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Cardiovascular risk markers in patients with primary aldosteronism: A systematic review and meta-analysis of literature studies



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ABSTRACT

Background/objectives: Several studies reported an increased cardiovascular (CV) morbidity and mortality in patients with primary aldosteronism (PA). We performed a meta-analysis on the impact of PA on major markers of CV risk.

Methods: Studies on the relationship between PA and common carotid artery intima-media thickness (CCA-IMT), prevalence of carotid plaques, flow-mediated dilation (FMD), nitrate-mediated dilation (NMD), pulse-wave velocity (PWV), augmentation index (AIx), and ankle-brachial index (ABI) were systematically searched in the PubMed, Web of Science, Scopus and EMBASE databases.

Results: 12 case–control studies (445 cases, 472 controls) were included. Compared to subjects with essential hypertension (EH), PA patients showed a higher CCA-IMT (MD: 0.12 mm; 95% CI: 0.09, 0.16; $P < 0.00001$), and a higher aortic-PWV (272 cases and 240 controls, MD: 1.39 m/s; 95% CI: 0.90, 1.87; $P < 0.00001$). In contrast, non-significant differences were found in AIx and AIx normalized to a heart rate of 75 beats per minute (AIx@75). When compared to normotensive subjects, PA patients showed significantly higher CCA-IMT (MD: 0.16 mm; 95% CI: 0.05, 0.27; $P = 0.004$), aortic-PWV (MD: 3.74 m/s; 95% CI: 3.43, 4.05; $P < 0.00001$), AIx@75 (MD: 8.59%; 95% CI: 0.69, 16.50; $P = 0.03$), and a significantly lower FMD (MD: -2.52% ; 95% CI: $-3.64, -1.40$; $P < 0.0001$). Sensitivity and subgroup analyses substantially confirmed our results. Metaregression models showed that male gender, diabetes, and smoking habit impact on the observed results.

Conclusions: PA appears significantly associated with markers of subclinical atherosclerosis and CV risk. These findings could help establish more specific CV prevention strategies in this clinical setting.

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Abbreviations: PA, Primary Aldosteronism; CV, Cardiovascular; EH, Essential hypertension; IMT, Intima-media thickness; FMD, Flow-mediated dilation; NMD, Nitrate-mediated dilation; PWV, Pulse-wave velocity; AIx, Augmentation index; ABI, Ankle-brachial index; PRISMA, Preferred Reporting Items for Systematic reviews and Meta-Analyses; Aortic-PWV, Carotid-femoral PWV; Brachial-PWV, Carotid-radial PWV; ba-PWV, Brachial-ankle PWV; AIx@75, AIx normalized to a 75 beats/min heart rate; NOS, Newcastle–Ottawa Scale; MD, Mean difference; 95% CI, 95% Confidence Interval; OR, Odds Ratio; mm, Millimeters; m/s, Meters per second; cm, Centimeters; BMI, Body-mass index; TC, Total cholesterol; LDLc, LDL-cholesterol; HDLc, HDL-cholesterol; TGs, Triglycerides; SMD, Standardized mean difference.

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1. Introduction

PA is the most frequent endocrine cause of secondary hypertension that affects 5–13% of hypertensive patients [1] and up to 20% of subjects with resistant hypertension [2]. It is characterized by autonomous aldosterone overproduction, which is caused in most cases by adrenocortical adenoma or by bilateral adrenal hyperplasia [3]. This results in potassium excretion, sodium reabsorption and fluid retention, thus leading to increased systolic and diastolic blood pressure [4].

In addition to these well known effects, it has been reported that patients with PA experience more CV events [3], with increased incidence of myocardial infarction and stroke, and increased prevalence of atrial fibrillation [5]. Moreover, both retrospective and prospective studies suggest that individuals with PA might be at a higher risk of CV mortality than patients with EH [6,7].

However, such an increased CV morbidity and mortality cannot be entirely explained by the increased blood pressure [8] and the

underlying mechanisms are not yet clearly understood. It has been suggested that the prolonged exposure to high aldosterone concentrations may result in renal and metabolic sequelae [9], with endothelial dysfunction and myocardial and/or vascular remodeling [10,11]. To further address this issue, a growing attention has been given to the assessment of the association between PA and subclinical atherosclerosis, a recognized marker of CV disease [12].

Carotid IMT assessment is a non-invasive imaging test for subclinical atherosclerosis [13,14], and it has been widely accepted as one of the strongest predictors of major CV events (stroke, myocardial infarction, or CV death) [15,16]. Similarly, FMD, NMD, PWV, Alx, and ABI are considered surrogate markers of subclinical atherosclerosis and independent predictors of CV events [17–20]. FMD and NMD are widely accepted as accurate and non-invasive methods to assess endothelial function in humans [21], while PWV and Alx are measures of peripheral and central arterial stiffness [22]. Thus, these CV risk markers provide important prognostic data over and above traditional CV risk factors.

During recent years, a series of case–control studies reported accelerated atherosclerosis [23,24] impaired endothelial function [25,26], and increased arterial stiffness [27,28] in patients with PA. However, the evidence is limited by small sample size and potential confounding factors and no meta-analytical data providing an overall information about this issue are currently available.

In order to provide a comprehensive overview of the relationship between PA and subclinical atherosclerosis, we performed a systematic review with meta-analysis of literature studies evaluating the impact of PA on the major markers of CV risk.

2. Methods

A protocol for this review was prospectively developed, detailing the specific objectives, the criteria for study selection, the approach to assess study quality, the outcomes, and the statistical methods.

2.1. Search strategy

To identify all available studies, a detailed search pertaining to PA and the markers of CV risk (i.e. IMT, FMD, NMD, PWV, Alx, and ABI) was conducted according to PRISMA guidelines [29]. A systematic search was performed in the electronic databases (PubMed, Web of Science, Scopus, EMBASE), using the following search terms in all possible combinations: *primary aldosteronism, hyperaldosteronism, Conn syndrome, intima-media thickness, carotid plaques, atherosclerosis, flow-mediated dilation, nitrate-mediated dilation, endothelium-dependent dilation, endothelium-independent dilation, endothelial dysfunction, pulse wave velocity, augmentation index, arterial stiffness, ankle-brachial index*. The last search was performed on 30th October 2015. The search strategy was developed without any language or publication year restriction.

In addition, the reference lists of all retrieved articles were manually reviewed. In case of missing data, study Authors were contacted by e-mail to try to retrieve original data. Two independent Authors (PA and MNDDM) analyzed each article and performed the data extraction independently. In case of disagreement, a third investigator was consulted (RL). Discrepancies were resolved by consensus. Selection results showed a high inter-reader agreement ($\kappa = 0.97$) and have been reported according to PRISMA flowchart (Fig. S1).

2.2. Data extraction and quality assessment

According to the pre-specified protocol, all studies evaluating the impact of PA on the markers of CV risk were included. Case-reports, case-series without a control group, reviews and animal studies were excluded. To be included in the analysis, a study had to provide values (means with standard deviation) of at least one variable among the following: common carotid artery IMT (CCA-IMT), brachial artery

FMD or NMD, aortic-PWV, brachial-PWV, ba-PWV, aortic Alx, aortic Alx@75, and ABI. Studies reporting the prevalence of carotid plaques were also included.

In each study, data regarding sample size, major clinical and demographic variables, values of CCA-IMT, FMD, NMD, aortic-PWV, brachial-PWV, ba-PWV, Alx, Alx@75, and ABI, and prevalence of carotid plaques in PA patients and controls were extracted. Controls were represented by patients with EH and/or subjects normal blood pressure.

Given the characteristics of the included studies, the evaluation of methodological quality of each study was performed with the Newcastle–Ottawa Scale (NOS), which is specifically developed to assess quality of non-randomized observational studies [30]. The scoring system encompasses three major domains (selection, comparability, exposure) and a resulting score range between 0 and 8, a higher score representing a better methodological quality. Results of the NOS quality assessment are reported in Table S1.

2.3. Statistical analysis and risk of bias assessment

Statistical analysis was carried out using Review Manager [Version 5.2, The Cochrane Collaboration, Copenhagen, Denmark] provided by The Cochrane Collaboration.

Differences among cases and controls were expressed as MD with pertinent 95% CI for continuous variables, and as OR with pertinent 95% CI for dichotomous variables.

CCA-IMT has been expressed in mm, FMD, NMD, Alx, and Alx@75 as percentage (%), aortic-PWV and brachial-PWV have been expressed in m/s, ba-PWV in cm/s, and ABI as absolute number.

The overall effect was tested using Z scores and significance was set at $P < 0.05$. Statistical heterogeneity between studies was assessed with chi square Cochran's Q test and with I^2 statistic, which measures the inconsistency across study results and describes the proportion of total variation in study estimates, that is due to heterogeneity rather than sampling error. In detail, I^2 values of 0% indicates no heterogeneity, 25% low, 25–50% moderate, and 50% high heterogeneity [31].

Publication bias was assessed by the Egger's test and represented graphically by funnel plots of the standard difference in means versus the standard error. Visual inspection of funnel plot asymmetry was performed to address for possible small-study effect, and Egger's test was used to assess publication bias, over and above any subjective evaluation. A $P < 0.10$ was considered statistically significant [32]. In case of a significant publication bias, the Duval and Tweedie's trim and fill method with the random-effect model was used to allow for the estimation of an adjusted effect size [33].

In order to be as conservative as possible, the random-effect method was used for all analyses to take into account the variability among included studies.

2.4. Sensitivity analyses

We repeated analyses by including only the studies judged as “high quality” according to NOS (i.e. NOS \geq to the median value found among included studies).

In order to avoid the risk of data overlap, a further sensitivity analysis was performed after excluding studies enrolling patients in the same period time from the same recruitment centers as other included studies.

2.5. Subgroup analyses

Given the potential influence of PA etiology on the outcomes, we planned to perform separate analyses of studies only including patients with aldosterone-producing adenoma.

2.6. Meta regression analyses

We hypothesized that differences among included studies may be affected by demographic variables (mean age, female gender) and clinical data related to the coexistence of traditional CV risk factors (smoking habit, diabetes mellitus, obesity, hyperlipidemia). To assess the possible effect of such variables in explaining different results observed across studies, we planned to perform meta-regression analyses after implementing a regression model with changes in CCA-IMT, FMD, NMD, aortic-PWV, brachial-PWV, ba-PWV, AIx, AIx@75, and ABI values, or presence of carotid plaques as dependent variables (y) and the above mentioned co-variables as independent variables (x). This analysis was performed with Comprehensive Meta-analysis [Version 2, Biostat, Englewood NJ (2005)].

3. Results

After excluding duplicate results, the search retrieved 236 articles. Of these studies, 189 were excluded because they were off the topic after scanning the title and/or the abstract, 32 because they were reviews/comments/case reports or they lacked of data of interest. Other 3 studies were excluded after full-length paper evaluation.

Thus, 12 articles (on 445 PA patients and 472 controls) were included in the final analysis [3,23,24,25,26,27,28,34–38] (Fig. S1). In detail, 10 studies [3,23,24,27,28,34–38] compared 384 PA patients with 333 EH subjects, of which 4 studies with data on CCA-IMT (5 data-sets on 118 cases and 118 controls), 7 reporting on aortic-PWV (on 272 patients and 240 controls), 2 on AIx (on 73 cases and 48 controls), and 2 on AIx@75 (on 50 cases and 61 controls). FMD has been evaluated in 1 study [39], while no study tested NMD, brachial-PWV, ba-PWV, ABI, and the prevalence of carotid plaques in PA patients and EH subjects.

In addition, 6 studies [23–27,36] compared 167 PA patients with 139 normotensive subjects, of which 3 studies with data on CCA-IMT (on 81 cases and 58 controls), 2 reporting on FMD (on 61 patients and 54 controls), 3 on aortic-PWV (on 73 cases and 52 controls), and 2 on AIx@75 (on 73 cases and 58 controls). NMD, ba-PWV, and AIx were evaluated in 1 study each [26,36]. Moreover, no study tested brachial-PWV, ABI, and the prevalence of carotid plaques in PA patients and normotensive subjects.

3.1. Study characteristics

All included studies had a case–control design. Major characteristics of populations are shown in Table 1.

The number of patients varied from 14 to 113, the mean age from 43.4 to 58.4 years, and the prevalence of male gender from 41.6% to 78.6%.

The presence of diabetes mellitus was reported by 0%–33% of patients, smoking habit by 0%–45%, and hyperlipidemia by 34.5%–34.8%.

Mean BMI varied from 22.4 kg/m² to 33.6 kg/m² but no study reported on the prevalence of obese subjects. Mean values of TC ranged from 4.83 to 5.04 mmol/l, of LDLc from 2.10 to 3.31 mmol/l, of HDLc from 1.20 to 1.55 mmol/l, and of TGs from 1.21 to 1.61 mmol/l.

The NOS for quality assessment of included studies showed a median value of 6.

3.2. Primary aldosteronism versus essential hypertension

In 4 studies (5 data-sets) [23–25,35], we found a significantly higher CCA-IMT in 118 PA patients than in 118 EH subjects (MD: 0.12 mm; 95% CI: 0.09, 0.16; $P < 0.00001$, Fig. 1A), without significant heterogeneity among studies ($I^2 = 37%$; $P = 0.18$).

Table 1

Demographic and clinical data of patients with primary aldosteronism (PA), essential hypertension (EH) and normal blood pressure (NBP) in included studies.

Author	Pop (n)	M/F	Age (years)	Smoking (%)	DM (%)	Ob (%)	HL (%)	BMI (kg/m ²)	TC (mmol/l)	LDLc (mmol/l)	HDLc (mmol/l)	TGs (mmol/l)	
Bernini 2008	PA	23	15/8	53.9	13	0	–	34.8	29.0	4.98	3.08	1.20	1.35
	EH	24	14/10	50.6	8.3	0	–	25	26.9	5.36	3.20	1.50	1.44
	NBP	15	10/5	49.9	13.3	0	–	0	25.8	5.07	3.10	1.34	1.38
Chang 2015	PA	37	17/20	43.4	–	–	–	–	–	–	–	–	–
	EH	20	8/12	45.0	–	–	–	–	–	–	–	–	–
Holaj 2007	PA	33	20/13	57.0	33	24	–	–	29.0	4.98	2.99	1.30	1.54
	EH	52	32/20	55.0	38	21	–	–	28.4	5.28	3.06	1.38	1.85
	NBP	33	17/16	54.0	24	0	–	–	26.6	5.59	3.19	1.61	1.73
Holaj 2015	PA	21	13/8	51.4	24	24	–	–	29.2	4.83	2.81	1.28	1.63
	PA	21	11/10	51.3	38	33	–	–	29.8	4.95	2.99	1.34	1.63
	EH	21	13/8	55.6	29	19	–	–	29.2	5.21	3.21	1.25	1.67
Lin 2012	PA	20	11/9	46.0	–	–	–	–	–	–	–	–	–
	EH	21	8/13	48.0	–	–	–	–	–	–	–	–	–
Mark 2014	PA	14	11/3	58.4	–	7.1	–	–	29.8	–	2.10	–	–
	EH	33	28/5	54.8	–	6.1	–	–	29.1	–	2.90	–	–
	NBP	17	10/7	51.8	–	0	–	–	25.8	–	–	–	–
Nishizaka 2004	PA	36	29/7	50.0	0	0	–	–	33.6	–	–	–	–
	NBP	44	24/20	55.0	0	0	–	–	34.5	–	–	–	–
Rosa 2012*	PA	49	36/13	51.0	45	–	–	–	29.7	4.9	3.0	1.20	1.30
	EH	49	33/16	50.0	31	–	–	–	30.1	4.8	2.9	1.20	1.40
Strauch 2006	PA	36	–	52.0	–	–	–	–	27.7	4.90	2.80	1.40	–
	EH	28	–	49.0	–	–	–	–	28.1	5.10	2.90	1.40	–
	NBP	20	–	46.0	–	–	–	–	25.3	5.30	3.10	1.68	–
Tsioufis 2008	PA	17	10/7	55.0	31	0	–	–	30.3	–	3.31	1.29	1.43
	EH	30	17/13	58.0	33	0	–	–	28.3	–	3.36	1.34	1.31
Tsuchiya 2009	PA	25	14/11	57.3	–	–	–	–	22.4	–	2.76	1.55	1.21
	NBP	10	5/5	63.6	–	–	–	–	21.2	–	3.13	1.47	1.30
Wu 2011	PA	113	47/66	49.6	18.6	11.5	–	34.5	24.9	5.04	–	1.21	1.61
	EH	55	20/35	50.2	10.9	7.3	–	34.5	24.3	5.04	–	1.24	1.47

PA: primary aldosteronism; EH: essential hypertension; Pop: population; M/F: male/female; HT: hypertension; DM: diabetes mellitus; Ob: obesity; HL: hyperlipidemia; BMI: body mass index; TC: Total Cholesterol; LDLc: LDL-cholesterol; HDLc: HDL-cholesterol; TGs: triglycerides; NA: not assessed. Age, BMI, TC, LDLc, HDLc, and TGs are expressed as mean values, unless otherwise indicated.

* TG values are expressed as median.

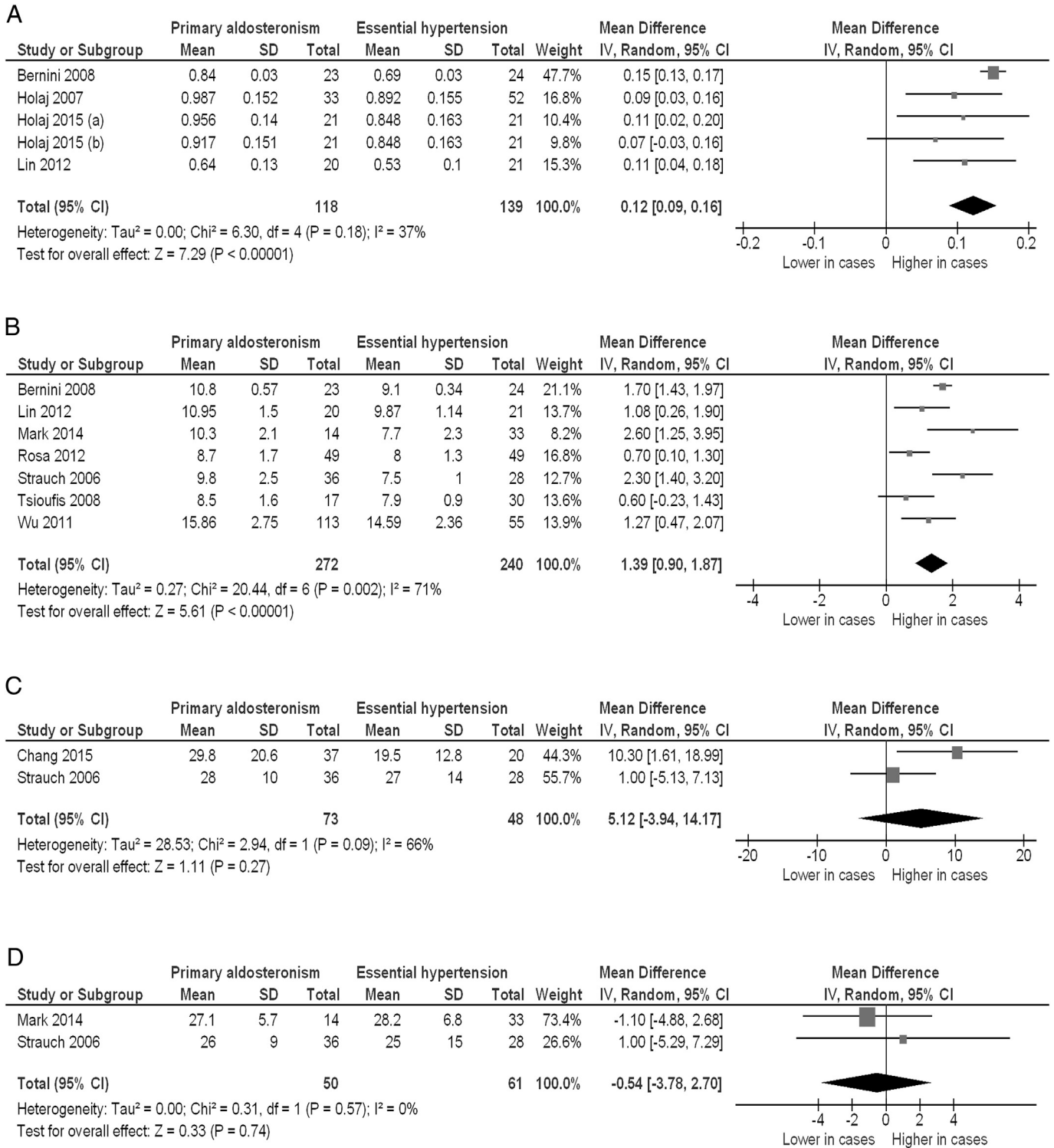


Fig. 1. Cardiovascular risk markers in patients with primary aldosteronism (PA) and in controls with essential hypertension (EH). Panel A: common carotid artery intima-media thickness (CCA-IMT). Panel B: carotid-femoral pulse-wave velocity (aortic-PWV). Panel C: augmentation index (Alx). Panel D: augmentation index normalized to a heart rate of 75 beats per minute (Alx@75).

Seven studies [23,27,28,35–38], evaluating a total of 272 cases and 240 controls, showed that PA patients have a significantly higher aortic-PWV as compared to EH subjects (MD: 1.39 m/s; 95% CI: 0.90, 1.87; P < 0.00001, Fig. 1B). The heterogeneity among studies was significant (I² = 71%; P = 0.002) and it was not reduced after excluding 1 study at time.

In contrast, no significant association between PA and an increased Alx was found in 2 studies [3,36] on 73 cases and 48 controls (MD:

5.12%; 95% CI: -3.94, 14.17; P = 0.27, Fig. 1C). Similarly, no differences in Alx@75 were reported among 50 PA and 61 EH subjects [27,36] (MD: -0.54%; 95% CI: -3.78, 2.70; P = 0.74, Fig. 1D).

3.3. Primary aldosteronism versus normal blood pressure

Three studies [23,24,26] on a total of 81 cases and 58 controls, showed a significantly higher CCA-IMT in patients with PA than in

normotensive controls (MD: 0.16 mm; 95% CI: 0.05, 0.27; $P = 0.004$, Fig. 2A). Significant heterogeneity among studies was found ($I^2 = 90\%$; $P < 0.00001$), not reduced by the one-study-at-time exclusion.

A significantly lower FMD was found in 2 studies [25,26] on 61 cases and 54 controls (MD: -2.52% ; 95% CI: $-3.64, -1.40$; $P < 0.0001$, Fig. 2B), without significant heterogeneity ($I^2 = 52\%$; $P = 0.15$).

In addition, a total of 3 studies [23,27,36] (73 cases and 52 controls) showed that PA patients have a significantly higher aortic-PWV as compared to subjects with normal blood pressure (MD: 3.74 m/s; 95% CI: 3.43, 4.05; $P < 0.00001$, Fig. 2C), with no heterogeneity among studies ($I^2 = 0\%$; $P = 0.89$).

Furthermore, 2 studies [27,36] showed significantly higher $Alx@75$ in 50 PA patients than 37 normotensive subjects (MD: 8.59%; 95% CI: 0.69, 16.50; $P = 0.03$, $I^2 = 72\%$; $P = 0.06$, Fig. 2D).

3.4. Publication bias

Because it is recognized that publication bias can affect the results of meta-analyses, we attempted to assess this potential bias using funnel plots analysis.

Visual inspection of funnel plots for studies on PA patients and EH subjects suggested the absence of publication bias and of small-study effect for aortic-PWV (Fig. S2), confirmed by the Egger's test ($P = 0.64$). In contrast, an asymmetric distribution of studies around the mean was found for studies evaluating CCA-IMT (Fig. S2), and the Egger's test confirmed a significant publication bias ($P = 0.008$). Interestingly, the adjusted effect size, estimated by the Duval and Tweedie's trim and fill method, substantially confirmed results for CCA-IMT (MD: 0.14 mm; 95% CI: 0.11, 0.17).

When considering the studies on PA patients and normotensive subjects, the distribution was rather symmetrical for CCA-IMT (Fig. S3) and

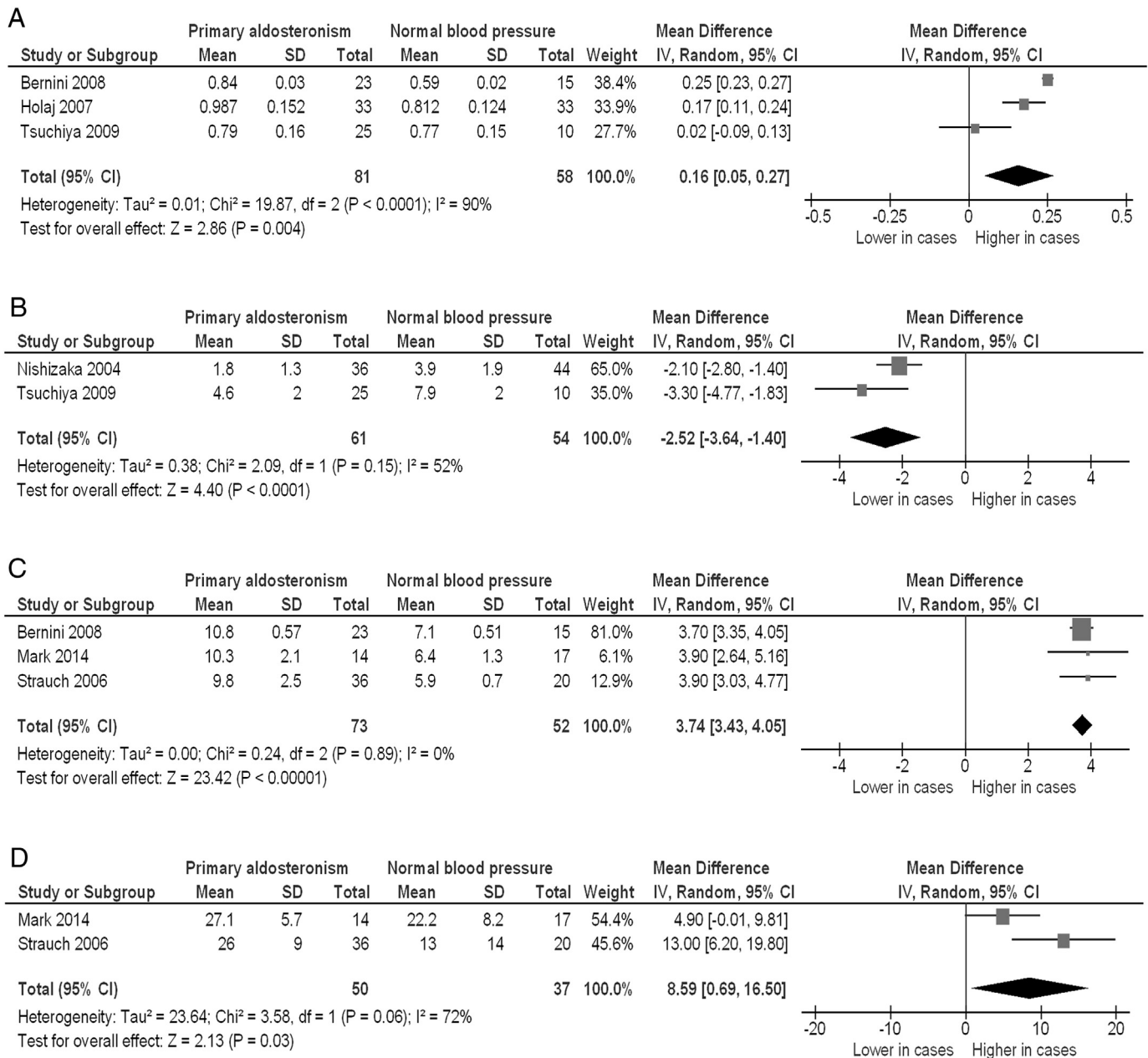


Fig. 2. Cardiovascular risk markers in patients with primary aldosteronism (PA) and in normotensive controls. Panel A: common carotid artery intima-media thickness (CCA-IMT). Panel B: flow-mediated dilation (FMD). Panel C: carotid-femoral pulse-wave velocity (aortic-PWV). Panel D: augmentation index normalized to a heart rate of 75 beats per minute ($Alx@75$).

no publication bias was found by the Egger's test ($P = 0.13$). In contrast, visual inspection of funnel plots of effect size versus standard error for studies evaluating aortic-PWV showed an asymmetric distribution of studies around the mean (Fig. S3), with significant publication bias (Egger's test $P = 0.03$). However, the adjusted effect size, estimated by the Duval and Tweedie's trim and fill method, confirmed our results on aortic-PWV (MD: 3.70 m/s; 95% CI: 3.39, 4.00).

The small sample-size and the low number of studies made publication bias assessment unlikely to be performed for all the other evaluated outcomes.

3.5. Sensitivity analyses

The median value of NOS quality assessment was 6. Thus, the analyses were repeated by including only the 8 studies classified as "high quality" (NOS ≥ 6) [3,23–25,28,34,37,38] (Table S1). Of interest, after excluding studies classified as "low quality" [26,27,35,36], our results were substantially confirmed (Table 2).

Similar results were confirmed also after excluding studies [24,35, 36] potentially reporting on the same population as other included studies [28,34,38] (Table 3).

3.6. Subgroup analysis

Given the potential influence of PA etiology on the evaluated outcomes, we planned to perform separate analyses of studies specifically enrolling patients with aldosterone-producing adenoma. However, considering the low number of studies ($n = 3$) [3,34,35], a subgroup analysis could be performed only for CCA-IMT. In particular, compared to EH subjects, a significantly higher CCA-IMT was confirmed in PA patients with adrenocortical adenoma (MD: 0.10 mm; 95% CI: 0.05, 0.15), without heterogeneity among studies ($I^2 = 0\%$; $P = 0.77$).

3.7. Meta-regression analyses

Regression models for studies comparing PA patients and EH subjects showed that the presence of diabetes and smoking habit significantly impacted on CCA-IMT. In particular, increasing percentages of diabetics and smokers were associated with a lower difference in CCA-IMT between PA patients and EH controls ($Z = -2.31$; $P = 0.02$, Fig. 3A, and $Z = -2.28$; $P = 0.02$, Fig. 3B, respectively).

Table 2
Sensitivity analyses on "high quality" studies (i.e. Newcastle–Ottawa Scale ≥ 6).

	N of studies	N of patients	Effect size MD [95% CI]
<i>Primary aldosteronism versus essential hypertension</i>			
CCA-IMT	3	98 patients	MD: 0.12 mm [0.08, 0.16]; $P < 0.00001$
	(4 data-sets)	97 controls	$I^2 = 46\%$; $P = 0.14$
Aortic-PWV	5	216 patients	MD: 1.29 m/s [0.69, 1.89]; $P < 0.0001$
		191 controls	$I^2 = 75\%$; $P = 0.003$
Alx	1	37 patients	MD: 10.30% [1.61, 18.99]; $P = 0.02$
		20 controls	$I^2 = \text{NA}$; $P = \text{NA}$
Alx@75	1	14 patients	MD: -1.10% [-4.88, 2.68]; $P = 0.57$
		33 controls	$I^2 = \text{NA}$; $P = \text{NA}$
<i>Primary aldosteronism versus normal blood pressure</i>			
CCA-IMT	2	56 patients	MD: 0.22 mm [0.15, 0.29]; $P < 0.00001$
		48 controls	$I^2 = 78\%$; $P = 0.003$
FMD	1	36 patients	MD: -2.10% [-2.80, -1.40]; $P < 0.00001$
		44 controls	$I^2 = \text{NA}$; $P = \text{NA}$
Aortic-PWV	2	37 patients	MD: 3.71 m/s [3.38, 4.35]; $P < 0.000001$
		32 controls	$I^2 = 0\%$; $P = 0.76$
Alx@75	1	14 patients	MD: 4.90% [-0.01, 9.81]; $P = 0.05$
		17 controls	$I^2 = \text{NA}$; $P = \text{NA}$

N: number; MD: mean difference; 95% CI: 95% Confidence Intervals; CCA-IMT: common carotid artery intima-media thickness; aortic-PWV: carotid-femoral pulse-wave velocity; Alx: augmentation index; Alx@75: augmentation index normalized to a 75 beats/min heart rate; FMD: flow-mediated dilation; NA: not applicable.

Table 3
Sensitivity analyses after exclusion of studies potentially reporting on the same population as other included studies.

	N of studies	N of patients	Effect size MD [95% CI]
<i>Primary aldosteronism versus essential hypertension</i>			
CCA-IMT	3	85 patients	MD: 0.13 mm [0.09, 0.16]; $P < 0.00001$
	(4 data-sets)	77 controls	$I^2 = 31\%$; $P = 0.23$
Aortic-PWV	5	216 patients	MD: 1.29 m/s [0.69, 1.89]; $P < 0.0001$
		191 controls	$I^2 = 75\%$; $P = 0.003$
Alx	2	73 patients	MD: 5.12% [-3.94, 14.17]; $P = 0.27$
		48 controls	$I^2 = 66\%$; $P = 0.09$
Alx@75	2	50 patients	MD: -0.54% [-3.78, 2.70]; $P = 0.74$
		61 controls	$I^2 = 0\%$; $P = 0.57$
<i>Primary aldosteronism versus normal blood pressure</i>			
CCA-IMT	3	81 patients	MD: 0.16 mm [0.05, 0.27]; $P = 0.004$
		58 controls	$I^2 = 90\%$; $P < 0.0001$
FMD	2	61 patients	MD: -2.52% [-3.64, -1.40]; $P < 0.0001$
		54 controls	$I^2 = 52\%$; $P = 0.15$
Aortic-PWV	3	73 patients	MD: 3.74 m/s [3.43, 4.05]; $P < 0.000001$
		52 controls	$I^2 = 0\%$; $P = 0.89$
Alx@75	2	50 patients	MD: 8.59% [0.69, 16.50]; $P = 0.03$
		37 controls	$I^2 = 72\%$; $P = 0.06$

N: number; MD: mean difference; 95% CI: 95% Confidence Intervals; CCA-IMT: common carotid artery intima-media thickness; aortic-PWV: carotid-femoral pulse-wave velocity; Alx: augmentation index; Alx@75: augmentation index normalized to a 75 beats/min heart rate; FMD: flow-mediated dilation; NA: not applicable.

Similarly, an increasing percentage of patients with smoking habit was also associated with a low effect size for aortic-PWV ($Z = -3.78$; $P < 0.001$, Fig. 4).

When analyzing regression models for studies on PA patients and normotensive subjects, male gender significantly impacted on CCA-IMT. An increasing percentage of males was associated with a higher difference in CCA-IMT between PA patients and normotensive controls ($Z = 2.83$; $P = 0.005$, Fig. 5).

All the other demographic and clinical data did not impact on the evaluated outcomes.

4. Discussion

Results of the present meta-analysis consistently show that PA is associated with subclinical atherosclerosis and increased arterial stiffness. In particular, compared to both hypertensive and normotensive subjects, we reported an increased CCA-IMT and aortic-PWV in patients with PA. In addition, when compared to controls with normal blood pressure, PA patients also showed increased Alx and Alx@75 and impaired endothelial function (as expressed by a reduced FMD). Our findings are strengthened by the sensitivity and subgroup analyses. Moreover, regression models were able to further refine results providing the evidence that male gender, diabetes, and smoking habit may significantly impact on the evaluated outcomes.

Overall, these data clearly show an increased CV risk in patients with PA and suggest the need for a strict monitoring of CV risk factors and of early signs of subclinical atherosclerosis in this clinical setting. Accordingly, previous published studies reported an increased risk of major CV events [3] and CV death in PA patients when compared to EH subjects [6]. The German Conn's Registry recently reported that CV mortality is the main cause of death in PA (50% versus 34% in EH) [7].

Many CV risk factors are thought to have a causal role in the atherosclerotic process [40]. Although PA patients are hypertensive subjects, the relationship between subclinical atherosclerosis and PA seems to be more complex and the presence of hypertension might not entirely explain the accelerated atherosclerotic process in this clinical setting [8]. Thus, other mechanisms have been proposed to explain the relationship between PA and atherosclerosis.

Growing evidence suggests that it is the long-term exposure to high aldosterone levels to determine a deleterious effect on the CV system by

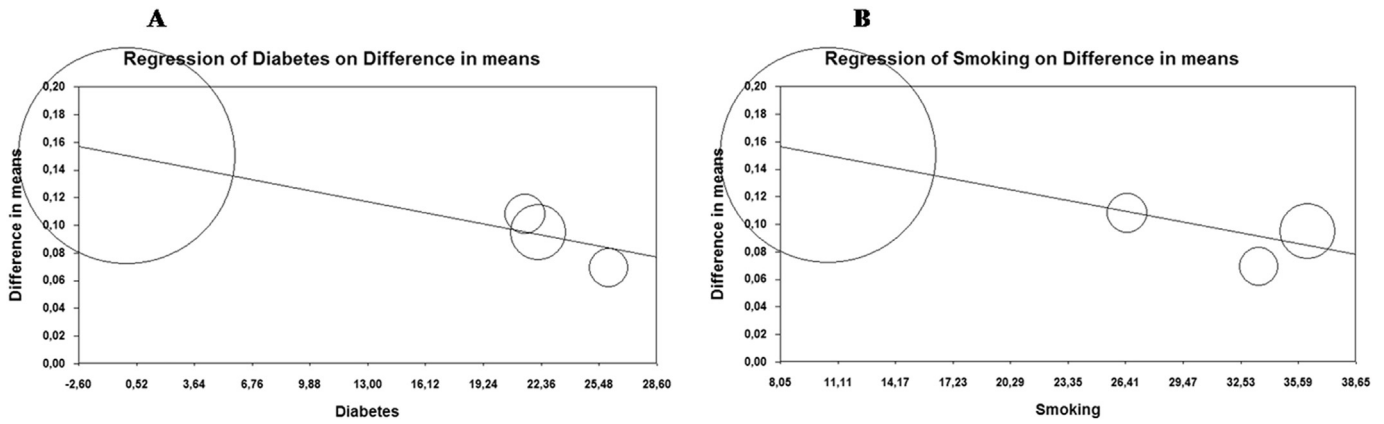


Fig. 3. Meta-regression analyses: impact of demographic and clinical variables on effect size assessed with regression-based techniques. Primary aldosteronism (PA) versus essential hypertension (EH): effects of diabetes (Panel A) and smoking habit (Panel B) on common carotid artery intima-media thickness (CCA-IMT). The area of each circle is proportional to the study weight in the meta-regression. Increasing percentages of diabetics and smokers are associated with a lower difference in CCA-IMT between PA patients and EH controls.

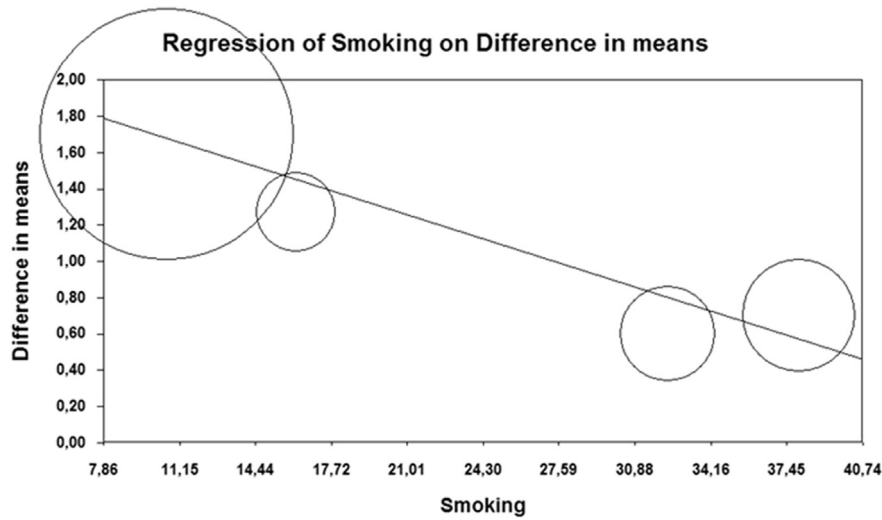


Fig. 4. Meta-regression analyses: impact of demographic and clinical variables on effect size assessed with regression-based techniques. Primary aldosteronism (PA) versus essential hypertension (EH): effect of smoking habit on carotid-femoral pulse wave velocity (aortic-PWV). The area of each circle is proportional to the study weight in the meta-regression. An increasing percentage of smokers is associated with a lower difference in aortic-PWV between PA patients and EH controls.

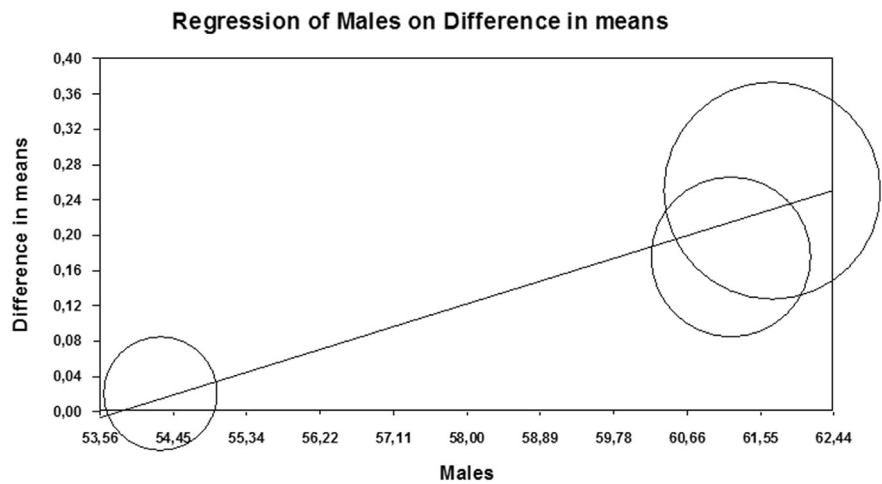


Fig. 5. Meta-regression analyses: impact of demographic and clinical variables on effect size assessed with regression-based techniques. Primary aldosteronism (PA) versus normal blood pressure: effect of male gender on CCA-IMT. The area of each circle is proportional to the study weight in the meta-regression. An increasing percentage of males is associated with a higher difference in CCA-IMT between PA patients and normotensive controls.

mechanisms other than sodium-water retention and hypertensive effects [41–43]. Aldosterone excess contributes to fibrosis and thickening of the arterial wall [24], and adversely affect endothelial function by enhancing oxidative stress and inflammation [44,45]. It has been suggested that aldosterone is able to induce vascular smooth muscle cell hypertrophy or hyperplasia with adventitial cell migration [46]. Moreover, aldosterone excess has been associated with hypertrophy of cardiac myocytes [47]. These aldosterone-induced changes result in vascular remodeling, accompanied by increased left ventricular mass [47] and increased IMT [48]. Aldosterone also induces changes in the extracellular matrix, leading to collagen deposition [49], and subsequently, to arterial stiffening [36] and myocardial fibrosis [41,50]. The role of aldosterone in the changes of vascular collagen accumulation is further confirmed by the evidence that the aldosterone antagonist spironolactone has been able to prevent the development of aortic fibrosis in animal models [51].

Overall, our findings are in line with all available experimental and clinical evidences, supporting the hypothesis that premature atherosclerosis may be one of the main features of PA and that vascular remodeling induced by long-term exposure to high aldosterone levels plays an important pathogenic role. Thus, aldosterone excess may act independently and/or synergistically with hypertension and traditional CV risk factors.

In order to provide a comprehensive overview of the relationship between PA and subclinical atherosclerosis, all the major recognized markers of CV risk were taken into account in the current meta-analysis. Moreover, with the aim to exclude the potential interference of PA-related hypertension on our results, we tried to compare PA patients to both hypertensive and normotensive subjects. As a matter of fact, our results on CCA-IMT and aortic-PWV were substantially confirmed both in comparison to EH and to normal blood pressure subjects, thus confirming an independent role of aldosterone excess on CV risk. In addition, we performed meta-regression analyses to evaluate whether clinical and demographic variables may impact on the observed results. As expected, regression models showed that results are conditioned by an increasing prevalence of traditional CV risk factors (i.e. smoking habit and diabetes mellitus) that was associated with a lower effect size. In addition, compared to normotensive subjects, PA patients also showed impaired FMD and increased Alx and Alx@75. The latter results on arterial stiffness parameters (Alx and Alx@75) could not be confirmed in the analyses versus EH. However, when interpreting this result, we should also consider the limited number of studies ($n = 2$) evaluating this outcomes. In keeping with this, the low number of available studies on the other searched markers of CV risk (i.e. NMD, brachial-PWV, ba-PWV, ABI) made any further meta-analytical evaluation unlikely to be performed.

The clinical relevance of these results and the need for a strict monitoring of subclinical signs of atherosclerosis in PA patients can be better understood if we consider that the risk of myocardial infarction increases of 43% every 0.163 mm increase in carotid IMT [52,53] while the risk of major CV events rises of about 14% each 1 m/s increase in aortic-PWV [19]. In addition, it is important to highlight that growing attention has been given to PA in the last years because recent data consistently suggest that its prevalence is not negligible, accounting for 5–13% of hypertensive patients of any causes [54].

Our results support the need for large long-term interventional trials with CV end-points to investigate whether disease remission obtained with appropriate surgical and/or medical treatments may modify the CV risk in PA patients.

5. Limitations

Some potential limitations of our study need to be discussed. First, studies included in our meta-analysis have different inclusion and exclusion criteria and most of patients included in the analysis had concomitant CV risk factors (smoking, obesity, diabetes mellitus,

hyperlipidemia). Since meta-analysis is performed on aggregate data and some missing information is present in each study, the multivariate approach allowed for the adjustment for some (but not all) potential confounders. Thus, although results of meta-regression analyses were able to refine analyses by assessing the influence of most clinical and demographic variables on the observed results, caution is necessary in overall results interpretation.

Second, heterogeneity among the studies was generally significant. Although it was not possible to conclusively ascertain sources of heterogeneity, all results were confirmed after adjustment for potential publication bias.

Another potential limitation is the presence of other clinical conditions potentially impacting on aldosterone levels (i.e. chronic kidney disease, atrial fibrillation) [55,56]. None of included studies reported the number of patients with any of these diseases. Thus, it was not possible to assess the impact on our outcomes. However, the present meta-analysis has been specifically designed to evaluate patients with PA, which is caused in most cases by adrenocortical adenoma or by lateral adrenal hyperplasia. Thus, we are confident that the impact of potential confounding factors may be marginal.

Moreover, at variance with IMT, measurement of PWV, Alx, and FMD may be influenced by many confounding factors, significantly limiting reproducibility of arterial stiffness and endothelial dysfunction assessment [57,58,59] and, in turn, the relevance of our results. Moreover, we have to consider that differences among assessment techniques and devices, as well as the lack of comparable age-adjusted normal values may limit the validity of these parameters as markers of early atherosclerosis. Thus, caution is necessary in overall results interpretation. However, in the attempt to overcome this potential limitation, we repeated the analyses by using SMD instead of MD, being this method designed to be used when different methods of measurement are analyzed together [31]. Interestingly, all results were confirmed with using SMD (data not shown).

6. Conclusion

PA is significantly associated with subclinical atherosclerosis and, in turn, with an increased CV risk. Thus, patients with PA may benefit from a periodic assessment of surrogate markers of CV risk and this could help establish more specific CV prevention strategies for these patients.

Authors contribution

Ambrosino P, Lupoli R and Di Minno MND conceived and designed the study, performed statistical analysis interpreted results and drafted the manuscript; Tortora A, Cacciapuoti M, Lupoli GA, Tarantino P, and Nasto A acquired clinical data, drafted the manuscript and performed critical revisions. All Authors read and approved the final version of the manuscript.

Di Minno MND, Ambrosino P, and Lupoli R had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of data analysis.

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Conflicts of interest

Dr. Matteo Nicola Dario Di Minno has acted as paid lecturer or board member and received grants and honoraria from Bayer, Biotest, Pfizer and Novo-Nordisk in the last 36 months for researches unrelated to the present study. All the other authors have nothing to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ijcard.2016.01.200>.

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