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Clinical Research Article

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A Multicenter Study of Neutrophil-to-Lymphocyte Ratio in Primary Aldosteronism

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Abbreviations: ACE, angiotensin converting enzyme; ARR, aldosterone-to-renin ratio; BMI, body mass index; CKD, chronic kidney disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; EH, essential hypertension; hsCRP, high-sensitivity C-reactive protein; MR, mineralocorticoid receptor; NLR, neutrophil-to-lymphocyte ratio; PA, primary aldosteronism; PAI-1, plasminogen activator inhibitor-1; SST, saline suppression test; WCC, white cell count.

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Abstract

Background: Hypertensive patients with primary aldosteronism (PA) have a higher risk of cardiovascular complications than those with blood pressure–matched essential hypertension. The excess cardiovascular consequences of PA can be attributed to the proinflammatory effect of excessive aldosterone and mineralocorticoid receptor activation in a range of peripheral tissues and cell types. The neutrophil-to-lymphocyte ratio (NLR) is a widely available marker of inflammation which has been shown to predict cardiovascular outcome in the general population. This study aims to evaluate the use of NLR as a potential biomarker of PA and PA severity.

Methods: Patients with PA (n = 355) were identified from 2 large PA databases in Australia and China, while controls (n = 222) were patients with hypertension who were referred for assessment but did not meet the diagnostic criteria for PA. The NLR was retrospectively collected from routine full blood examination, prior to commencement of targeted treatment for PA.

Results: The NLR did not differ between PA patients and hypertensive controls (median 2.3 and 2.4, $P = 0.563$). However, among patients with PA, the NLR was positively correlated with baseline and post-saline aldosterone levels ($r = 0.22$ and $P < 0.001$ for both) and negatively correlated with serum potassium ($r = -0.15$, $P = 0.006$). Furthermore, in a logistic regression analysis of data from patients with PA, the NLR predicted the presence

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of comorbid chronic kidney disease (CKD) (defined as estimated glomerular filtration rate <60 mL/min/1.73m^2) with an odds ratio of 1.5 ($P = 0.003$).
Conclusion: While the NLR did not distinguish PA from controls, it was a marker of PA severity, being associated with aldosterone concentration as well as the presence of CKD. A prospective study is needed to further clarify the role of NLR in predicting end-organ damage associated with PA.

Key Words: primary aldosteronism, biomarker, neutrophils, lymphocytes

Primary aldosteronism (PA) is an important cause of secondary hypertension, accounting for approximately 5% of hypertensive cases in the general community and up to 30% of hypertensive cases referred to tertiary centers [1, 2]. PA is associated with worse cardiovascular prognosis than essential hypertension (EH) matched for age, sex, and blood pressure [3, 4] but targeted treatment with either mineralocorticoid receptor (MR) antagonist or adrenalectomy can mitigate many of these adverse consequences if instituted in a timely manner [5, 6]. However, PA is substantially underdiagnosed due to the lack of distinct clinical features in the majority and the absence of the aldosterone-to-renin ratio (ARR) from first-line investigations recommended by current hypertension guidelines [7-9]. The ARR is infrequently ordered by clinicians, with one study reporting that only 7% of primary care physicians ever ordered ARR to investigate their hypertensive patients, and another study reporting that the ARR was only measured in 2% of patients with resistant hypertension [10, 11]. A widely used, routine marker, that might alert clinicians to the need to screen for PA and assess cardiovascular risk would be very useful.

Studies have demonstrated that the higher cardiovascular risk associated with PA can be attributed to the proinflammatory effects of inappropriate aldosterone-induced MR activation [12, 13]. Inflammatory markers such as high-sensitivity C-reactive protein (hsCRP) have been shown to be elevated in patients with PA compared with normotensive controls [14] but are not routinely measured. The neutrophil-to-lymphocyte ratio (NLR) is a widely available inflammatory marker that can be calculated from a routine full blood examination and is a promising marker for prognostication in cardiovascular disease [15]. In a post hoc analysis of the National Health and Nutrition Examination Survey-III, the NLR independently predicted cardiovascular mortality in the general population, above and beyond the traditional Framingham risk factors [16]. Furthermore, in large population studies, NLR in the highest quantile predicted incident hypertension [17, 18]. Another facet of PA and MR-mediated organ damage is kidney disease. The large Primary Aldosteronism Prevalence in Italy (PAPY) study reported a significantly higher rate of microalbuminuria in patients with PA than with EH [19]. Given that the NLR has been shown to predict the development of end-stage kidney failure in patients with chronic kidney disease [20, 21] it may also have prognostic utility for PA-induced kidney disease.

Given the proinflammatory nature of excessive aldosterone and MR activation found in PA, we hypothesized that the NLR would be distinctly higher in patients with PA than those with EH and may serve as a useful biomarker of PA and associated end-organ damage. We systematically evaluated patients with hypertension from 2 dedicated PA research centers in Australia and China to examine the relationship between the NLR and ARR as well as with markers of PA severity.

Methods

Data were retrospectively extracted from 2 large PA databases established by the Endocrine Hypertension Group at Hudson Institute of Medical Research / Monash Health in Australia and the CONPASS (Chongqing PA Study) at The First Affiliated Hospital of Chongqing Medical University in China [22, 23]. Relevant information for the current study were extracted for the periods of 2013 to April 2020 (China) and 2016 to April 2020 (Australia). Patients were referred to these centers for management of hypertension and underwent detailed investigations into secondary causes of hypertension. Baseline characteristics, including patient demographics, medications (prior to switching to noninterfering agents for measurement of plasma renin and aldosterone concentrations), cardiovascular comorbidity (including coronary artery disease, stroke, and atrial fibrillation), and diabetes were collected from hospital records. The presence of left ventricular hypertrophy was qualitatively assessed by transthoracic echocardiogram. Chronic kidney disease (CKD) was defined as estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2.

The neutrophil and lymphocyte counts were extracted from full blood counts that patients routinely had as part of their clinical care prior to commencement of PA-specific treatment. Patients with baseline white cell count (WCC) > 12 × 10^9/L or <3 × 10^9/L, or

CRP > 10 mg/L were excluded. Patients who were not able to be withdrawn from interfering medications for further assessment of PA were also excluded from the study.

PA diagnosis

The diagnosis of PA was made in accordance with the Endocrine Society guidelines [24]. Briefly, a screening test was performed on all patients by measuring the ARR after cessation of interfering medications such as diuretics, beta blockers, dihydropyridine calcium channel blocker, angiotensin converting enzyme (ACE) inhibitor, angiotensin II receptor blocker or MR antagonists. If needed, a non-dihydropyridine calcium channel blocker, prazosin, hydralazine, or moxonidine was used to maintain blood pressure, since these agents were less likely to interfere with aldosterone and renin measurements [24]. ARR > 70 pmol/mU was considered a positive screening result at the Australian center, while the cutoff used in the Chinese center was ARR > 55 pmol/mU (>20 ng/mU) and plasma aldosterone > 277 pmol/L (>10 ng/dL). These patients proceeded to confirmatory testing with the saline suppression test (SST). Two liters of saline was infused over 4 hours and the diagnosis of PA was confirmed at the Australian center if the plasma aldosterone concentration remained >140 pmol/L after a recumbent SST, or >170 pmol/L after a seated SST [23]. The cutoff used by the Chinese center was plasma aldosterone >210 pmol/L (>8 ng/dL) after a recumbent SST, or >235 pmol/L (>8.5 ng/dL) after a seated SST [22]. Patients who did not meet the criteria for PA served as hypertensive control subjects.

Laboratory assessment

Samples for the full blood count were collected in EDTA. WCC, including the differential count, was measured using automated hematology analyzers. The absolute neutrophil count was divided by the absolute lymphocyte count to generate the NLR. Blood samples for aldosterone, renin and their ratio were collected in the morning between 0800 and 1000 AM after the patient had been in the upright position for at least 2 hours. In both centers, plasma aldosterone and direct renin concentrations were measured using chemiluminescent immunoassays on a DiaSorin Liaison analyzer (DiaSorin, Saluggia, Italy). The within-run coefficients of variation for aldosterone were 3.5% at 188 pmol/L and 1.8% at 798 pmol/L. The total coefficients of variation were 9.6% at 188 pmol/L and 5.6% at 798 pmol/L. For renin, the within-run coefficients of variation were 6.6% at 24 mU/L and 1.4% at 92.4 mU/L. The total coefficients of variation for renin were 10.0% at 24 mU/L and 4.5% at 92.4 mU/L.

Statistical analysis

IBM SPSS Statistics for Windows version 26 (IBM Corp., Armonk, NY, USA) was used to perform all statistical analyses. The Kolmogorov-Smirnov test was used to analyze continuous data for normality. Continuous variables were presented as either mean ± standard deviation or median (interquartile range) as appropriate. Comparison between groups was performed using the Student *t* test or the nonparametric Mann-Whitney U test. Categorical data were presented as count (percentage) and compared using chi-square test. The correlation between continuous variables were assessed using Spearman correlation. A multivariate logistic regression analysis was performed to determine the predictors of CKD as a dichotomous dependent variable (defined as eGFR <60 mL/min/1.73m^2) among patients with PA. Cases with any missing values (n = 29) were excluded from the regression analysis. A 2-sided *P* value of < 0.05 was considered statistically significant within a 95% confidence interval.

Results

The cohort consisted of 355 patients with PA (117 from Australia and 238 from China) and 222 hypertensive control subjects (55 from Australia and 167 from China) in whom PA had been excluded. Baseline characteristics are summarized in Table 1. Patients in the PA group had a longer duration of hypertension and higher office blood pressure, despite being on greater number of antihypertensive medications. Transthoracic echocardiogram was performed in 527 patients. There was a higher prevalence of end-organ damage in the PA group, evidenced by a higher proportion of patients with left ventricular hypertrophy on echocardiogram and a higher proportion with CKD. As expected, the serum potassium level was lower, the aldosterone was higher, and renin was lower in the PA group.

The median NLR was 2.4 in the PA group and 2.3 in the hypertensive control group (*P* = 0.563, Fig. 1). There was no significant difference in the NLR irrespective of the post-saline plasma aldosterone concentration threshold used to make the diagnosis of PA at each of the research centers (i.e., 140, 170, 210, or 235 pmol/L). The NLR was also compared between patients with screening ARR < 50 pmol/mU and those with ARR > 100 pmol/mU, which represent the most lenient and most stringent screening criteria for PA. There was again no significant difference between the 2 groups (median NLR 2.5 and 2.4, respectively, *P* = 0.756).

Table 1. Baseline Characteristics

	Essential hypertension (n = 222)	PA (n = 355)	*P* value
Age (years)	49 ± 12	50 ± 12	0.691
Females	152 (69%)	196 (55%)	0.002
BMI (kg/m^2)	26 (23, 28)	26 (23, 29)	0.788
Smoking	45 (21%)	79 (24%)	0.434
Ethnicity			
- East Asian	173 (78%)	247 (70%)	0.097
- Caucasian	31 (14%)	68 (19%)	
- Others	11 (5%)	31 (9%)	
- Unknown	7 (3%)	9 (3%)	
Family history of hypertension	132 (60%)	209 (59%)	0.921
Duration of hypertension (years)	3 (0.5, 9)	6 (2, 11)	<0.001
Office systolic BP (mmHg)	146 (133, 157)	152 (139, 165)	<0.001
Office diastolic BP (mmHg)	89 (80, 97)	95 (86, 101)	<0.001
Cardiovascular disease	33 (15%)	74 (21%)	0.072
Diabetes	41 (19%)	61 (17%)	0.694
Left ventricular hypertrophy	21 (10%)	54 (17%)	0.043
Chronic kidney disease	4 (2%)	33 (9%)	<0.001
Medications			
Number of antihypertensive medications	1 (0, 2)	2 (1, 3)	<0.001
ACE inhibitor / angiotensin receptor blocker	67 (30%)	129 (36%)	0.129
Calcium channel blocker	102 (46%)	216 (61%)	<0.001
Beta blocker	24 (11%)	54 (15%)	0.133
Diuretics	24 (11%)	75 (21%)	0.001
Alpha blocker	79 (36%)	118 (33%)	0.563
Other antihypertensive	11 (5%)	32 (9%)	0.071
Biochemistry			
Serum potassium (mmol/L)	4.0 (3.7, 4.3)	3.6 (3.1, 4.0)	<0.001
Creatinine (umol/L)	64 (57, 75)	68 (58, 83)	0.004
eGFR (mL/min)	90 (82, 101)	90 (78, 100)	0.050
CRP (mg/L)	0.9 (0.4, 1.7)	0.9 (0.4, 2.0)	0.639
Aldosterone (pmol/L)	385 (284, 500)	684 (490, 1050)	<0.001
Renin (mU/L)	7.6 (3.1, 18.1)	2.9 (1.3, 6.5)	<0.001
ARR (pmol/mU)	52 (23, 121)	195 (91, 620)	<0.001
Aldosterone post saline suppression (pmol/L)	147 (109, 188)	416 (282, 680)	<0.001
Blood count			
Hemoglobin	132 (125, 143)	135 (125, 146)	0.332
White cell count	6.1 (5.1, 7.3)	6.1 (5.1, 7.2)	0.850
Neutrophils	3.9 (3.1, 4.8)	3.9 (3.1, 4.7)	0.988
Lymphocytes	1.6 (1.3, 2.1)	1.6 (1.3, 2.1)	0.385
NLR	2.3 (1.8, 3.2)	2.4 (1.8, 3.2)	0.563
Monocytes	0.4 (0.3, 0.5)	0.4 (0.3, 0.5)	0.329
Platelet	217 (178, 256)	219 (182, 259)	0.472

Continuous data are presented as mean ± standard deviation or median (interquartile range). Categorical data were presented as count (percentage).
Abbreviations: ARR, aldosterone-to-renin ratio; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; NLR, neutrophil-to-lymphocyte ratio.

The NLR was higher in males than in females (2.6 versus 2.3, $P = 0.002$). However, when the data were re-analyzed for males and females separately, there was no difference in NLR between PA and controls. The NLR was higher in the overall Chinese cohort than the Australian cohort (median of 2.5 versus 2.1, $P < 0.001$), and in the Chinese PA cohort than the Australian PA cohort (median of 2.5 versus 2.2, $P = 0.003$). However, no difference was found between the PA and control patients in each of these cohorts (Table 2). Compared to the Australian cohort, the Chinese cohort was younger and had lower body mass index (BMI) but higher baseline ARR.

The data were also analyzed based on tertiles of NLR. Patients' age and baseline blood pressure were comparable

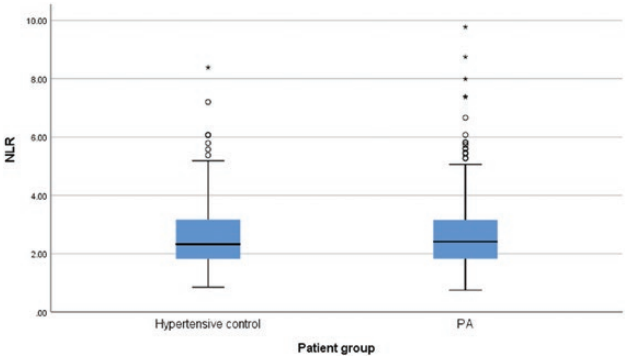

Figure 1. NLR in PA and hypertensive controls. The line inside the box represents the median while the length of the box represents the interquartile range. The whiskers extend to the smallest and largest values in each group, while the circles and asterisk denote outliers.

across the tertiles, but there were more males in the highest, compared to the lowest, NLR tertile (data not shown). The proportion of patients with PA was similar between the tertiles, and no significant differences in the aldosterone, renin, or ARR levels were found between the NLR tertiles. Adrenal vein sampling was performed in 172 (48%) of PA patients, 104 of whom were found to have unilateral disease. In these 172 PA patients, the median NLR was 2.5 in those with unilateral disease and 2.1 with bilateral disease ($P = 0.098$). The NLR was comparable between either subtype with controls ($P > 0.1$ for both comparisons). There was also no difference in the WCC or monocyte count of PA and control groups. CRP was measured in 67% of the patients and it did not correlate with NLR ($r = 0.071$, $P = 0.164$).

Among patients with PA, the NLR was positively correlated with plasma aldosterone concentration both pre- and post-saline infusion ($r = 0.22$ and $P < 0.001$ for both) and negatively correlated with serum potassium ($r = -0.15$, $P = 0.006$), but no correlation was seen with renin or ARR (Fig. 2). Furthermore, the NLR was correlated with serum creatinine and CKD ($P < 0.05$ for both). Compared to PA patients without CKD, those with CKD had significantly higher NLR (2.9 versus 2.4, $P = 0.001$). A logistic regression analysis was performed to assess the ability of NLR to predict comorbid CKD in PA patients, adjusting for age, sex, BMI, blood pressure, duration of hypertension, smoking, and diabetes. In this model, the NLR was an independent predictor of CKD in patients with PA with an odds ratio of 1.5 ($P = 0.003$, Table 3). The ability of NLR in predicting CKD was second only to diabetes. However, there was no significant correlation between NLR and the presence of left ventricular hypertrophy in the PA group. Similarly, in the control group, the NLR did not correlate with aldosterone, potassium, CKD, or left ventricular hypertrophy.

Discussion

PA is an important cause of hypertension and is associated with worse cardiovascular prognosis than EH [3]. The excess aldosterone and MR activation in epithelial tissues promotes sodium reabsorption and hypertension, while in nonepithelial tissues in the heart and vasculature they induce proinflammatory effects, an increase in oxidative stress, adverse cardiovascular remodeling, and atherosclerotic processes [12]. At present, PA is significantly underdiagnosed and the recommended screening test, the ARR, is utilized infrequently, with a recent study showing that only 2.1% of 4660 resistant hypertensive patients were appropriately screened for PA [11]. An accessible inflammatory marker, the NLR, that can be calculated from a routine full blood count to differentiate PA from EH would be very helpful. While our results did not reveal a distinct NLR profile in patients with PA compared to those without PA, the NLR was found to be a marker of PA severity, being correlated with baseline and post-saline aldosterone level, serum potassium, and CKD.

In the effort to identify distinguishing features of aldosterone excess from other forms of hypertension, several inflammatory markers have been investigated. Patients with higher 24-hour urinary aldosterone excretion (from 33 to 66 nmol/day) and suppressed plasma renin activity were found to have higher hsCRP, serum amyloid A, homocysteine, fibrinogen, and plasminogen activator inhibitor-1 (PAI-1) than those with lower 24-hour urinary aldosterone (<33 nmol/day) [25]. A study derived from the German Conn's Registry found higher level of hsCRP in patients with PA versus normotensive controls [14]. Furthermore, hsCRP level decreased significantly following treatment of PA [26]. Other inflammatory markers reported to be elevated in PA include malondialdehyde (MDA), amino terminal propeptide type I (PINP), and plasma osteopontin [27, 28], but not IL-6 and TNFa [27, 29]. However, most of these inflammatory markers have not been validated by other groups and are not routinely measured in clinical practice [29]. In the current study, we did not find a significant difference in the CRP levels of the PA and control groups. However, CRP was only measured in 67% of the cases and patients with CRP >10 mg/L were excluded from the study as they may have had an infection or other acute causes of inflammation. These factors would compromise the detection of differences between the groups.

In contrast to many of the markers studied previously, NLR is a cheap and widely available inflammatory that which has been shown to predict outcome in patients with cardiovascular disease [30, 31]. NLR is thought to be superior in predicting adverse outcome than the neutrophil or lymphocyte count individually as it takes into account

Table 2. Baseline Characteristics Stratified by Centers

	Australian center			Chinese center		
	Essential hypertension (n = 55)	PA (n = 117)	*P* value	Essential hypertension (n = 167)	PA (n = 238)	*P* value
Age (years)	47 ± 14	55 ± 12	<0.001	50 ± 12	47 ± 12	0.009
Females	33 (60%)	55 (47%)	0.112	119 (72%)	141 (59%)	0.013
BMI (kg/m^2)	29 (25, 34)	29 (26, 33)	0.840	25 (23, 27)	25 (22, 27)	0.034
Smoking	11 (22%)	13 (13%)	0.181	34 (20%)	66 (28%)	0.090
Family history of hypertension	39 (71%)	73 (63%)	0.305	93 (56%)	136 (57%)	0.771
Duration of hypertension (years)	2 (0, 11)	10 (2, 17)	<0.001	3 (0.5, 8)	5 (1, 10)	0.002
Office systolic BP (mmHg)	137 (128, 153)	153 (138, 165)	0.001	148 (137, 158)	152 (139, 165)	0.006
Office diastolic BP (mmHg)	89 (81, 95)	93 (86, 100)	0.001	89 (80, 99)	96 (86, 102)	<0.001
Number of antihypertensives	1 (0, 3)	2 (1, 3)	0.036	1 (1, 2)	2 (1, 2)	0.009
Cardiovascular disease	5 (9%)	18 (15%)	0.258	28 (17%)	56 (24%)	0.098
Chronic kidney disease	2 (4%)	15 (13%)	0.062	2 (1%)	18 (8%)	0.004
Diabetes	7 (13%)	31 (27%)	0.042	34 (20%)	30 (13%)	0.035
Left ventricular hypertrophy	6 (11%)	19 (16%)	0.498	15 (9%)	35 (15%)	0.085
Serum potassium (mmol/L)	4.2 (4.0, 4.5)	4.0 (3.6, 4.2)	<0.001	3.9 (3.6, 4.2)	3.4 (3.0, 3.7)	<0.001
Creatinine (umol/L)	68 (63, 78)	72 (60, 85)	0.723	62 (56, 73)	68 (56, 83)	0.006
eGFR (mL/min)	90 (86, 90)	90 (76, 90)	0.033	93 (82, 110)	91 (78, 110)	0.374
Aldosterone (pmol/L)	314 (219, 438)	522 (388, 734)	<0.001	396 (294, 504)	801 (567, 1105)	<0.001
Renin (mU/L)	16.0 (8.2, 26.0)	3.6 (2.1, 5.8)	<0.001	5.2 (2.8, 14.2)	2.4 (1.0, 7.4)	<0.001
ARR (pmol/mU)	23 (11, 44)	151 (92, 297)	<0.001	69 (33, 159)	254 (91, 871)	<0.001
Aldosterone post saline suppression (pmol/L)	113 (88, 130)	254 (175, 361)	<0.001	150 (111, 191)	518 (315, 885)	<0.001
Blood count						
Hemoglobin	142 (129, 151)	141 (131, 152)	0.766	131 (122, 141)	132 (123, 141)	0.772
White cell count	6.7 (5.5, 7.6)	6.3 (5.4, 7.5)	0.458	5.9 (5.0, 7.3)	6.0 (5.1, 7.0)	0.976
Neutrophils	3.8 (3.1, 4.5)	3.8 (3.0, 4.9)	0.971	4.0 (3.1, 4.8)	3.9 (3.2, 4.7)	0.993
Lymphocytes	2.0 (1.5, 2.1)	1.7 (1.4, 2.2)	0.159	1.6 (1.2, 2.0)	1.6 (1.2, 1.9)	0.505
NLR	1.9 (1.6, 2.7)	2.2 (1.7, 2.9)	0.231	2.5 (1.9, 3.4)	2.5 (1.9, 3.3)	0.767
Platelet	237 (204, 281)	234 (194, 280)	0.497	206 (173, 247)	210 (176, 248)	0.468

Abbreviations: ARR, aldosterone-to-renin ratio; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; NLR, neutrophil-to-lymphocyte ratio.

Table 3. Predictors of CKD Among PA Patients

Variable	Odds ratio	95% confidence interval	*P* value
Presence of diabetes	4.3	1.6–11.9	0.005
NLR	1.5	1.2–2.0	0.003
Duration of hypertension (years)	1.1	1.0–1.2	<0.001
Age (years)	1.0	0.9–1.0	0.315
Female sex	1.2	0.4–3.4	0.721
Systolic blood pressure (mmHg)	1.0	0.9–1.0	0.943
Diastolic blood pressure (mmHg)	1.0	0.9–1.0	0.759
BMI (kg/m^2)	1.0	0.9–1.1	0.384
Ever smoked	0.9	0.3–3.0	0.814

Abbreviations: BMI, body mass index; NLR, neutrophil-to-lymphocyte ratio.

2 different arms of the immune system [32]. Neutrophils play a role in active inflammation through secretion of inflammatory mediators and oxygen free radicals, while lymphocytes represent the regulatory pathway of the immune system [30, 32]. Therefore, in states of physiological stress, neutrophilia and lymphopenia are observed, which results in elevated NLR [30, 32]. In healthy subjects, the mean NLR was reported to be 1.65 with a range between 0.78 and 3.53 [33]. Female gender, age <60 years and never smoking were associated with lower NLR [34]. Consistent with this observation, the current study found higher NLR in males. There is a higher proportion of females in our control group because the majority of patients referred to our centers have an elevated ARR at baseline and females have higher ARR than males [35]. Therefore, it is more likely to find women with "falsely" elevated ARR who subsequently tested negative on the SST and these patients contributed to the control group. However, this did not affect the results as analyses stratified by sex still revealed no difference in NLR between PA and hypertensive controls.

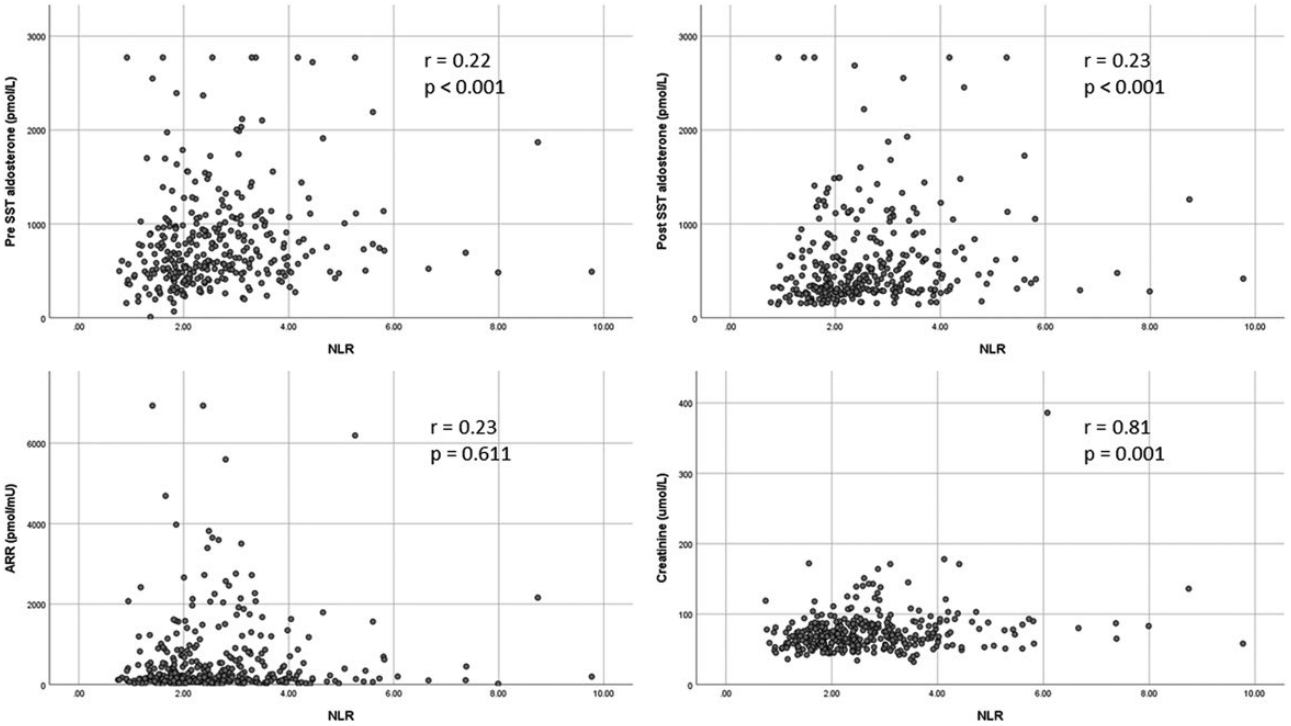

Figure 2. Relationships between NLR with aldosterone, ARR, and serum creatinine in patients with PA. A: relationship between NLR and pre-SST aldosterone; B: NLR with post-SST aldosterone; C: NLR with ARR; C: NLR with serum creatinine.

We hypothesized that the NLR would be higher in patients with PA than in those with EH, as pathological MR activation by aldosterone has been shown to cause a proinflammatory phenotype which is linked with atherosclerosis and cardiovascular disease [12, 36]. There is evidence that aldosterone directly stimulates differentiation of CD34 + hematopoietic stem cells into granulocyte-macrophage colonies [37]. Studies in cardiovascular diseases have demonstrated the superiority of NLR in predicting cardiovascular outcome compared to WCC or other leucocyte parameters [30]. NLR >2.2 predicted left ventricular hypertrophy in patients with hypertension [38] while an even higher level of >4.5 predicted cardiovascular mortality [16, 32]. In hypertension studies, increasing NLR (in particular, at levels >2) predicted incident hypertension, especially in the elderly male population [17, 18]. Hypertensive patients with impaired nocturnal dipping had higher NLR than those with preserved nocturnal dipping (2.3 vs 1.8, respectively) [39]. However, the current study did not show any difference in NLR between PA patients and hypertensive controls. It is possible that while PA represents a state of inflammation, the inflammation is related to end-organ damage in one or more tissues and is not reflected systemically by differences in NLR.

However, we found evidence to suggest a possible association between a higher NLR with PA severity. In patients with PA, a higher NLR was correlated with higher pre- and post-saline aldosterone and with lower potassium. Our study design also enabled comparison of 2 PA cohorts. The Chinese cohort had a more severe PA phenotype, evidenced by the higher baseline aldosterone and post-saline aldosterone levels, than the Australian cohort. The PA patients from the Chinese cohort demonstrated higher NLR than the PA patients from the Australian cohort. Furthermore, we observed a higher NLR in patients with aldosterone-producing adenoma, who often have a more severe PA phenotype, than those with bilateral adrenal hyperplasia. This difference did not reach statistical significance but was limited by the fact that only 50% of the PA cohort underwent adrenal vein sampling.

The NLR was further found to be an independent predictor of CKD in PA patients after adjusting for potential confounders. It was a stronger predictor than blood pressure per se and only second to diabetes as a predictive factor. An elevated NLR has been shown to predict progression to end-stage renal disease among patients with stage 1 to 4 CKD. It is postulated that NLR elevation in the setting of renal impairment is caused by a distortion of the hematopoietic process in the bone marrow with an increase in the number of myeloid progenitors and a decrease in the lymphoid progenitors [20, 40]. CKD is frequently masked in PA patients by hyperaldosteronism-mediated glomerular hyperfiltration. While an eGFR decline following PA treatment is expected, the extent of

decline is unpredictable. A prospective study is needed to further clarify the role of NLR in accurately characterizing current renal function and predicting the renal response to PA-specific treatment.

We did not find an association between NLR and left ventricular hypertrophy as another marker of PA severity, but left ventricular hypertrophy was only assessed qualitatively in the current study as either present or absent. Correlating the NLR with a quantitative echocardiogram measurement such as the left ventricular mass index could offer more precise information, but this data is not available in the current study.

The strengths of the current study lie in the large sample size and inclusion of 2 different ethnic groups, which contribute to the generalizability of the findings. Meanwhile, a limitation inherent with retrospective studies is the lack of control of factors which can affect the NLR, such as infections, autoimmune diseases, and other physiological stressors. However, we excluded patients with abnormal baseline WCC or CRP. Another limitation is the lack of full blood examination data after the targeted treatment of PA. The data could help to clarify if hypertension per se has an effect on the NLR, after the biochemical cure or control of aldosterone excess. The recruitment of patients from our dedicated PA centers may have also introduced a selection bias as the majority of referred patients have either an elevated ARR or are considered at risk of having PA. Therefore, the non-PA group may not entirely be consistent with a EH population. However, subanalysis of patients with baseline ARR <50 and ARR >100 (reflecting the most lenient and most stringent cutoff in the screening test for PA) showed no difference in NLR. Similarly, no difference in NLR was found between PA and control groups regardless of the cutoff used for the post-saline aldosterone level.

In conclusion, the current study did not demonstrate a difference in the NLR between PA and hypertensive controls and thus, NLR is therefore not a useful marker to differentiate PA from essential hypertension. The ARR remains the gold standard for screening hypertensive patients for PA and clinicians need to be encouraged to order this test in the appropriate setting. However, the NLR was a marker of PA severity and of the presence of CKD. Further studies are needed to evaluate the use of NLR in predicting the effect of PA-specific treatment on changes in renal function and potential unmasking of CKD.

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Additional Information

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