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Title

Comparative effectiveness of 4th line anti-hypertensive agents in resistant hypertension; A systematic review and meta-analysis

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Abstract

Aim

We assessed the effectiveness of 4th line mineralocorticoid receptor antagonists in comparison to other 4th line anti-hypertensive agents in resistant hypertension.

Methods and Results

We systematically searched Medline, EMBASE and the Cochrane library from database inception until January 2016. We included randomised and non-randomised studies that compared mineralocorticoid receptor antagonists to other 4th line anti-hypertensive agents in patients with resistant hypertension. The outcome was change in systolic blood pressure, measured in the office, at home or by ambulatory blood pressure monitoring. Secondary outcomes were changes in serum potassium and occurrence of hyperkalaemia. We used random effects models and assessed statistical heterogeneity using the I^2 test and corresponding 95% confidence intervals.

From 2,506 records, 5 studies met our inclusion criteria with 755 included patients. Two studies were randomised and three were non-randomised. Comparative fourth line agents included bisoprolol, doxazosin, furosemide and additional blockade of the renin angiotensin-aldosterone system. Using data from randomised studies, mineralocorticoid receptor antagonists reduced blood pressure by 7.4mmHg (95% CI 3.2 – 11.6) more than the active comparator. When limited to non-randomised studies, mineralocorticoid receptor antagonists reduced blood pressure by 11.9mmHg (95% CI 9.3 – 14.4) more than the active comparator.

Conclusion

On the basis of this meta-analysis, mineralocorticoid receptor antagonists reduce blood pressure more effectively than other 4th line agents in resistant hypertension. Effectiveness stratified by ethnicity and comorbidities, in addition to information on clinical outcomes such as myocardial infarction and stroke now needs to be determined.

Keywords

Resistant hypertension, blood pressure, mineralocorticoid receptor antagonists, spironolactone, comparative effectiveness research, meta-analysis.

Introduction

Hypertension is a leading cause of mortality worldwide. It occurs in 1 out of 4 people and is responsible for 9.4 million deaths annually.[1, 2] Of those affected, approximately 14% are said to have resistant hypertension (RH)[3], defined as blood pressure (BP) that remains ≥140/90mmHg despite being treated with maximum doses, or best tolerated doses, of three or more antihypertensive agents, one of which should be a diuretic.[4] The prevalence of RH is equally distributed between men and women, but is more common in older people (mean age 60yrs).[3] Those with diabetes and chronic kidney disease (CKD), along with those who are obese, are over-represented in the RH population.[5] Patients with RH generally have a poorer prognosis than those whose hypertension is controlled, with a 50% increased risk of a cardiovascular event.[6]

The pathophysiology of RH remains poorly understood. Once adherence and white coat hypertension have been ruled out, over activation of the renin-angiotension-aldosterone system (RAAS), over activation of the sympathetic nervous system, sodium retention leading to volume expansion and/or vascular stiffening have all been suggested as potential pathological mechanisms.[7-10] Given the mixed pathologies and a historical dearth of evidence for the treatment of RH[11], current clinical guidance from international sources is slightly discordant. For example, NICE guidelines in the UK suggest the use of either spironolactone (a mineralocorticoid receptor antagonist (MRA) with potassium sparing diuretic activity), or increasing the dose of the thiazide diuretic in the case of high serum potassium as potential 4th line options on top of an angiotensin-converting enzyme inhibitor (ACEI)/angiotensin receptor blocker (ARB), a calcium channel blocker, and a diuretic.[4] The European Society of Hypertension/European Society of Cardiology guidelines refer to the use of fourth-line MRA, amiloride or an alpha-blocker. [12] In the USA, both the American Heart Association and the Eighth Joint National Committee guidance specify adding a beta-blocker or a MRA as fourth-

line agents and/or seeking specialist advice. [13, 14] Despite these disparities, the general message from all is to enhance diuretic treatment. [4, 12-14]

Two recent systematic reviews have pointed to the effectiveness of MRAs versus placebo in lowering BP in those with RH.[15, 16] While this is important evidence, it would now be useful to establish how MRAs compare to other potential 4th line agents.

Hence, we assessed the effectiveness, in terms of systolic BP reductions, of MRAs in comparison to alternative 4th line anti-hypertensive agents in patients with RH.

Methods

Data sources and searches

We searched Medline, Embase and the Cochrane Library from inception up to January 2016 with no language restriction. The search terms used in Medline were 'resistant hypertension' AND "Hypertension/drug therapy"[Mesh] AND "Antihypertensive Agents" [Pharmacological Action]; we constructed analogous searches in the other databases. We searched Clinicaltrials.gov for ongoing or completed trials of anti-hypertensive agents in RH. We also searched the reference lists of included articles and recent clinical guidelines. Where relevant abstracts were found without corresponding full papers, we contacted study authors for full text papers. If a full text paper did not exist at that time, the record was excluded. We also contacted study authors to clarify any questions on their reported results.

Study selection

Definition of RH

We included studies that defined RH as systolic BP ≥140mmHg despite being on ≥3 anti-hypertensive agents.

Study types

Full texts of both randomised studies and non-randomised studies were eligible for inclusion. Letters, editorials and opinion pieces were excluded.

Intervention and comparator

The intervention was the addition of an MRA. The comparator was the addition of an alternative fourth-line anti-hypertensive agent. There was no restriction on agent, dose, duration of treatment or length of follow up. Studies that examined drugs that are not available on the market or not currently being tested in phase 2 or phase 3 trials were excluded.

Outcome

The outcome was change in systolic BP in the intervention group relative to the comparator group. We used systolic BP, as opposed to both systolic and diastolic BP for two reasons. First, because systolic hyperetnsion is much more common in populations aged >50yrs than diastolic BP.[17] Second, because systolic hypertension contributes more to the global cardiovascular disease burden than diastolic hypertension.[17] There were no restrictions on how BP was measured; office, home or ambulatory blood pressure monitoring (ABPM) measurements were all included. In studies where more than one type of measurement was reported, ABPM was the preferred outcome for inclusion in the meta-analysis. Secondary outcomes included mean changes in serum potassium and the number of cases of hyperkalaemia in each treatment group.

Data extraction and quality assessment

SJS carried out the searches. After exclusion of duplicates and irrelevant titles and abstracts, four study authors (SJS, AR, RM and KM) independently assessed full texts for eligibility, and carried out data extraction and quality assessment in duplicate. Any differences of opinion were discussed and a third reviewer was available to arbitrate any issues that remained unresolved. We used a standardised data extraction form to collect information for each study on: the definition of RH used, including whether due consideration was given to white coat hypertension, adherence and secondary causes of hypertension; the type of study design and analysis used; and details on population characteristics for example, number of people included, mean age, proportion of females, mean body mass index (BMI), proportion of diabetic patients and mean estimated Glomerular Filtration Rate (eGFR). We extracted detailed data on baseline systolic BP, systolic BP at the end of follow up and change in systolic BP between the treatment arms for each study along with information on how BP

was measured. We collected adverse event data specifically for mean changes in serum potassium and hyperkalaemia.

We assessed the quality of included studies using a modified Downs and Black checklist, which can be used for randomised studies and non-randomised studies.[18] This checklist assesses quality across four domains: internal validity (bias and confounding), external validity and general quality of study reporting. Included studies were scored out of a potential 21 points across these four domains.

Data synthesis and statistical analysis

We used the difference in mean reductions in systolic BP between treatment arms and the standard error in DerSimonian-Laird random effects models. Statistical heterogeneity was assessed using the I^2 test and corresponding 95% confidence intervals estimated using the formula proposed by Higgins and Thompson.[19] An I^2 threshold of >60% indicated substantial heterogeneity. We analysed randomised and non-randomised studies separately. We did not formally test for the presence of publication bias due to the small number of included studies.[20] Rather, we visually inspected the funnel plot. Secondary outcomes were qualitatively assessed.

Sensitivity analyses

Three methods of measuring BP were reported in the included studies; 1) office BP, 2) home BP and 3) ABPM. We conducted sensitivity analyses to assess whether combining different types of BP measurements in a meta-analysis gave substantially different result. We ran all analyses in Revman Version 5.3.[21] We referred to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for reporting (*Supplementary Information 1*).[22]

Results

From 2506 citations, after exclusion of duplicates and irrelevant titles, 22 full texts were assessed for eligibility. Seventeen of these were excluded (**Figure 1**). Thus, five articles were included in the review.[8, 23-26]

*****Insert Figure 1**

Gap in text maintained above to preserve order of referencing

This included 755 patients with a mean age of 62 years and 30% female. Diabetes was highly prevalent at 45.6%, while eGFR was 83.9 ml/min, likely due to exclusion of patients with chronic kidney disease in some studies.[8, 23, 25, 26] Mean BMI was 30.7 kg/m^2 **(Table 1).**

Insert Table 1

Of the included studies, two were randomised controlled trials[26] [23] and three were non-randomised[24, 25] [8] The intervention was spironolactone in all studies. The comparator drugs included doxazosin, bisoprolol, furosemide and additional RAAS blockade **(Table 2)**.

Insert Table 2

There was substantial heterogeneity across the included studies in terms of how RH was defined and identified. Four studies referred to adherence to medication regimen before including patients as RH cases, but the reported detail on how this was examined was variable.[8, 24-26] Bobrie *et al*.referred to adherence measurement during the study by pill count, but the threshold for adherence was not reported.[23] The results of on treatment adherence assessment by urinalysis in the PATHWAY-2 trial is yet to be published.[26] One study did not clearly define the BP thresholds used to define RH[8] and two studies did not define how long a patient should be on 3 or more anti-hypertensive agents before being defined as having RH.[8, 25]

Two studies measured the outcome, systolic BP, both in the office and with ABPM monitoring [8, 23], one study each used office and ABPM monitoring respectively[24, 25] and one study used home monitoring and office measurements.[26] Follow up ranged from eight weeks to six months.

Non-randomised studies were of much lower quality than randomised studies **(Table 3).** They achieved lower scorings on internal validity due to baseline characteristics being non-comparable, statistical tests that did not account for confounding, not accounting for losses to follow up, not being adequately powered and not tracking adherence to the intervention or comparator drug.

Insert Table 3

Results of meta-analysis

We included two studies, including a total of 502 patients, in a meta-analysis of randomised studies. Using a random effects model, the overall pooled estimate for reduction of systolic BP by MRAs was 7.4mmHg (95% CI 3.2 – 11.6) more than the active comparator **(Figure 2a)**. Heterogeneity was measured as I^2 = 76% (95% CI 0 – 95.5). There was one ABPM measurement in this analysis[23] and one home measurement.[26]

Insert Figure 2A and 2B

We included three studies, including a total of 253 patients, in a meta-analysis of non-randomised studies. Using a random effects model, the overall reduction in systolic BP was 11.9mmHg (95% CI 9.3 – 14.4) more in spironolactone users than the active comparator **(Figure 2b)**. Heterogeneity was measured as I^2 = 0% (95% CI 0 - 40). There were two ABPM measurements[8, 25] in this analysis and one office measurement.[24]

Sensitivity analyses

Office measurements in non-randomised studies

In the main analysis using randomised and non-randomised studies, ABPM measurements were included where reported. In a sensitivity analysis, we included office BP, where reported, to assess the influence of measurement types on pooled results. For randomised studies, this analysis included two office BP measurements as opposed to one ABPM measurement[23] and one home measurement in main analysis.[26] Using a random effects model, the overall effect measure estimated that spironolactone reduced systolic BP by 7.3mmHg (95% CI 0.9 – 13.8) more than the active comparator (**Figure 3a**). Heterogeneity was measured as I^2 = 87% (95% CI 24.8 -97.8). For non-randomised studies, the sensitivity analysis included two office BP measures[8, 24] and one ABPM measurement.[25] Using a

random effects model, the overall effect measure estimated that spironolactone reduced systolic BP by -13.4mmHg (95% CI 8.4 – 18.3) more than the active comparator (**Figure 3b**). Heterogeneity was measured as I^2 = 66% (95% CI 0 – 94).

Insert Figure 3

Changes in serum potassium and hyperkalaemia

All five included studies reported changes in serum potassium or cases of hyperkalaemia.[8, 23-26] From **Table 4,** there were 12 cases of hyperkalaemia in 424 patients treated with MRAs, in comparison to 0 events in 471 patients treated with another fourth-line agent. Mean serum potassium values increased to a greater extent in patients treated with MRAs than patients treated with another fourth-line agent (**Table 4)**.

Insert Table 4

Publication bias

There was some visual evidence of asymmetry in the funnel plot, suggesting a small study bias **(***Supplementary Information 3***)**.

Conclusions

This meta-analysis, encompassing five separate studies and 755 patients, found that when MRAs were compared with another fourth-line agent or strategy in the treatment of RH, MRAs achieved larger reductions in systolic BP, in the order of 7 to 12mmHg.

Three previous reviews have indicated the effectiveness of MRAs versus placebo, in addition to its' safety.[15, 16, 44] The reduction in systolic BP achieved by MRAs in previous reviews averaged at approximately 20mmHg. This is roughly double the reduction in BP shown in our review. This difference was not unexpected considering we included studies with an active comparator only, whereas previous reviews included studies where placebo was the comparator group. Whether this magnitude of reduction in systolic BP will translate to a decrease in cardiovascular outcomes in patients with RH remains to be examined. It might be reasonably expected that clinical relevance is likely given recent evidence that, in a general hypertensive population, a 10mmHg reduction in systolic BP was associated with an approximate 20% reduction in risk of cardiovascular and coronary heart disease events, and an approximate 30% reduction in risk of stroke and heart failure.[45]

Our sensitivity analysis for randomised studies demonstrated little difference in the magnitude of reductions gained in systolic BP when measured using office measurements *versus* home or ABPM measurements. The randomised nature of these studies likely preserved the relative difference between treatment arms. In contrast, when the majority of non-randomised studies reported office BP rather than the majority reporting ABPM measurements larger reductions in systolic BP were found (-13.8mmHg versus -11.9mmHg). Although the difference in these findings was not significant, the trend towards greater reductions *via* office measurements is in line with current knowledge on the contribution of white coat hypertension in RH, and indeed in hypertension more broadly.[46, 47] This finding

also points to the importance of home BP or ABPM monitoring in detecting BP levels that are ultimately predictive of clinical events and mortality.[48]

In all studies, where reported, the average increase in serum potassium was larger in the MRA group compared with other 4th line agents. The magnitude of mean changes appeared to be larger in non-randomised studies than randomised studies. Similar findings were reported in a recent systematic review whereby the increase in serum potassium, found in non-randomised studies, was 0.46mmol/L higher than in placebo treated patients.[16] However, in randomised studies, the mean change between the groups was 0.15mmol/L, and this was non-significant.[16] A second review, encompassing a meta-analysis of mixed randomised and non-randomised studies, showed an increase of 0.33mmol/L (95% CI, 0.27-0.39) in serum potassium in users of MRAs.[15]

Our review also points to an increased number of hyperkalaemia-related events in patients treated with MRAs in comparison to patients treated with other 4th line agents. The systematic review authored by Dahal et al. reports an event rate of 46/1000 for hyperkalaemia in patients treated with MRAs in comparison to placebo, but this was solely in non-randomised studies and the same finding of increased risk was not found in randomised studies.[16] The difference in biochemical parameters reported by randomised and non-randomised studies may reflect differences in how patients are monitored in different study settings. For example, in clinical trials frequent follow up visits allow opportunity to identify changes in serum potassium before advancement to hyperkalaemia. In contrast, non-randomised studies are often conducted in routine care and reflect the true frequency/infrequency of laboratory testing, and thus the real world safety implications of treatments for patients.[49] Discordant findings between randomised and non-randomised studies aside, the risk of hyperkalaemia related events, especially in people using both and

ACEI/ARB and spironolactone, remains a worry and frequent lab monitoring is recommended.[50]

Our review provides evidence that on average, MRAs are more efficient in lowering systolic BP than other potential fourth-line agents such as bisoprolol, doxazosin and additional RAAS blockade. This may be explained by the main pathophysiology associated with RH; volume expansion secondary to salt sensitivity/retention.[10]

MRAs' antagonism of aldosterone at the distal tubule, resulting in the removal of sodium in exchange for potassium thus increasing diuresis, reduces the problem of volume expansion.[51] While the use of an ACEI or an ARB should block the production of aldosterone at an earlier stage in the RAAS, a phenomenon referred to as "aldosterone synthesis escape" requires direct blockade of aldosterone at the mineralocorticoid receptor to ensure lowering of blood pressure, thus providing a functional and productive role for spironolactone on top of other anti-hypertensive agents.[52]

While other pathophysiologies can be implicated in RH, such as over-activation of the sympathetic nervous system[10], the success of MRAs in RH may be due to volume expansion being the most prevalent mechanism underpinning the disease. A second reason for the benefit of MRAs above other 4th line agents is that, in addition to its' action at the distal tubule, there is evidence to suggest that MRAs also work on the vasculature reducing BP by other mechanisms. For example, spironolactone has been found to increase vascular compliance in rats[52], inhibit vasoconstriction in the arterioles [53]and eplenerone has been found to improve endothelial function and inhibit Rho-associated kinases, which are involved in the contracture of vascular smooth muscle cells.[54]

We observed several important sources of heterogeneity between the studies included in the review, for example; study authors rarely discussed how long their included populations

were on ≥3 anti-hypertensive agents before being classified as RH. Not all studies sought to exclude white coat hypertension, nor did all studies examine insufficient adherence to anti-hypertensive medication regimens during the study. This points to a requirement for a more stringent application of a standardised definition of resistant hypertension to avoid mixed samples of patients, leading to results that do not apply to the actual RH population. We noted some evidence of publication bias in the funnel plots. This was likely associated with poor methodological quality in the included non-randomised studies.[55]

Our review has multiple strengths. First, we used a comprehensive search strategy yielding more than 2,500 records that we screened for inclusion. Second, we carried out study selection and data abstraction in duplicate to enhance the reliability of our findings. Third, this review provides a quantitative estimate of the effectiveness of MRA in comparison to other antihypertensive agents that could be used as fourth-line agents in RH, improving on other reviews that examined placebo as the comparison group.[15 16, 44] Information on comparative effectiveness is constructive in that MRAs will not suit every patient with RH, for example in patients where a drug-drug interaction is expected or adverse events such as hyperkalaemia could reasonably occur.[56] In such cases, information on the effectiveness of alternative pharmacologic options is required.

Our review is limited in that it we did not assess individual level patient data. This would have allowed comprehensive subgroup analyses according to sex, age, diabetes status and renal function. The number of included studies in each meta-analysis was low. While more studies would have been preferable, it was still appropriate to carry out a meta-analysis. This was for reasons of transparency in the processes employed to reach a summary conclusion, and also because combining the results of studies added information beyond what was held in each individual study.[57] A small number of included studies meant it was also challenging to accurately assess between-study heterogeneity. We attempted to ameliorate this limitation by

297 presenting 95% confidence intervals around the point estimate for I^2 value.[58,19] A further
298 limitation is that the included studies were of varying quality. Non-randomised studies, in
299 particular, often include an amount of confounding by indication, and the studies included in
300 this review mostly used methodology not designed to address this, for example simple
301 statistical analyses such as t-tests or Wilcoxon tests. Nonetheless, a meta-analysis of these
302 studies was useful for the reasons of transparency and combining information as mentioned
303 above.[57] In addition, for a topic area where not many trials exist, it seems efficient to use all
304 available evidence, with due appreciation for its' limitations. The non-randomised studies we
305 included found a similar overall effect to the randomised studies in this review suggesting
306 confounding may not have been strong in this instance. This is likely to arise if the choice
307 between different drugs is not driven by strong evidence and could indicate a perception of
308 equipoise in many cases. It therefore appears that observational data may be of further use for
309 investigating the comparative effects of different drug choices for RH. However, our nuanced
310 summary of deficits noted in the literature should be addressed in future studies.

311 While quantitative estimates of the benefits of MRAs in reducing BP in RH are now
312 available, it would be helpful to stratify these changes in BP by patient characteristics such as
313 ethnicity, and co-morbidities such as diabetes and renal function.[15, 59] Future meta-analyses
314 might endeavour to stratify by different classes of comparator agents, e.g., beta-blockers,
315 diuretics and alpha-blockers to enable a more nuanced understanding of the comparative
316 effectiveness of MRA. It is now important that an assessment of effects on clinical outcomes
317 such as stroke and myocardial infarction is conducted. A rough calculation using information
318 on outcome parameters from the SPRINT trial indicates that an RCT of approximately 15,000
319 patients with 2 years follow up would be required to detect a 20% difference in
320 cardiovascular outcomes for RH patients on spironolactone versus other 4th line agents.[60] The
321 practical challenges of recruiting this number could be sidestepped by conducting a well-

designed and appropriately powered observational study. From the data presented in this study, it appears that observational studies can detect similar effect sizes to randomised trials in studies of RH, and thus, if designed appropriately offer a useful and practical way forward.

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Conflicts of Interest

There are no conflicts of interest to report.

Authorship

SJS contributed to the conception or design of the work. SJS, ID, LT, RM, KM, AR and LS contributed to the acquisition, analysis, or interpretation of data for the work. SJS drafted the manuscript. ID, LT, RM, KM, AR and LS critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

References

1. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. The lancet 2005;**365**(9455):217-223.
2. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, AlMazroa MA, Amann M, Anderson HR, Andrews KG. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. The lancet 2013;**380**(9859):2224-2260.
3. Achelrod D, Wenzel U, Frey S. Systematic review and meta-analysis of the prevalence of resistant hypertension in treated hypertensive populations. American journal of hypertension 2015;**28**(3):355-61.
4. National Institute for Health and Care Excellence. Hypertension in adults: diagnosis and management. NICE guidelines [CG127]. In; 2011.
5. Myat A, Redwood SR, Qureshi AC, Spertus JA, Williams B. Resistant hypertension. BMJ (Clinical research ed) 2012;**345**(7884):e7473.
6. Daugherty SL, Powers JD, Magid DJ, Tavel HM, Masoudi FA, Margolis KL, O'Connor PJ, Selby JV, Ho PM. Incidence and prognosis of resistant hypertension in hypertensive patients. Circulation 2012;**125**(13):1635-1642.
7. Yiannakopoulou EC, Papadopulos JS, Cokkinos DV, Mountokalakis TD. Adherence to antihypertensive treatment: a critical factor for blood pressure control. European Journal of Cardiovascular Prevention & Rehabilitation 2005;**12**(3):243-249.
8. Alvarez-Alvarez B, Abad-Cardiel M, Fernandez-Cruz A, Martell-Claros N. Management of resistant arterial hypertension: role of spironolactone versus double blockade of the renin-angiotensin-aldosterone system. Journal of hypertension 2010;**28**(11):2329-35.
9. Tsioufis C, Kordalis A, Flessas D, Anastasopoulos I, Tsiachris D, Papademetriou V, Stefanadis C. Pathophysiology of resistant hypertension: the role of sympathetic nervous system. International journal of hypertension 2011.
10. Rimoldi SF, Messerli FH, Bangalore S, Scherrer U. Resistant hypertension: what the cardiologist needs to know. European heart journal 2015;**36**(40):2686-2695.
11. Williams B. Resistant hypertension: an unmet treatment need. The Lancet 2009;**374**(9699):1396-1398.
12. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F, Burnier M, Ambrosioni E, Caufield M, Coca A, Olsen MH, Tsioufis C, Van De Borne P, Zamorano JL, Achenbach S, Baumgartner H, Bax JJ, Bueno H, Dean V, Deaton C, Erol C, Ferrari R, Hasdai D, Hoes AW, Knuuti J, Kolh P, Lancellotti P, Linhart A, Nihoyannopoulos P, Piepoli MF, Ponikowski P, Tamargo JL, Tendera M, Torbicki A, Wijns W, Windecker S, Clement DL, Gillebert TC, Rosei EA, Anker SD, Bauersachs J, Hitij JB, Caulfield M, De Buyzere M, De Geest S, Derumeaux GA, Erdine S, Farsang C, Funck-Brentano C, Gerc V, Germano G, Gielen S, Haller H, Jordan J, Kahan T, Komajda M, Lovic D, Mahrholdt H, Ostergren J, Parati G, Perk J, Polonia J, Popescu BA, Reiner Z, Ryden L, Sirenko Y, Stanton A, Struijker-Boudier H, Vlachopoulos C, Volpe M, Wood DA. 2013 ESH/ESC guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). European Heart Journal 2013;**34**(28):2159-2219.
13. Go AS, Bauman MA, King SMC, Fonarow GC, Lawrence W, Williams KA, Sanchez E. An effective approach to high blood pressure control: a science advisory from the American Heart Association, the American College of Cardiology, and the Centers for Disease Control and Prevention. Journal of the American College of Cardiology 2014;**63**(12):1230-1238.
14. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, Lackland DT, LeFevre ML, MacKenzie TD, Ogedegbe O. 2014 evidence-based guideline for the management of

high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). Jama 2014;**311**(5):507-520.
15. Liu G, Zheng X, Xu Y, Lu J, Hui R, Huang X. Effect of aldosterone antagonists on blood pressure in patients with resistant hypertension: a meta-analysis. Journal of human hypertension 2015;**29**(3):159-166.
16. Dahal K, Kunwar S, Rijal J, Alqatahni F, Panta R, Ishak N, Russell RP. The Effects of Aldosterone Antagonists in Patients With Resistant Hypertension: A Meta-Analysis of Randomized and Nonrandomized Studies. American journal of hypertension 2015;**28**(11):1376-85.
17. Williams B, Lindholm LH, Sever P. Systolic pressure is all that matters. The Lancet 2008;**371**(9631):2219-2221.
18. Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. Journal of epidemiology and community health 1998;**52**(6):377-384.
19. Higgins J, Thompson SG. Quantifying heterogeneity in a meta-analysis. Statistics in medicine 2002;**21**(11):1539-1558.
20. Lau J, Ioannidis JP, Terrin N, Schmid CH, Olkin I. The case of the misleading funnel plot. BMJ (Clinical research ed) 2006;**333**(7568):597-600.
21. Review Manager (RevMan). Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration. In; 2014.
22. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Annals of internal medicine 2009;**151**(4):264-269.
23. Bobrie G, Frank M, Azizi M, Peyrard S, Boutouyrie P, Chatellier G, Laurent S, Menard J, Plouin PF. Sequential nephron blockade versus sequential renin-angiotensin system blockade in resistant hypertension: a prospective, randomized, open blinded endpoint study. Journal of hypertension 2012;**30**(8):1656-64.
24. Rodilla E, Costa JA, Perez-Lahiguera F, Baldo E, Gonzalez C, Pascual JM. Spironolactone and doxazosin treatment in patients with resistant hypertension. Revista espanola de cardiologia 2009;**62**(2):158-66.
25. Verdalles Guzman U, Garcia De Vinuesa S, Goicoechea M, Verde E, Perez De Jose A, Macias N, Santos A, Luno J. Management of resistant hypertension (RHT): Aldosterone antagonists or intensification of diuretic therapy? Journal of Hypertension 2015;**33**:e360.
26. Williams B, MacDonald TM, Morant S, Webb DJ, Sever P, McInnes G, Ford I, Cruickshank JK, Caulfield MJ, Salsbury J. Spironolactone versus placebo, bisoprolol, and doxazosin to determine the optimal treatment for drug-resistant hypertension (PATHWAY-2): a randomised, double-blind, crossover trial. The Lancet 2015;**386**(10008):2059-2068.
27. Achrafi H. Action of nitroprusside in multidrug-resistant hypertension. Lancet 1989;**2**(8667):867.
28. Ambrosio GB. Minoxidil in resistant hypertension. Lancet 1977;**2**(8047):1087.
29. Frank M, Bobrie G, Azizi M, Peyrard S, Boutouyrie P, Chatellier G, Laurent S, Menard J, Plouin PF. Superiority of low dose diuretics combination over renin-angiotensin blockers combination in resistant hypertension. Journal of Hypertension 2010;**28**(Supplement A):276.
30. Garcia-Donaire JA, Cerezo C, Guerrero L, Fernandez L, Ruilope LM, Segura J. Long term evolution of blood pressure control in resistant hypertension. Journal of Clinical Hypertension 2012;**14**(Supplement S1).
31. Krieger EM, Drager LF, Giorgi DM, Krieger JE, Pereira AC, Barreto-Filho JA, Rocha Nogueira A, Mill JG. (2014) Resistant hypertension optimal treatment trial: a randomized controlled trial. *Clinical cardiology,* 10.1002/clc.22228.
32. Bassan FB, Rodrigues ML, Valenca D, Carvalho R, Soares E, Torres M, Neves MF, Oigman W, Sanjuliani AF. Behavior of endothelial function and blood pressure after use of spironolactone or clonidine in patients with resistant hypertension. Hypertension 2012;**60**(3 Meeting Abstracts).

33. Colina-Chourio JA, Godoy N, Oliveros-Palacios M, Arocha I. Long-term (3 years) sustained antihypertensive and metabolic actions of nitrendipine in severe, complicated, and resistant hypertension. Journal of cardiovascular pharmacology 1991;**18 Suppl 1**:S84-90.
34. Dargie HJ, Dollery CT, Daniel J. Minoxidil in resistant hypertension. Lancet 1977;**2**(8037):515-8.
35. Russell GI, Swart S, Bing RF. Combined prazosin and hydralazine in resistant hypertension. Lancet 1980;**1**(8167):543.
36. Weinberger MH. Treatment-resistant hypertension: Efficacy of angiotensin-converting enzyme inhibition with captopril. Preventive Medicine 1980;**9**(3):459.
37. White NJ, Rajagopalan B, Yahaya H, Ledingham JG. Captopril and frusemide in severe drug-resistant hypertension. Lancet 1980;**2**(8186):108-10.
38. Segura J, Cerezo C, Garcia-Donaire JA, Schmieder RE, Praga M, de la Sierra A, Ruilope LM. Validation of a therapeutic scheme for the treatment of resistant hypertension. Journal of the American Society of Hypertension : JASH 2011;**5**(6):498-504.
39. Cuesta V, Cannata J, Herrera J, Maisueche I, Peral V, Redondo C. [Prazosin in the treatment of resistant arterial hypertension associated with nephropathy]. Revista clinica espanola 1981;**161**(1):37-9.
40. Kuriyama S, Yokoyama K, Hara Y, Sugano N, Yokoo T, Hosoya T. Effect of aliskiren in chronic kidney disease patients with refractory hypertension undergoing hemodialysis: a randomized controlled multicenter study. Clinical and Experimental Nephrology 2013;**18**(5):821-830.
41. Ramsay LE, Silas JH, Freestone S. Diuretic treatment of resistant hypertension. British medical journal 1980;**281**(6248):1101-3.
42. Mann SJ, Parikh NS. A simplified mechanistic algorithm for treating resistant hypertension: efficacy in a retrospective study. Journal of clinical hypertension (Greenwich, Conn) 2012;**14**(4):191-7.
43. Karns AD, Bral JM, Hartman D, Peppard T, Schumacher C. Study of aldosterone synthase inhibition as an add-on therapy in resistant hypertension. Journal of clinical hypertension (Greenwich, Conn) 2013;**15**(3):186-92.
44. Guo H, Xiao Q. Clinical efficacy of spironolactone for resistant hypertension: a meta analysis from randomized controlled clinical trials. International journal of clinical and experimental medicine 2015;**8**(5):7270.
45. Ettehad D, Emdin CA, Kiran A, Anderson SG, Callender T, Emberson J, Chalmers J, Rodgers A, Rahimi K. Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. Lancet 2016;**387**(10022):957-67.
46. Myers MG, Godwin M, Dawes M, Kiss A, Tobe SW, Grant FC, Kaczorowski J. Conventional versus automated measurement of blood pressure in primary care patients with systolic hypertension: randomised parallel design controlled trial. BMJ (Clinical research ed) 2011;**342**:d286.
47. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, White A, Cushman WC, White W, Sica D, Ferdinand K, Giles TD, Falkner B, Carey RM, American Heart Association Professional Education C. Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. Circulation 2008;**117**(25):e510-526.
48. Dolan E, Stanton A, Thijs L, Hinedi K, Atkins N, McClory S, Den Hond E, McCormack P, Staessen JA, O'Brien E. Superiority of ambulatory over clinic blood pressure measurement in predicting mortality the Dublin outcome study. Hypertension 2005;**46**(1):156-161.
49. Cohen AT, Goto S, Schreiber K, Torp-Pedersen C. Why do we need observational studies of everyday patients in the real-life setting? European Heart Journal Supplements 2015;**17**(suppl D):D2-D8.
50. MHRA. Drug Safety Notice. Spironolactone and renin angiotensin system drugs in heart failure: risk of potentially fatal hyperkalaemia. Available at https://www.gov.uk/drug-safety-

update/spironolactone-and-renin-angiotensin-system-drugs-in-heart-failure-risk-of-potentially-fatal-hyperkalaemia 2016.
51. Horisberger JD, Giebisch G. Potassium-sparing diuretics. Ren Physiol 1987;**10**(3-4):198-220.
52. Delyani JA. Mineralocorticoid receptor antagonists: the evolution of utility and pharmacology. Kidney international 2000;**57**(4):1408-1411.
53. Roush GC, Kaur R, Ernst ME. Diuretics: a review and update. Journal of cardiovascular pharmacology and therapeutics 2014;**19**(1):5-13.
54. Fujimura N, Noma K, Hata T, Soga J, Hidaka T, Idei N, Fujii Y, Mikami S, Maruhashi T, Iwamoto Y. Mineralocorticoid Receptor Blocker Eplerenone Improves Endothelial Function and Inhibits Rho-Associated Kinase Activity in Patients With Hypertension. Clinical Pharmacology & Therapeutics 2012;**91**(2):289-297.
55. Egger M, Juni P, Bartlett C, Holenstein F, Sterne J. How important are comprehensive literature searches and the assessment of trial quality in systematic reviews? Empirical study. Health Technol Assess 2003;**7**(1):1-76.
56. Bianchi S, Bigazzi R, Campese V. Long-term effects of spironolactone on proteinuria and kidney function in patients with chronic kidney disease. Kidney international 2006;**70**(12):2116-2123.
57. Valentine JC, Pigott TD, Rothstein HR. How many studies do you need? A primer on statistical power for meta-analysis. Journal of Educational and Behavioral Statistics 2010;**35**(2):215-247.
58. Evangelou E, Ioannidis JP, Patsopoulos NA. Uncertainty in heterogeneity estimates in meta-analyses. BMJ: British Medical Journal 2007;**335**(7626):914-916.
59. Rossignol P, Massy ZA, Azizi M, Bakris G, Ritz E, Covic A, Goldsmith D, Heine GH, Jager KJ, Kanbay M. The double challenge of resistant hypertension and chronic kidney disease. The Lancet 2015;**386**(10003):1588-1598.
60. The SPRINT Research Group. Systolic Blood Pressure Intervention Trial (SPRINT) - Protcol Version 4.0. Available from https://www.sprinttrial.org/public/Protocol_Current.pdf. 2012.

Figures

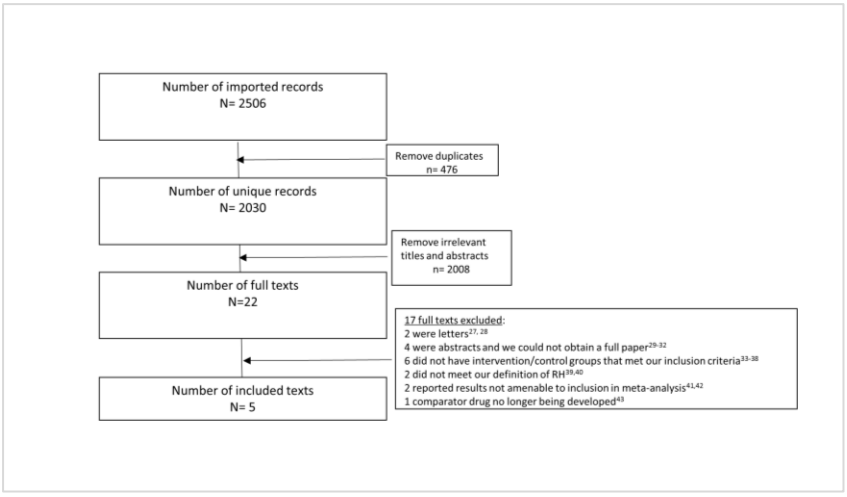

Figure 1: Flowchart of results

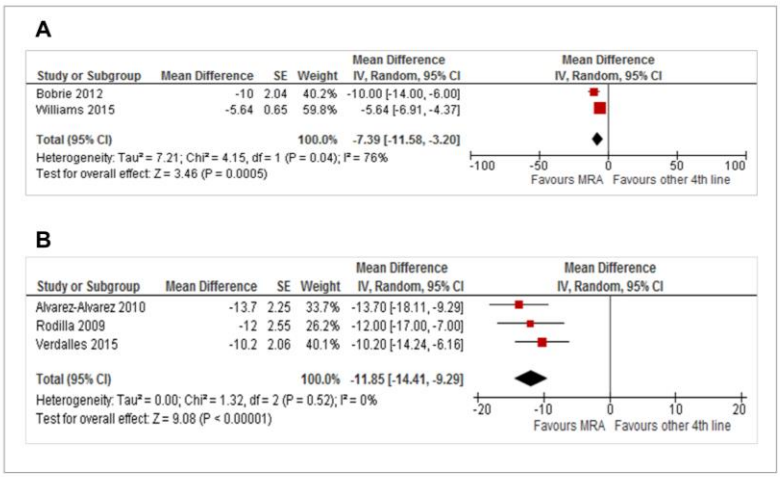

Figure 2A (upper panel): Meta-analysis of changes in systolic BP for randomised studies.

Figure 2B (lower panel): Meta-analysis of changes in systolic BP for non-randomised studies

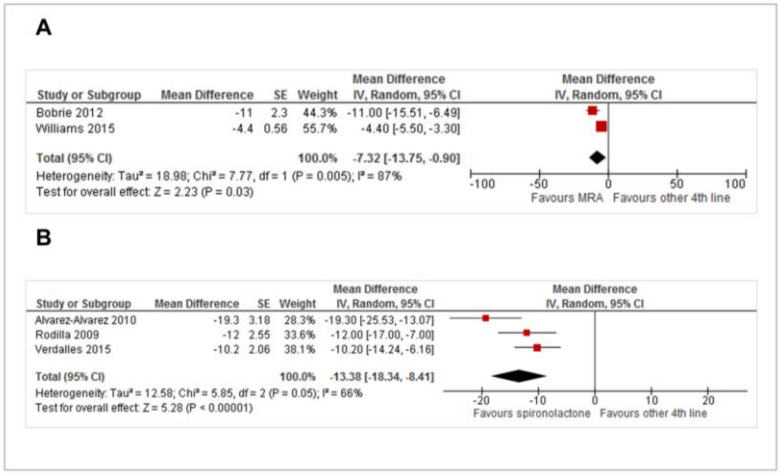

Figure 3: Meta-analysis of changes in systolic BP for non-randomised studies, using office BP measurements where reported

Tables

Table 1: Description of participants in included studies

	n	Mean Age	% Female	% Diabetes	Mean eGFR	% Smoking	Mean BMI	Mean no. of drugs	Baseline systolic BP	Outcome measurement 1	Outcome measurement 2
Randomised studies											
Bobrie 2012[23]	167	55.87	24.51	19.96	83.44^	51.91	28.36	3.00	146.00	24 hr ABPM	Office BP
Williams 2015[26]	335	61.40	31.00	41.00	91.00[#]	7.80	NR	NR	147.60	Home BP	Office BP
Non-randomised studies											
Alvarez-Alvarez 2010[8]	42	66.85	50	NR	83.08~	10.3	31.79	4.10	141.00	24 hr ABPM	Office BP
Rodilla 2009[24]	181	65.49	29.00	76.09	76.09^	9.41	32.45	NR	165.43	Office BP	Office BP
Verdalles 2015[25]	30	66.30	30.00	56.70	55.85*	NR	31.35	3.80	162.80	24 hr ABPM	NR
Total	755	61.65	30.1	45.64	83.92	18.51	30.68	3.29	151.76	~	~

eGFR – estimated Glomerular Filtration Rate, ABPM – Ambulatory Blood Pressure Monitoring, BMI – Body Mass Index, BP –Blood Pressure

^GFR calculated with MDRD equation, [#]GFR calculated with unknown method, *GFR calculated with CKD EPI equation ~Creatinine Clearance given

NR- not reported

Table 2: Description of included studies

Study	Study Design	Location	n	Intervention	Comparator	Assessment of white coat hypertension	Assessment of adherence prior to inclusion	Assessment of adherence during trial	Follow up
Randomised studies									
Bobrie 2012[23]	RCT	France	165	Nephron blockade: spironolactone 25mg, followed by furosemide 20mg/day, titrated to 40mg/day, followed by addition of amiloride.	Block of RAS: ramipril5mg/day, titrated to ramipril 10mg/day, followed by bisoprolol 5mg/day titrated to bisoprolol 10mg/day	Yes	No details	Yes - pill counts	12 weeks
Williams 2015[26]	RCT	UK	335	Spironolactone (25mg-50mg)	Bisoprolol (5 – 10mg) or doxazosin (4-8mg)	Yes	Yes - pill counts and directly observed therapy	Urinalysis	12 weeks
Non-randomised studies									
Alvarez-Alvarez 2010[8]	Prospective crossover	Spain	39	Spironolactone 25mg increased to 50mg	Addition of ACEI/ARB	Yes	No details	No details	12 weeks
Rodilla 2009[24]	Cohort study	Spain	181	Spironolactone 14mg (average)	Doxazosin 4mg (average)	Yes	Yes, but no details how	No details	3 months for spironolactone and 6 months for doxazosin
Verdalles 2015[25]	Cohort study	Spain	30	Spironolactone 25mg	Furosemide 40mg	Yes	Yes, but no details how	No details	6 months

RCT – randomised controlled study, RAS – renin-angiotensin system, RH – resistant hypertension, ABPM- ambulatory blood pressure monitoring. ACE – angiotensin converting enzyme, ACEI/ARB - angiotensin converting enzyme inhibitor/angiotensin receptor blocker

Table 3: Description of quality of included studies

	Internal Validity - Bias	Internal Validity - Confounding	External Validity	Adverse event reporting
Randomised studies				
Bobrie 2012[23]	6.5/8	6/10	2/2	1/1
Williams 2015[26]	8/8	8/10	1/2	1/1
Non-randomised studies				
Alvarez-Alvarez 2010[8]	5/8	4/10	0/2	1/1
Rodilla 2009[24]	3/8	3/10	0/2	1/1
Verdalles 2015[25]	5/8	4/10	0/2	1/1

Notes: *A detailed scoring sheet along with description of quality assessment form is included in* ***Supplementary Information 2.***

Table 4: Number of cases of hyperkalaemia and mean changes in serum potassium in patients treated with spironolactone and other 4th line agents

	Spironolactone		Other 4th line agents	
	Cases of hyperkalaemia	Mean change in serum potassium (SE)	Cases of hyperkalaemia	Mean change in serum potassium (SE)
Bobrie 2012[23]	3/85	0.30 (0.80)	0/82	0.00 (0.13)
Williams 2015[26]	6/285	0.42*	0/335	0.15^*/0.08#*
Subtotal events for randomised studies	**9/370**	~	**0/417**	~
Alvarez-Alvarez 2010[8]	1/39	0.53 (0.09)	0/39	0.09 (0.08)
Rodilla 2009[24]	NR	0.41 (0.05)	NR	0.11 (0.08)
Verdalles 2015[25]	2/15	NR	0/15	NR
Subtotal events for non-randomised studies	**3/54**	~	**0/54**	~
Total events	**12/424**	~	**0/471**	~

Notes: NR = not reported. Verdalles reported two cases of "mild" hyperkalaemia defined as serum potassium 5.0-5.5mmol/L. * *Variance for serum potassium changes not reported. ^Bisoprolol as comparator. #Doxazosin as comparator.

Supplementary Information 1

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title page
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3+4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	No, attempted to register at PROSPERO however, our work had begun so our protocol could not be included in PROSPERO
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5+6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5+6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5+6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5+6

Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6+7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1 and page 8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1 and page 9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 2 and page 10
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figures 2 and 3.
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Figures 2, and 3
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Appendix 3 and page 14.
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Figure 3 and Table 3, also pages 13 and 14.
DISCUSSION			

Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	15-18
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Pg 18
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Pg 18
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Pg 19

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Supplementary Information 2

Quality Assessment

Table S2: A detailed scoring across quality indicators as assessed using a modified Downs and Black quality assessment tool

	Internal Validity - Bias								Internal Validity - Confounding										External Validity		Misc - study quality	**Total**
Question #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**21**
Alvarez-Alvarez	0	0	1	1	1	1	0	1	1	1	1	0	0	0	1	0	0	0	0	0	1	10
Bobrie	0	1	1	1	1	1	0.5	1	1	0	1	1	1	1	0	1	0	0	1	1	1	15.5
Rodilla	0	0	1	0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	6
Verdalles	0	0	1	1	1	1	0	1	1	0	1	1	0	0	1	0	0	0	0	0	1	10
Williams	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	18

Supplementary Information 3

Publication bias

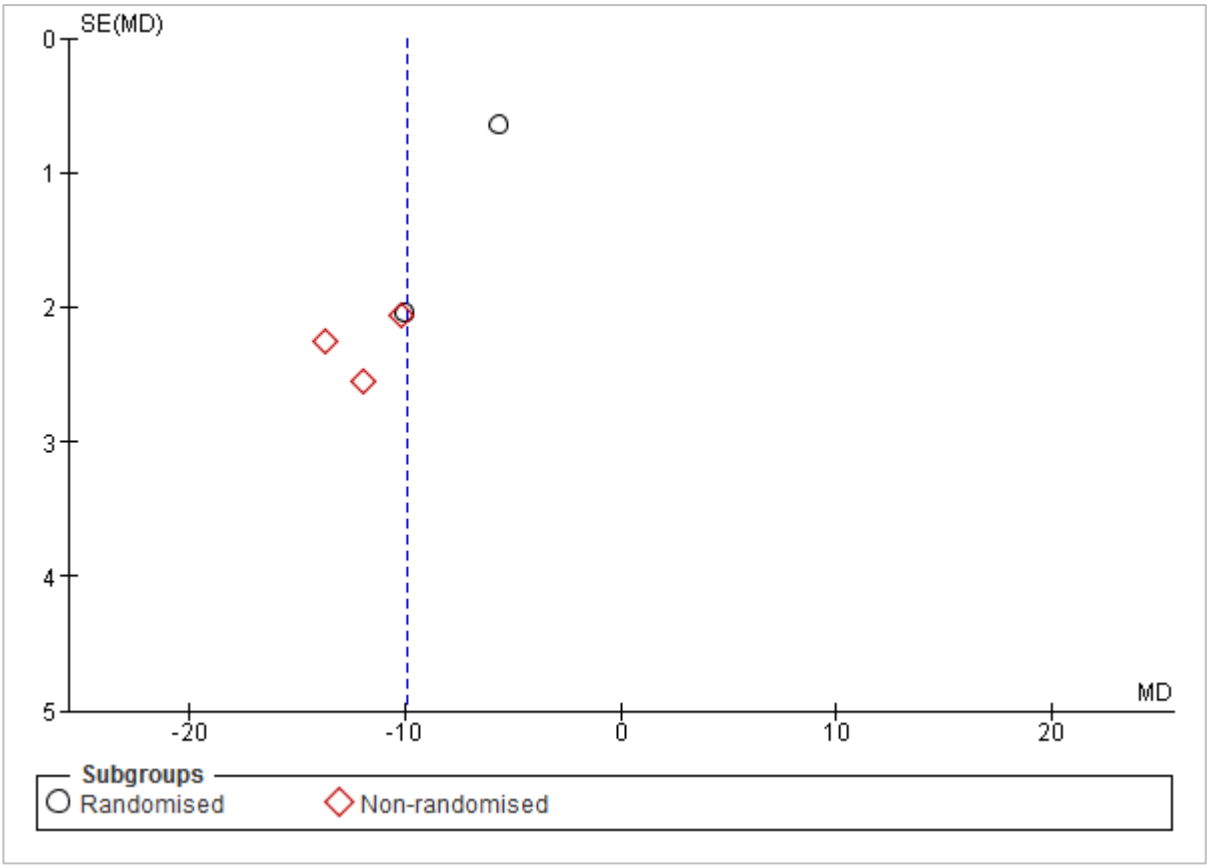

Figure S1: A funnel plot demonstrating the direction and size of effects in Randomised Studies and non-Randomised Studies.

- Largest study (Williams, n=335) is at the top of the graph, with a smaller effect size than the mean estimated effect.
- Note, all the NRS lie to the left of the mean effect estimate. This indicates that the effect of MRA is more beneficial in NRS than in RS.
- The likelihood of publication bias is small for two reasons.
    - First, the most commonly used MRA, spironolactone, is an off-patent medicine and investigators would have little financial incentive to not publish negative results. Second, the small study effects are likely due to poor methodological quality. Asymmetry in the graph is caused by the distribution of NRS. The methodological quality of all the NRS was quite low, as recorded in quality assessment forms.