriptomics and epigenetics might unravel novel potential biomarkers which contribute to the variation in BP response in this population.
In AA subjects, correlations were assessed between log-transformed PRA and SBP responses to:
- MET monotherapy (n=165)
- CTD monotherapy (n=139)

PRA cut point was estimated from PEAR-2 using multivariable linear regression models

The estimated cut point was tested in 2 independent cohorts:
- PEAR-1 (n=303)
- GERA (n=551) → Meta-analysis (n=854)

Sensitivity, specificity, PPV and NPV of estimated PRA cut point was determined using:
- β-blockers' observations from combined PEAR-1 & GERA (n=417)

PRA's predictive value was assessed by ROC analysis using:
- Atenolol observations from combined PEAR-1 (n=152)
- PEAR-2 paired data (n=118)

Figure 3-1. Flow chart showing the analytic steps in this study.
Table 3-1. Definitions of true positives, true negatives, false positives and false negatives to determine the sensitivity, specificity, PPV and NPV of the PEAR2-derived PRA cut point

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>True positives</strong></td>
<td>number of subjects with PRA ≥ 1.3 ng/mL/hr who had a fall in SBP ≥ 5 mmHg post RAS blockers</td>
</tr>
<tr>
<td><strong>False positives</strong></td>
<td>number of subjects with PRA ≥ 1.3 ng/mL/hr who had a fall in SBP &lt; 5 mmHg post RAS blockers</td>
</tr>
<tr>
<td><strong>False negatives</strong></td>
<td>number of subjects with PRA &lt; 1.3 ng/mL/hr who had a fall in SBP ≥ 5 mmHg post RAS blockers</td>
</tr>
<tr>
<td><strong>True negatives</strong></td>
<td>number of subjects with PRA &lt; 1.3 ng/mL/hr who had a fall in SBP &lt; 5 mmHg post RAS blockers</td>
</tr>
</tbody>
</table>
Figure 3-2. Flow diagram showing the AA participants included in the final analysis from the PEAR-2 cohort.
Figure 3-3. Flow diagram showing the AA participants included in the final analysis from the PEAR cohort.
Figure 3-4. Flow diagram showing the AA participants included in the final analysis from the GERA cohorts.
Table 3-2. Baseline characteristics of AA participants in the PEAR and GERA cohorts

<table>
<thead>
<tr>
<th></th>
<th>PEAR-2 (n=139)</th>
<th>MET (n=165)</th>
<th>HCTZ (n=151)</th>
<th>Atenolol (n=152)</th>
<th>HCTZ (n=286)</th>
<th>Candesartan (n=265)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>50.2 (8.7)</td>
<td>50.1 (9.3)</td>
<td>47.5 (8.8)</td>
<td>47.2 (8.5)</td>
<td>47.8 (6.1)</td>
<td>48.9 (6.4)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.0 (5.2)</td>
<td>30.9 (5.1)</td>
<td>31.4 (5.3)</td>
<td>31.6 (6.3)</td>
<td>31.5 (6.4)</td>
<td>30.4 (4.5)</td>
</tr>
<tr>
<td>Female no. (%)</td>
<td>71 (51.1%)</td>
<td>86 (52.1%)</td>
<td>94 (62.3%)</td>
<td>108 (71.1%)</td>
<td>147 (51.4%)</td>
<td>156 (58.9%)</td>
</tr>
<tr>
<td>PRA, ng/mL/hr</td>
<td>0.4 (0.2-0.7)</td>
<td>0.4 (0.3-0.7)</td>
<td>0.4 (0.2-0.6)</td>
<td>0.4 (0.2-0.7)</td>
<td>0.6 (0.3-1.1)</td>
<td>0.2 (0.1-0.6)</td>
</tr>
</tbody>
</table>

All values are expressed as mean (SD) except that for PRA (non-normally distributed), which are expressed as median (lower and upper quartiles).
Table 3-3. Baseline and post-treatment BP of AA participants in the PEAR and GERA cohorts

<table>
<thead>
<tr>
<th></th>
<th>PEAR-2</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTD (n=139)</td>
<td>MET (n=165)</td>
<td>HCTZ (n=151)</td>
<td>Atenolol (n=152)</td>
<td>HCTZ (n=286)</td>
<td>Candesartan (n=265)</td>
</tr>
<tr>
<td>Pre-SBP, mmHg</td>
<td>148.8 (12.0)</td>
<td>150.8 (13.0)</td>
<td>151.6 (13.5)</td>
<td>151.2 (12.1)</td>
<td>150.0 (15.4)</td>
<td>147.6 (12.4)</td>
</tr>
<tr>
<td>Post-SBP, mmHg</td>
<td>133.7 (12.1)</td>
<td>144.3 (16.5)</td>
<td>135.9 (14.4)</td>
<td>142.2 (16.6)</td>
<td>132.4 (15.8)</td>
<td>137.7 (14.8)</td>
</tr>
<tr>
<td>Delta SBP, mmHg</td>
<td>-15.1 (14.3)</td>
<td>-6.5 (15.0)</td>
<td>-15.7 (14.3)</td>
<td>-9.0 (16.8)</td>
<td>-17.6 (13.4)</td>
<td>-9.9 (13.5)</td>
</tr>
<tr>
<td>Pre-DBP, mmHg</td>
<td>97.8 (5.1)</td>
<td>98.7 (6.1)</td>
<td>99.3 (6.2)</td>
<td>98.9 (6.8)</td>
<td>96.8 (5.3)</td>
<td>95.6 (5.2)</td>
</tr>
<tr>
<td>Post-DBP, mmHg</td>
<td>88.7 (8.1)</td>
<td>91.6 (8.7)</td>
<td>90.0 (8.6)</td>
<td>90.9 (10.2)</td>
<td>87.4 (9.3)</td>
<td>88.5 (9.3)</td>
</tr>
<tr>
<td>Delta DBP, mmHg</td>
<td>-9.1 (8.0)</td>
<td>-7.1 (8.1)</td>
<td>-9.3 (8.6)</td>
<td>-8.0 (9.9)</td>
<td>-9.4 (8.5)</td>
<td>-7.2 (8.2)</td>
</tr>
</tbody>
</table>

Data are summarized as mean (SD).
Figure 3-5. Scatter plots showing the relationships between the log-transformed baseline PRA (Log$_{10}$ PRA) and SBP responses (A) post MET, and (B) post CTD in the PEAR-2 study. Pearson’s correlation coefficients, adjusted for baseline SBP are shown with their corresponding $P$ values. PRA values were log-transformed since they were not normally distributed.
Table 3-4. Multivariable linear regression models for SBP responses to MET monotherapy and to CTD monotherapy in PEAR-2

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Model for SBP response post MET</th>
<th></th>
<th>Model for SBP response post CTD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>55.27 ± 12.98</td>
<td>&lt;0.0001</td>
<td>78.34 ± 12.14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Log₁₀ PRA</td>
<td>-10.18 ± 3.56</td>
<td>0.0048</td>
<td>10.06 ± 2.73</td>
<td>0.0003</td>
</tr>
<tr>
<td>Baseline SBP</td>
<td>-0.43 ± 0.09</td>
<td>&lt;0.0001</td>
<td>-0.61 ± 0.08</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Figure 3-6. Scatter plot with regression lines showing the relationship between the baseline PRA on a logarithmic scale and SBP responses to MET monotherapy and to CTD monotherapy in the PEAR-2 study. The green regression line represents the SBP response to MET (n=165), whereas blue regression line represents the SBP response to CTD (n=139). The intersecting point between the two regression lines represents the PRA cut point at which both drugs were associated with the same response.
Figure 3-7. Scatter plots of PC1 vs. PC2 from the PCA ancestry of ancestry differences between subjects with PRA < 1.3 and those with PRA > 1.3 ng/mL/hr in (A) PEAR-1 (n=303) and (B) PEAR-2 (n=103) cohorts.
Figure 3-8. Adjusted mean differences in clinic SBP response (mean, 95% confidence interval (CI)) to (A) HCTZ monotherapy vs. atenolol monotherapy in PEAR-1 cohort and (B) HCTZ vs. candesartan in GERA cohort by the different PRA subgroups. All the values are adjusted for baseline SBP.
Figure 3-9. Adjusted clinic SBP in response to (A) HCTZ and to atenolol in the PEAR-1 and to (B) HCTZ and to candesartan in the GERA by PRA subgroups based on the PEAR2-derived cut point. All the values are adjusted for baseline SBP.
Figure 3-10. Meta-analysis of PEAR-1 and GERA cohorts showing the pooled adjusted mean differences in clinic SBP response (mean, 95% confidence interval (CI)) to HCTZ vs. atenolol/ candesartan (ARB) (RAS blockers) by the different PRA subgroups. All the values were adjusted for baseline SBP.
Table 3-5. Sensitivity, specificity, PPV and NPV of the PEAR2-derived PRA cut point using the RAS blockers’ observations from combined PEAR-1 and GERA (n=417)

<table>
<thead>
<tr>
<th>PRA cut point</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3 ng/mL/hr</td>
<td>10% (7-15%)</td>
<td>97% (93-99%)</td>
<td>88% (71-96%)</td>
<td>38% (33-43%)</td>
</tr>
</tbody>
</table>

All the values are shown with their corresponding 95% confidence interval (CI).
Table 3-6. Number of AA participants within each PRA subgroup based on the PEAR2-derived cut point according to their SBP response to RAS blockers in combined PEAR-1 and GERA cohorts

<table>
<thead>
<tr>
<th></th>
<th>Fall in SBP ≥ 5 mmHg post RAS blockers’ treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>PRA ≥ 1.3 ng/mL/hr</td>
<td>28\textsuperscript{a}</td>
</tr>
<tr>
<td>PRA &lt; 1.3 ng/mL/hr</td>
<td>239\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} true positives, \textsuperscript{b} false positives, \textsuperscript{c} false negatives, \textsuperscript{d} true negatives.
Figure 3-11. The ROC curves of PRA as a potential predictive biomarker of SBP response in (A) PEAR-1 (n=152) where the vertical axis shows whether or not there was a fall in the SBP ≥ 5 mmHg post treatment with atenolol. The area under the curve (AUC) was 0.59 (95% confidence interval [95% CI], 0.51-0.67, \( P = 0.06 \)) and in (B) subjects who were sequentially treated with both MET monotherapy and CTD monotherapy (paired data) in PEAR-2 (n=118) where the vertical axis shows whether or not the difference in SBP to MET compared with that to CTD was at least 5 mmHg. The AUC was 0.60 (95% confidence interval [95% CI], 0.51-0.69, \( P = 0.07 \)).
Role of PRA Biomarker in Personalizing Antihypertensive Therapy in EAs

Several previous studies showed that EA patients are overall likely to have a better antihypertensive response to RAS blockers, including β-blockers, ACE inhibitors, and ARBs, than to diuretics, calcium channel blockers and α-blockers.\textsuperscript{43-47, 99} The better response to RAS blockers is mainly explained by the high PRA levels found in EA patients, reflecting an over-activity of the RAAS.\textsuperscript{26, 48, 49} However, among EA hypertensive patients, a variability in BP response to various drugs exists.\textsuperscript{93} Therefore, identifying the subset of EA patients who are likely to respond better to thiazide diuretics, as opposed to RAS blockers, can help individualize the antihypertensive therapy. Measuring PRA, which assesses RAAS activity, has been suggested as a methodology to identify this subset of EA patients.\textsuperscript{65}

When we compared the DBP responses to MET (β-blocker) and to CTD (thiazide-like diuretic) among EA subjects who were sequentially treated with both drug monotherapies by PRA subgroups in the PEAR-2 study, we found that EA subjects with PRA < 0.65 ng/mL/hr had a significantly greater DBP response to CTD than to MET. This is inconsistent with the results from two previous studies where EA subjects with lower PRA had similar BP responses to HCTZ (thiazide) monotherapy and atenolol (β-blocker) monotherapy,\textsuperscript{47} and where EA subjects with lower PRA had a greater DBP response to candesartan (ARB) than to HCTZ.\textsuperscript{46} This inconsistency could be due to the higher potency of CTD, compared with that of HCTZ. Previous studies demonstrated that CTD was associated with significantly greater SBP and DBP reduction, compared with HCTZ.\textsuperscript{88, 89, 128} In contrast, our EA subjects with PRA ≥ 0.65 ng/mL/hr had a
significantly greater response to MET than to CTD, which is consistent with other studies. Based on the observed strong relationship between PRA and BP response among our EA population, we further tested the predictive performance of PRA biomarker in this population. We demonstrated that PRA has a good discriminating power between EA subjects based on their BP response to CTD and MET. Our data also suggest that PRA at a cut point of 0.60-0.65 ng/mL/hr, a level that had been derived from a cohort of EA patients decades ago, is an informative predictive biomarker of both SBP and DBP responses. It demonstrates a sensitivity of approximately 50% and a specificity of 85%, thus can be used to personalize antihypertensive therapy in this population.

**PRA-Based Versus Age-Based Strategy in Personalizing Antihypertensive Therapy in EA**

Assessing age as a potential factor for personalizing antihypertensive therapy, we found that younger EA subjects had a greater DBP response to MET, whereas older EA subjects had a similar response to both MET and CTD. Our data demonstrate that younger subjects have higher PRA levels compared with the older subjects, and this explains the difference in response between the two different age subgroups. The difference in BP response between the age subgroups is also consistent with the results observed in previous studies, and is elucidated by the fact that as age increases, BP tends to increase, which in turn leads to decreased release of renin from the kidneys. Accordingly, Preston and colleagues suggested that antihypertensive treatment can be personalized in patients with stage 1 and stage 2 HTN using an age-based strategy, instead of measuring PRA. They concluded that the PRA-based treatment strategy did not provide additional information for selecting antihypertensive therapies.
compared with the age-based strategy which is cheaper and simpler. However, we found that there are subsets of EA patients whose PRA levels were inconsistent with their age subgroup: 25% of younger EA patients had PRA < 0.65 ng/mL/hr (lower-PRA), and 61% of older EA subjects had PRA ≥ 0.65 ng/mL/hr (higher-PRA). Consistent with our results, Alderman et al. suggested that although age was significantly negatively correlated with the PRA levels in a cohort of 4,170 hypertensive patients, age is not an accurate estimation of RAAS activity. Thus, Alderman and associates concluded that PRA measurement is necessary to precisely individualize antihypertensive therapy. Moreover, in a retrospective analysis of GERA study, where EA hypertensive subjects were treated with either HCTZ or candesartan, Schwartz and associates showed that the strategy of drug selection based on baseline PRA levels was associated with a BP control rate of 76.3% versus 67.1% with the age-based strategy in EA subjects ($P<0.001$). A recently published retrospective study also demonstrated that BP response to HCTZ and atenolol was significantly greater based on the PRA strategy than the age-based strategy in EA subjects. Therefore, the PRA-guided treatment approach is more compelling and promising than the age-based treatment approach in guiding treatment decisions in EA patients.

**Clinical Utility of the PRA-Guided Treatment Approach**

Few prospective clinical trials have been conducted to compare a PRA-guided treatment approach with the usual clinical care in patients with essential HTN. A randomized clinical trial, which recruited treated hypertensive patients with primarily European ancestry, who had persistently elevated BP despite antihypertensive medication treatment, showed that the PRA-guided treatment approach was associated with a greater reduction in SBP of 10 mmHg, a greater rate of BP control, and use of
less antihypertensive medications, when compared with usual treatment.\(^{84}\) Also, in a prospective study where 73 resistant hypertensive subjects were followed up for one year, BP control was achieved in about 60% of the subjects using the PRA-based treatment strategy. Moreover, there was a significant decrease in the number of medications needed to achieve BP control, with a 20% reduction in the total cost of medication per patient when treatment decisions were based on pretreatment PRA levels.\(^{129}\) To further assess the utility of the PRA-guided treatment approach as compared to the usual treatment regimen, additional clinical trials which assess BP control, number of medications needed to successfully treat the patient, and adverse event rates in EA hypertensive patients are necessary. Since previous studies demonstrated that CTD is more potent than HCTZ in EA patients,\(^{88, 89, 128}\) we suggest utilizing CTD as the diuretic (anti-V drug) in these future clinical trials.

The cost associated with the clinical implementation of the PRA-guided treatment approach is also an important aspect to be considered by clinician, patient, and insurance companies. The cost of the PRA test has substantially decreased over the past several years. Presently, it is estimated to be US $100-150 and it is reimbursed by Medicare and many insurance companies in the U.S, yet clinicians consider it unnecessary and costly.\(^{66, 130}\) Using a simulation model and different case scenarios based on the available data from a clinical trial of PRA-guided treatment,\(^{84}\) the costs and the QALYs of the PRA-guided approach have been estimated and compared with that of the standard treatment clinical care in poorly controlled hypertensive patients.\(^{130}\) The PRA-guided approach has been found to increase the QALYs and to be more cost-effective than the standard treatment care since it has been estimated to be associated
with less complications such as heart failure, stroke, acute myocardial infarction and renal disease. This was especially true for younger patients and those with more cardiovascular risk factors. The authors in this study also suggested that future further reductions in the cost of the PRA test would result in more cost savings and additional improvement in quality of life. This study did not include the medication costs since it assumed that they are similar between the two treatment strategies.

Since Laragh and colleagues suggested that the PRA-guided strategy would be associated with less antihypertensive drugs to control BP and less treatment costs, further pharmacoeconomic studies are needed to further evaluate the cost-effectiveness of this strategy, including the costs of antihypertensive therapies.

**Remaining Challenges of Clinical Implementation of a PRA-Guided Approach for Treatment of HTN**

A PRA-guided HTN treatment approach still encounters various challenges which might render it impractical for implementation in clinical settings. These challenges include the questionable reproducibility of the PRA test among different laboratories, as well as its long turnaround time, which might be extended to 7 days. The DiaSorin company has recently developed a direct renin assay which has been shown to have good sensitivity and to have better intra and inter-laboratory reproducibility compared with the PRA test. Also, this new assay, which was approved by the US FDA in 2013, has an incubation time of 30 minutes versus 18 hours for the PRA test. Despite these promising findings and despite the fact that both assays have the same blood sample collection procedures, the PRA test is still more widely used in research studies in the U.S. Therefore, we suggest future studies should be designed to further investigate and compare the sensitivity and reproducibility of the two assays.
Improving the Predictability of BP Response in EA

Finally, we speculate that developing a predictive model which combines PRA with other genetic factors that contribute to the variability in BP response (complex trait) among EA hypertensive patients would be more informative, and would increase the predictability of the most effective antihypertensive drug class for each patient. Strong evidence suggests that genetic variants in the *NEDD4L*\(^{131-133}\) and in the *ADRB1*\(^{134-137}\) genes are associated with BP response to thiazides and β-blockers respectively in EA population. Previous studies showed that the G allele of the rs4149601 single SNP in the *NEDD4L* gene\(^{131, 132, 138}\) is associated with a greater response to thiazides, whereas the Arg389 allele and the Ser49Arg389 haplotype in the *ADRB1* gene\(^{134, 135, 139}\) are associated with a greater response to β-blockers. Other promising genetic variants which have been shown to be associated with response to antihypertensive drugs in EA patients include those in the *PRKCA*, *FGF5*, *SH2B*, *EBF1*, *CHIC2*, *MOV10*, *HFE*, *GNB3* and *GRK4* genes.

Role of PRA as a Biomarker in Personalizing Antihypertensive Therapy in AAs

AAs develop HTN at an earlier age, have more cardiovascular complications, are more likely to be aggressively treated, yet less likely to have controlled BP compared to other populations.\(^{109, 121, 143-145}\) Overall, AA patients tend to have better responses to anti-volume (anti-V) antihypertensive agents including diuretics, α-blockers, and calcium channel blockers than to RAS blockers.\(^{20, 40, 41, 99, 104-106}\) This is likely explained by the lower PRA levels found in AA subjects, reflecting a high sodium retention, thus a better response to drugs that enhance sodium excretion (anti-V drugs).\(^{43-49, 67}\) Since inter-patient variability in BP response to various antihypertensive drugs exists in this population,\(^{93-95}\) PRA has been suggested as a tool to identify the subset of AA patients.
who could be successfully treated with RAS blockers, thus helping clinicians to individualize antihypertensive therapy and improve the patients' BP control.\textsuperscript{65}

Consistent with previous studies,\textsuperscript{46, 47} we did not find a strong relationship between PRA and the DBP responses to MET and CTD in AA subjects who were sequentially treated with both drug monotherapies. Since the 0.65 ng/mL/hr PRA cut point was determined based on nomograms constructed using normotensive and hypertensive individuals of primarily EA ancestry and since there is a paucity of data regarding PRA level and the associated BP response in AA population,\textsuperscript{23} we investigated whether an optimal PRA cut point could be identified among a cohort of hypertensive AAs. Accordingly, we conducted a study that aimed to determine and validate a PRA cut point that could predict antihypertensive drug response amongst AAs. We estimated the PRA cut point from a cohort of AA individuals enrolled in the PEAR-2 study, tested the derived cut point (1.3 ng/mL/hr) in two independent AA cohorts of individuals enrolled in the PEAR-1 and GERA studies, and assessed its predictive performance. Although the derived cut point was able to differentiate between the AA subjects based on SBP responses to HCTZ and to atenolol or candesartan (RAS blockers) in a meta-analysis of the two independent cohorts, we found that the PEAR-2 derived cut point (1.3 ng/mL/hr) had low sensitivity (10\%) to identify the subset of AA patients who could be successfully treated with RAS blockers. Hence, using the data available, we were not able to identify a PRA cut point that was predictive of BP response with good sensitivity in this population of AA hypertensives. Moreover, we demonstrated that the PRA biomarker did not discriminate among AAs based on their
SBP response. Therefore, our data suggest that PRA may not be a useful predictive biomarker in individuals of AA ancestry.

**A Considerable Proportion of AA Could Be Successfully Treated with RAS Blockers**

The low sensitivity of the PEAR-2 derived PRA cut point reflects the high percentage (about 62%) of the lower-PRA (< 1.3 ng/mL/hr) AA subjects who had a good response (a reduction in SBP of at least 5 mmHg from baseline) to the RAS blockers (atenolol and candesartan). Moreover, our data suggest that about 27% of the AA subjects with PRA < 1.3 ng/mL/hr who were sequentially treated with MET, followed by CTD had an even better SBP response to MET than to CTD. There was no significant difference between the pre-MET SBP and pre-CTD SBP among these AA subjects. Our study is the first to report such a high proportion of AA patients who are successfully treated with RAS blockers. In a randomized controlled clinical trial where AA subjects with stage 1-2 HTN were randomized to either enalapril or candesartan, showed that 25% and 29% of the subjects had a reduction in SBP of at least 10 mmHg from baseline in response to enalapril and candesartan respectively. Based on these observations, we suggest that the empiric use of diuretics in all AA patients with uncomplicated HTN - as recommended by the Eighth Joint National Committee guidelines may not be an optimal treatment approach.

A possible explanation for the observed good response to RAS blockers among AA patients with PRA < 1.3 ng/mL/hr is that these patients might have an activated RAAS at the tissue level which is not correlated with the plasma levels of renin as observed in a previous study. Also, Weir et al. demonstrated that BP reduction in response to trandolapril (an ACE inhibitor) in AA patients was not correlated with the
degree of inhibition of ACE activity. Similarly, Weir and associates found that despite both EA and AA patients having had a similar degree of inhibition of ACE activity in response to trandolapril at doses of 1 and 2 mg, AA patients did not have a significant BP reduction until they were given a higher dose (4 mg). Their observations could reflect pathways other than ACE inhibition which are responsible for the mean reduction of 10 mmHg in SBP in response to ACE inhibitors in AA patients in this study. We also speculate that candesartan (an ARB) which was included in our validation cohort, might act through a different mechanism of action to effectively reduce BP in AA patients with lower PRA. This mechanism might involve renal vasodilatation through blocking the action of the vasoconstrictor angiotensin II, thus decreasing the release of aldosterone and enhancing sodium excretion in these patients. Moreover, we suggest that the observed good BP response to MET and atenolol (β-blockers) in our AA patients could be caused by the other well-known mechanisms of β-blockers, rather than solely inhibition of renal renin secretion. These mechanisms include decreasing heart rate by blocking β1-adrenergic receptors in the heart, which in turn would decrease the CO (CO is the product of two variables: stroke volume and heart rate).

**Variability in BP Response among AA Remains Unexplained**

When we investigated the association between several clinical factors (other than PRA) such as age, gender, baseline BP, smoking status, alcohol drinking status, height and waist, and BP response to MET and to CTD, only baseline BP was consistently associated with BP response to these drugs. Additionally, the genetic variants which have been associated with BP response in AA hypertensive patients in previous candidate gene or genome-wide association studies only account for less than 5% of the variability. Therefore, future studies which utilize metabolomics, transcriptomics,
and epigenetics are necessary to unravel novel potential biomarkers and to improve our understanding of the variation in BP response in AA population.

**Conclusion**

According to the AHA, HTN has a prevalence in 2016 of 39% in the U.S, which is expected to reach 42% by 2035.\(^{149}\) It is the leading risk factor for cardiovascular adverse events, including angina, myocardial infarction, heart failure and cardiac arrest.\(^3\) Despite the availability of many antihypertensive drug classes with multiple agents within each class, only half of hypertensive patients have controlled BP.\(^2\) The high rate of uncontrolled BP likely reflects the high inter-individual variability in BP response to various antihypertensive agents, which is mainly caused by the heterogeneity in the pathophysiologic pathways underlying HTN.\(^{10}\)

RAAS is one of the physiologic mechanism which plays a significant role in regulating BP.\(^{11}\) PRA provides an assessment of RAAS activity, which has been found to vary among hypertensive patients.\(^{12}\) Based on PRA levels, patients can be categorized into either volume-dependent (lower-PRA) or vasoconstriction-dependent (higher-PRA) hypertensives.\(^{27}\) Several studies demonstrated that patients in each category respond differently to various antihypertensive drug classes: patients with lower PRA are likely to respond better to thiazides, whereas those with higher PRA are likely to respond better to RAS blockers.\(^{28, 29, 47}\) Accordingly, PRA has been suggested to be a useful biomarker for predicting BP response, thus for personalizing antihypertensive therapy.\(^{13, 65}\)

Using data from PEAR-2 study, we investigated the association between PRA and DBP responses to MET and CTD in EA and AA subjects who were sequentially treated with both drug monotherapies. Our results demonstrate a strong relationship
between PRA and DBP responses in our EA population. Furthermore, our data showed that PRA has a good discriminating power between the EA subjects based on their DBP and SBP responses to MET and CTD. We were also able to validate the PRA cut point (0.60-0.65 ng/mL/hr), which had been derived from a cohort of EA subjects several decades ago.\textsuperscript{20,21} PRA of this cut point demonstrates good sensitivity and specificity, thus can be used to personalize antihypertensive drug selection in the EA population.

However, after categorizing the PEAR-2 AA subjects who were sequentially treated with MET and CTD monotherapies based on the 0.65 ng/mL/hr PRA cut point, we did not observe a strong relationship between PRA and DBP responses. Since the 0.65 ng/mL/hr PRA cut point was determined based on subjects of primarily EA ancestry and since there are limited data regarding PRA level and antihypertensive BP response,\textsuperscript{23} we investigated whether an optimal PRA cut point could be identified among a cohort of hypertensive AAs. Accordingly, we estimated the PRA cut point from a cohort of AA subjects enrolled in the PEAR-2 study, tested the derived cut point (1.3 ng/mL/hr) in two independent AA cohorts, and assessed its predictive performance. Although we found an association between PRA using the derived cut point (1.3 ng/mL/hr) and SBP responses to HCTZ and RAS blockers (atenolol and candesartan) in a meta-analysis of the two independent cohorts, we found that the derived cut point had very low sensitivity to identify the subset of AA subjects who could be effectively treated with RAS blockers. Additionally, ROC analyses demonstrate that PRA did not discriminate between the AA subjects based on their SBP response. Thus, our data suggest that PRA may not be a useful predictive biomarker in AA population.
Our findings also indicate that a considerable proportion of AA subjects with PRA < 1.3 ng/mL/hr could be successfully treated with RAS blockers. Therefore, we suggest that the empiric use of diuretics in all AA patients with uncomplicated HTN-as recommended by the current treatment guidelines—\(^8\) is not the optimal treatment approach. The mechanisms for variation in BP response in this population still remain poorly understood. Previous studies suggested that the genetic variants which have been associated with BP responses in AA only account for < 5% of the variability.\(^\text{111}\) Therefore, the future utility of metabolomics, transcriptomics and epigenetics might unravel novel potential biomarkers which contribute to the variation in BP response in this population.
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BIOGRAPHICAL SKETCH

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