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STUDY OF SEVERAL ISSUES RELATED TO HYPERTENSION THROUGH CLASSICAL AND MODERN META-ANALYTICAL METHODS

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SUMMARY

1 INTRODUCTION

Hypertension is a clinical condition characterized by high blood pressure (BP) affecting about a quarter of the adult population worldwide (8 million in Italy, 60 million in the USA, 1 billion people in the world) [1]. This condition is one of the leading cause of death in the Western countries due to its complications and it is one of the most important risk factor for cardiovascular (CV) diseases. Indeed, hypertension significantly increases the incidence of stroke, myocardial infarction, heart failure, atrial fibrillation, peripheral arterial disease, nephroangiosclerosis and diseases of the aorta. Hypertension is a global problem and the reduction of high blood pressure is an effective strategy to decrease the CV risk, but its success depends on the achievement of the target values defined by the guidelines [2]. In patients with prehypertension or hypertension, lifestyle modifications are recommended to achieve the blood pressure goals. These modifications include, among others, physical activity, weight loss and limited alcohol consumption. If the lifestyle modifications do not adequately reduce BP levels, drug therapy is necessary. First-line medications used in the treatment of hypertension include diuretics, angiotensin-converting enzyme inhibitors (ACEs) or angiotensin receptor blockers (ARBs), beta-blockers (BBs), and calcium channel blockers (CCBs). The diuretics help to increase the elimination of liquids through urine increasing the diuresis and helping the reduction of blood volume and its pressure inside the vessels and the elimination of sodium from the body. BBs act directly on the heart, blocking the action of the receptors beta of the sympathetic nervous system, reducing the force of the contraction and the cardiac output. In this way the blood is pumped with less energy in the circulatory system and blood pressure is reduced. The CCBs inhibit the accesss of calcium in the smooth muscles of the arteries obtaining the dilation of blood vessels which allows the reduction of pressure values and the heart rhythm. The renin-angiotensin system regulates, through different mechanisms, the blood pressure values, circulating plasma volume and the arterial musculature. The main active drugs acting on the renin-angiotensin system are ARBs and ACEs. ARBs operate directly on the angiotensin II

hormone blocking the receptors that bind this hormone reducing blood pressure. ACEs block the action of the angiotensin converting enzyme that promotes the formation of angiotensin II from angiotensin I. The block of this enzyme causes a dilation of the vascular walls and therefore a reduction of blood pressure. In the last years, several trials have been conducted in order to study the efficacy of these drugs in reducing the BP levels [2]. Two aspects concerning BP are under debate: (i) the quantification of the differences in BP reduction measured with different techniques, (ii) the estimation of the association between antihypertensive treatment (or specific classes) and the risk of several specific clinical endpoints considered as potential surrogates of CV events.

(i) In the clinical practice, blood pressure is measured considering: i) the peak pressure due to ventricular contraction during systole (systolic blood pressure SBP) and ii) the pressure during ventricular relaxation in diastole (diastolic blood pressure DBP). Both BP measurements have specific cut-off in order to define an hypertensive patient:140 mmHg (millimeters of mercury) for SBP and 90 mmHg for DBP [3]. Usually, BP is measured with at least one measurement (generally three) in the clinical setting by an experienced heath care professional (so-called Office or Clinic measurement). However this technique may not show the true blood pressure level due to several phenomenons. The most known problem is the white coat effect, that is the situation in which patients have a much higher blood pressure reading than real blood pressure value. This is thought to be due to the anxiety that they experience when encountering a health care professional [4]. For example, an individual with white coat hypertension may have true blood pressure values lower than 135/85 mmHg, but office values greater or equal to 140/90 mmHg [5]. Another phenomenon related to Office techinique is the masked hypertension. Masked hypertension is defined as a normal blood pressure in the clinic reading (<140/90 mmHg), observed in patients actually characterized by high blood pressure or hypertension. The most important causes of masked hypertension are: sedentary behaviour, unhealthy lifestyle like the addictions of alcohol, cardiovascular disease, kidney disease ecc that may cause fluctuations of BP level during the

day[2]. Other potential problems related to Office technique are: i) systematic bias due to the instrument used to measure BP (e.g. incorrect calibration of the instrument), ii) "digit preference" rounding off, most of the time, to one decimal place and iii) systematic bias related to the observer, based on the preconceived notion of what the pressure values should be in the subject that the doctor is visiting [6]. Since the early 1990, another technique to measure blood pressure was introduced: the 24-hour ambulatory blood pressure monitoring (ABPM). ABPM is a method in which the patient wears blood pressure equipment continuously for 24 hours. Readings are taken automatically throughout the day and night, generally every 15 minutes. The resulting BP level is obtained averaging the values of all readings. This method requires a follow-up of 24 hours and offers several advantages over the Office blood pressure measurements to study the efficacy of antihypertensive drugs. For example, unlike Office, ABPM is not modified by the 'white coat' effect. This means that, whatever blood pressure reduction is measured during treatment, it can be safely ascribed to the drug(s) used rather than to an attenuation of the initial white-coat response overtime. Finally, 24-h average blood pressure can be up to three times more reproducible than Office blood pressure values. The ABPM method has, however, some limitations: first of all, during the 24 hours some measurements may be missed due to technical problems of the instrument and secondly, it is a costly procedure compared to the Office technique [6]. In the latest years, many randomized clinical studies, aimed to evaluate the effects of antihypertensive drugs in reducing BP, used indifferently these techniques to identify patient BP levels, while the choice of which tool to use has relevant implications.

(ii) The effectiveness of antihypertensive treatment in the prevention of CV events can be evaluated only after a long follow-up period. In order to avoid long trials, it has been suggested to assess the treatment efficacy/effectiveness considering organ damages as surrogate endpoint. Once organ damage is detected, the CV risk is usually high. For example Patel et al. showed that the 10-year CV risk is 20% higher in patients with organ damage respect to patients without it [7]. The ESH

(European Society of Hypertension) and ESC (European Society of Cardiology) guidelines recommend the asymptomatic assessment of organ damage in the diagnostic control of hypertensive patients. Moreover, they recommend to locate all the damaged organs because the CV risk increases as more organs are involved [8]. So another goal when treating high blood pressure is to reduce the incidence of organ damage (consequently preventing CV events) and thus to decrease the incidence of premature death. The most frequent organ damages caused by hypertension regard: i) brain, ii) heart and iii) kidney.

The objectives of this thesis were to quantify, in patients treated with antihypertensive, the BP changes (i.e. the difference of the measure of BP between baseline and end of follow-up) measured with both Office and ABPM techniques and to investigate the association between antihypertensive use and risk of two important organ damages: dementia and left ventricular hypertrophy. These aims were addressed through a meta-analytic approach. Generally meta-analyses of randomized clinical trials (RCTs) are preferred to those including observational studies because the RCT design represent the "gold standard" to evaluate the efficacy of a therapy or an intervention intended to improve outcome. However, meta-analysis of observational studies could improve the conclusions based on RCTs, because this type of studies can be useful to provide information about the risk/benefit profile of a drug or to observe the effects of the drug in the clinical practice [9]. In this thesis both type of study design will be considered.

In particular, in the study on BP measurements, we performed a meta-analysis of RCTs. In this study a methodological problem about application of meta-analytic in presence of correlated effect estimates was faced. Specifically we treated this problem by means of the linear mixed models and building a general user friendly SAS macro. The studies on organ damage were based on observational studies and in one of these we dealt with the problem of the potential bias in summary estimates due to the presence of a unmeasured confounder.

The chapter 2 provides a description of the methods used while the chapter 3 shows the main results. Finally, in the last chapter another methodological issue related to a meta-analysis is described, that is the optimization of the process of identification of the papers to include in a meta-analysis. This process is usually very expensive in terms of time and the probability of missing some relevant articles may be not negligible. This problem was addressed by designing a prototype based on machine-learning method in collaboration with Dr. Mirko Cesarini, a researcher in informatics of Milano-Bicocca University.

2 METHODS

2.1 META-ANALYSIS

Meta-analysis is the part of the review process concerning the analysis of the data extracted from the primary research studies. The meta-analysis uses quantitative methods (i) to explore the heterogeneity of study results, (ii) to estimate overall measures of association or effect and (iii) to assess the sensitivity of the results to possible threats to validity such as publication bias and study quality. A strength of the meta-analysis is the increased study power and the precision of the estimates compared with those of the individual studies included. When the results of several studies regarding the same research question (i.e. the effectiveness of a treatment) are compared, it is possible to observe contrasting evidence, some studies may show results favoring the treatment while others no benefits. Meta-analyses, including all available studies on a specific issue, artificially increase the number of patients included and allow the identification of effects unobservable in small studies, leading to more precise estimate of the treatment effect. Indeed, this explains the relevance of using meta-analyses in order to study rare events and secondary outcomes. The meta-analysis can be useful to identify patient subgroups at high risk of adverse events or those patients with a best response to treatment. Meta-analyses can include studies carried out in different geographic areas and so may also produce more generalizable results [10]. Moreover, this kind of study: i) can help in the clinical decision making process, ii) be auxiliary in new study designing, iii) may show that in prior studies one outcome index had proven to be more sensitive than others, or even highlight the uselessness of additional studies for a specific treatment [10].

The measure of interest in meta-analyses is the summary estimate ($\overline{Y_{\cdot}}$) of the effect of interest that is basically the mean of the study- specific estimates (y_i) weighted for a function of the variability of each estimate (s^2_i). To combine the estimates from each study in order to yield an overall result are mainly used two methods: i) fixed effect model and ii) random effect model. The fixed effect model assumes that each estimate y_i is a realization from a unique population with mean θ and variance σ_i^2.

$$y_i \sim N(\theta, \sigma_i^2)$$

So a fixed-effect meta-analysis assumes that the effects of all studies aim to estimate the same true effect θ (homogeneity assumption). The most common method used to calculate the fixed effect summary estimate is the so called "inverse variance-weighted" method, where weights (w_i) are represented by the reciprocal of the variance. Assuming to perform a meta-analysis of *k* studies, the weights and the summary estimate are calculated respectively as in the equations below [10].

$$w_i = \frac{1}{s_i^2}$$

$$\bar{Y}_{\cdot} = \frac{\sum_{i=1}^{k} w_i y_i}{\sum_{i=1}^{k} w_i}$$

However the homogeneity assumption may be difficult to be satisfied in several meta-analysis and so, in such cases, the random effect models is preferable. The random effect model assumes that the effects of each study estimate a different true effect θ_i. Therefore, each estimate y_i is derived from a normal distribution with mean θ_i and variance σ_i^2 and each mean θ_i derives from a normal distribution with mean μ and variance τ^2.

$$y_i \sim N(\theta_i, \sigma_i^2)$$

$$\theta_i \sim N(\mu, \tau^2)$$

Where τ^2 is a parameter that quantifies the heterogeneity between study estimates and it is calculated as [11]:

$$\hat{\tau}^2 = min\left\{0, \frac{Q-(k-1)}{\sum_{i=1}^{k} w_i - \frac{\sum_{i=1}^{k} w_i^2}{\sum_{i=1}^{k} w_i}}\right\}$$

Q is the heterogeneity test statistic defined by the equation

$$Q = \sum_{i=1}^{k} w_i (y_i - \bar{Y}_{\cdot})^2$$

Q is approximately distributed as a χ^2 distribution on $k - 1$ degrees of freedom under the null hypthesis of homogeneity.

Once τ^2 is calculated, it is possible to estimate the new weights w_i^* for each studies and the summary estimate using DerSimonian and Laird method [12] as:

$$w_i^* = \frac{1}{[s_i^2 + \hat{\tau}^2]}$$

$$\bar{Y}_{\cdot} = \frac{\sum_{i=1}^{k} w_i^* y_i}{\sum_{i=1}^{k} w_i^*}$$

Moreover, in order to study the total variation across studies that is due to heterogeneity rather than chance, the I^2 statistic is calculated as:

$$I^2 = \frac{Q - k}{Q}$$

The I^2 is the proportion of between study variability due to heterogeneity raher than chance. Thi index varies from 0% to 100%, High values of I^2 indicate high between studies heterogeneity (usually presence of heterogeneity is considered with $I^2 \geq 50\%$) [13].

2.2 DEPENDENT MEASURES IN META-ANALYSIS

The methods previously explained are used in the "classic" meta-analysis where the included estimates are independent. However, in the conduction of meta-analysis, dependent measures are frequently encountered when: i) the association measures (i.e odds ratio (OR), relative risk (RR) or hazard ratio (HR)) have the same patient group as the reference (for example in studies evaluating dose-response relatinships); ii) several events are studied jointy; iii) the same outcome is measured with different techniques on the same patients. If in each i^{th} included study two continuous outcome measures (Y_{1i} and Y_{2i}) are considered, we assume that the response vector is distributed as a multivariate normal distribution which considers the correlation between outcomes as follows:

$$\begin{pmatrix} y_{1i} \\ y_{2i} \end{pmatrix} \sim N \begin{pmatrix} \theta_{1i} \\ , R_i \\ \theta_{2i} \end{pmatrix} \; where \; R_i = \begin{pmatrix} \sigma_{1i}^2 & \sigma_{1i}\sigma_{2i}\rho_{wi} \\ \sigma_{1i}\sigma_{2i}\rho_{wi} & \sigma_{2i}^2 \end{pmatrix}$$

and

$$\begin{pmatrix} \theta_{1i} \\ \\ \theta_{2i} \end{pmatrix} \sim N \begin{pmatrix} \mu_1 \\ , G \\ \mu_2 \end{pmatrix} \; where \; G = \begin{pmatrix} \tau_1^2 & \tau_1\tau_2\rho_b \\ \tau_1\tau_2\rho_b & \tau_2^2 \end{pmatrix}$$

The corresponding model is a bivariate model with random effects used to account for between study variability. R_i is the within study variance-covariance matrix while G is the between-studies variance-covariance matrix. σ_i^2 is the variance of the outcome Y_i , τ^2 is the between studies variance and ρ_{wi} and ρ_b are the correlations within and between studies respectively. Usually, R_i matrix is known while G is estimated by likelihood method. The extension of this method allows to maximize the restricted likelihood function trough the Newton-Raphson algorithm but the G matrix has to be defined non-negative [14]. A bivariate (or multivariate) model can be implemented using the Linear Mixed Models (LMMs). LMMs can include both fixed and random effects as independent variables. The fixed-effect parameters represents the association between the

covariates and dependent variable and they are unknown constant parameters associated with either continuous covariates or the levels of categorical variables. The random effect parameters are specific to cluster or subjects within a population.

The equation of LMM is the following:

$$Y_i = X_i\beta + Z_i u_i + \epsilon_i$$

Y_i represents the continuous response variable represented by the vector of dependent variable of the i-th unit (i ϵ 1….n). X_i is the matrix of the know values of the k covariates. The β is a vector of k unknown regression coefficients related to the k covariates. The Z_i matrix includes the known values of the q covariates included as random effect in the model and u_i is the vector of the correspoding q random effects of the q covariates in the Z_i matrix. Finally, ε_i is the vector of residuals [15]. We assume that the q random effect coefficients of the u_i vector follow a multivariate normal distribution with mean equal to 0 and a variance-covariance matrix D_i:

$$u_i \sim N(0, D_i)$$

In the D_i matrix, the variances of the random effects are on the main diagonal and the covariances between two random-effects on the off-diagonal elements. If we consider q random effects for the i-th unit, D_i is a q×q matrix symmetric and positive-definite.

$$D_i = Var(u_i) = \begin{pmatrix} Var(u_{1i}) & \cdots & cov(u_{1i}, u_{qi}) \\ \vdots & \ddots & \vdots \\ cov(u_{1i}, u_{qi}) & \cdots & Var(u_{qi}) \end{pmatrix}$$

In the LMM, the residuals associated with repeated observations can be correlated and then we assume that the residuals ε_i for i-th subject, are random variables that follow a multivariate normal distribution with a mean vector 0 and a positive-definite symmetric variance-covariance matrix Ri:

$$\varepsilon_i \sim N(0, R_i)$$

$$R_i = Var(\varepsilon_i) = \begin{pmatrix} Var(\varepsilon_{1i}) & \cdots & cov(\varepsilon_{1i}, \varepsilon_{ni}) \\ \vdots & \ddots & \vdots \\ cov(\varepsilon_{1i}, \varepsilon_{ni}) & \cdots & Var(\varepsilon_{ni}) \end{pmatrix}$$

Moreover, the LMM assumes that (i) the residuals of different units are independent and (ii) the vectors ε_i and u_i are independent [15].

Up to this point, we showed a LLM for a specific i-th subject. If we wanted a specification based on all subjects the equation is:

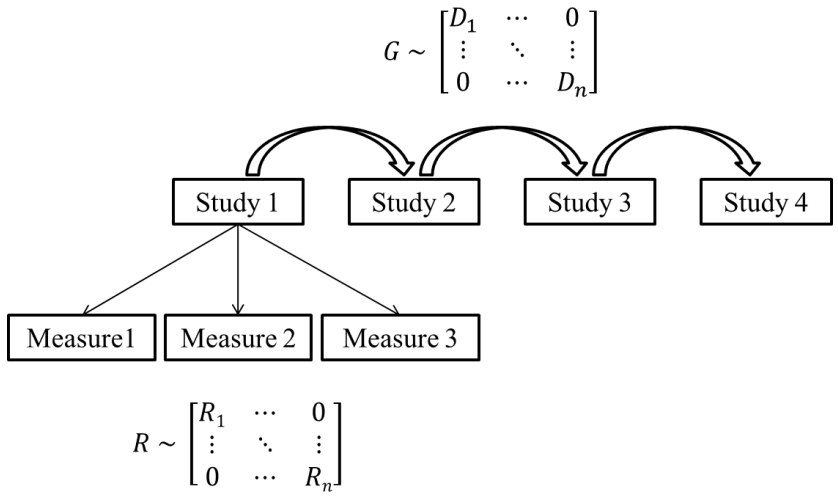
$$Y = X\beta + Z\mu + \varepsilon$$

$$\mu \sim N(0, G)$$

$$\varepsilon \sim N(0, R)$$

Vector Y represents the "vertical union" of all Y_i vectors (one vector for each unit). The matrix X (n×p) is the "vertical union" of all X_i matrices as well. The Z matrix is a block diagonal matrix where each block represents the Z_i matrices. Finally, u and ε are vectors that join all u_i and ε_i respectively vectors vertically. The G and R are block-diagonal matrices. In G matrix, the blocks on the main diagonal are represented by the D_i matrices while in R matrix the blocks on the main diagonal are represented by the R_i matrices. Indeed, the G matrix is the the variance-covariance matrix for random effects (n x n) and R matrix is the variance-covariance matrix for residuals (n x n). In a meta-analysis matrix G represents the variability between studies and the matrix R represents the variability within studies (Figure) [15].

Figure of the G and R matrices



2.3 UNMEASURED CONFOUNDER IN META-ANALYSIS

In contrast to RCT, the effect estimates obtained from observational studies are usually affected by confounding. A confounder is a factor related to both exposure and outcome that may influence the observed relationship between them. In the planning of observational studies, the researcher should identify all potential confounding factors and collect the related data to adjust the estimate of interest. However, some potential confounders could be unknown or not available leading to estimates affected by residual confounding [16]. As the results of observational studies could be prone to the effect of unmeasured confounder, the summary estimates of the meta-analyses based on observational studies should be, in turn, affected by this bias. It is therefore desirable to account for these confounders to obtain summary estimates as similar as possible to the meta-analytic estimates based on RCTs.

Several methods were developed in order to adjust the study estimates for the presence of unmeasured confounder, among them, the Monte Carlo Sensitivity Analysis (MCSA) is often used.

The basic concept of sensitivity analyses is to use external information (available from the literature or from other data sources) to quantify the effect of an unmeasured confounder on the exposure-outcome association estimates. Usually epidemiological studies aim to assess the association between a factor and the presence of disease (dichotomous variable). The measures of interest are the Relative Risk (RR), the Hazard Ratio (HR) or the Odds Ratio (OR) depending on the study design. Assuming that β_x^* is the biased estimate of the association between a binary exposure and a dichotomous outcome.To adjust this measure for a binary unmeasured confounder, it is necessary, firstly, to quantify the bias parameter. Lin et al. [17] proposed the following formulation of the bias parameter:

$$\Omega(RR\ \ , p_1, p_0) = \log \frac{RR\ \ * p_1 * (1 - p_1)}{RR\ \ * p_0 * (1 - p_0)}$$

Where RR is the association measure between unmeasured confounder and the outcome of interest , p0 is the prevalence of the unmeasured confounder among the unexposed subjects and p1 the prevalence of the unmeasured confounder among the exposed subjects. After the calculation of the bias factor, it is possible to obtain the estimate of the exposure-outcome association measure corrected for the unmeasured confounder β_x as $\beta_x^* - \Omega(RR_k, p_{1k}, p_{0k})$. MCSA consists in sampling *M* times the components of the bias factor directly from the prior distributions and then calculate i) the bias factor for each sample and ii) the adjusted estimates. For RR the prior distribution will be $logRR \sim N(\mu_{RR}, \tau_{RR}^2)$ while the prior distributions for p1 and p0 will be $logit(p_1) \sim N(\mu_{p1}, \tau_{p1}^2)$ and $logit(p_0) \sim N(\mu_{p0}, \tau_{p0}^2)$. Using the sensitivity analysis by Lin et al [17] and considering β_x^* as the biased estimate, SE(β_x^*) its standard error and *M* as the number of the simulations, the phases to adjust the estimate are:

(i) Sample bias parameters from the prior distributions;

(ii) Calculate the bias factor;

(iii) Calculate the adjusted estimate β_x as $\beta_x^* - \Omega(RR_k, p_{1k}, p_{0k})$;

(iv) Sample a value from the distribution $N\left(\beta_x\ ,\ SE^2(\beta_x^*)\right)$ to incorporate random error

This process is repeated *M* times and in this way it is possible to obtain the distribution of the adjusted β_x . The median of this distribution can be interpreted as the point estimate of the adjusted association for the unmeasured confounder while the 2.5th and 97.5th percentiles of the distribution are respectively the lower and upper limits of the confidence intervals for this estimate.

We decided to apply the MCSA in the field of the meta-analysis. Indeed, if the researcher apply the MCSA for each *i-th* study included in the meta-analysis can obtain *i* adjusted estimate and so he/she can calculate the adjusted summary estimate. Clearly for RR the prior distribution will be $logRR_i \sim N(\mu_{RR}, \tau_{RR}^2)$ for ($i \in$ 1:k). In this way strength of the association between the unmeasured confounder and outcome can vary from study to study, we consider the RR_i as exchangeable. Finally, the prior distributions for the components of the bias factor p_1 and p_0 will be

$logit(p_{1i}) \sim N(\mu_{p1}, \tau_{p1}^2)$ and $logit(p_{0i}) \sim N(\mu_{p0}, \tau_{p0}^2)$. Also in this situation if the standard deviation will be different to 0 the strength of the association between the unmeasured confounder and exposure will differ from study to study.

In 2012, McCandless proposed a Bayesian method to control the effect of unmeasured confounder in a meta-analysis of observational studies [18] when the unmeasured confounder is dichotomous. This approach assumes that each effect estimate β_{xi}^* derived from the individual studies included in the meta-analysis (β_{xi}^* represents logRR or logHR or logRR) has been adjusted for a set of covariates measured and available in the *i-th* study and that y_i follows a Normal distribution:

$$\beta_{xi}^* \sim N(\theta_i^*, \sigma_i^2)$$

The mean θ_i^* is the logarithm of the association measure provided by the *i-th* study of the relationship between a dichotomous exposure and dichotomous outcome and σ_i^2 is the related known variance. This method exploits the algebraic adjustment formula described by Lin et al [17] that demonstrated the algebraic relationship between the parameter θ_i estimated in the full model (including the unmeasured confounders as adjustment) and θ_i^* from the model without the unmeasured confounder. Lin shows that

$$\theta_i^* \approx \theta_i + \Omega(RR_i, p_{0i}, p_{1i}) \quad for\ i \in 1{:}\,k$$

where

$$\Omega(RR_i, p_{1i}, p_{0i}) = \log \frac{RR_i * p_{1i} * (1 - p_{1i})}{RR_i * p_{0i} * (1 - p_{0i})}$$

Where RR_i, p_{0i}, and p_{1i} are previous explained. Using the bias model, the equation $\beta_{xi}^* \sim N(\theta_i^*, \sigma_i^2)$ becomes:

$$\beta_{xi} \sim N(\theta_i + \Omega(RR_i, p_{1i}, p_{0i}), \sigma_i^2)$$

Where σ_i^2 is known. Lin et al demonstrated that, when the magnitude of the bias factor is noted, the adjustment for the unmeasured confounder in an observational study lead to a relocation of the point estimate but the corresponding standard error will remain unchanged. However, this is singular because it is known that covariate adjustment modify the precision of the estimates [19]. From an operative point of view, priors distribution are assigned to each parameter so that θ and (*RR*, p_1, p_0) are marginally independent. The prior distributions will be assigned to $\theta_1..\theta_k$: $\theta_i \sim N(\mu, \tau)$ where μ is the mean of the distribution of exposure effects and τ is the standard deviation (for the hyperparameters μ τ will be assigned these distributions: $\mu \sim N(0, 10^3)$ and $\tau \sim Uniform(0, 10^3)$). For *RR*, p_1 and p_0 the priors are the same used in MCSA. According to Bayes theorem, the posterior distribution is calculated as the likelihood function multiplied by the prior distribution. In this case given the bias parameters (RR_i, p_{1i}, p_{0i}) and data (y_i, σ_i) the posterior distribution of θ_i is a multivariate normal with diagonal covariance matrix:

$$\theta_i | RR_i, p_{1i}, p_{0i}, y_i, \sigma_i, \mu, \tau^2 \sim N\left(\frac{\tau^2\{y_i - \Omega(RR_i, p_{1i}, p_{0i})\} + \sigma_k^2\mu}{\sigma_i^2 + \tau^2}, \frac{\sigma_i^2\tau^2}{\sigma_i^2 + \tau^2}\right)$$

where the mean is a weighted average of the prior mean of θ_i (which is μ) and the bias-corrected exposure effect estimate, which is $y_i - \Omega(RR_i, p_{1i}, p_{0i})$. Markov chain Monte Carlo (MCMC) will be used to sample from the posterior distribution and the vectors *RR*, p_1, p_0 will update using a random walk Metropolis Hastings step a multivariate normal with mean zero and covariance matrix equal to identity matrix multiplying by a tuning parameters. The tuning parameter is adjusted using MCMC simulation runs to ensure satisfactory convergence. In the section 3.4 we made a simulation to compare the performance of Bayes and MCSA methods.

In order to compare the Bayes and MCSA method we retrieved a data simulation carried out by Mccandless [18]. He simulated the data of 6 studies (*i*), in each study were included 5000 subjects (*j*). He considered Y_{ij} and X_{ij} two dichotomous variables. Y_{ij} is the outcome and X_{ij} is the exposure

variable. Moreover, he built U_{ij} that represents the dichotomous unmeasured confounder. So he generated data using the following distributions:

$$Y_{ij}|X_{ij,}U_{ij} \sim Bernoulli\{exp(-4 + \theta_i X_{ij} + logRR_i U_{ij})\}$$

$$U_{ij}|X_{ij} = 1 \sim Bernoulli\{p_{i1}\}$$

$$U_{ij}|X_{ij} = 0 \sim Bernoulli\{p_{i1}\}$$

$$X_{ij} \sim Bernoulli\{0.5\}$$

He decided to put y-intercept equal to -4 ensures that outcome is rare. In order to simulate the data he needed four parameters for each study:

$$\theta_i \sim N(0,0.25)$$

$$logRR_i \sim N(1,0.25)$$

$$logitp_{0i} = 0$$

$$logitp_{1i} \sim N(1,0.25)$$

Therefore, he could generate six databases (one for each study) and he analysed each dataset using the log linear model $log[P(Y_{ij} = 1|X_{ij})] = \alpha^* + \theta_{ij}^* X_{ij}$ and he obtained 6 $\beta_1 \ldots \beta_6$ log relative risk estimates with of $\theta_1^* \ldots . \theta_6^*$ where the $\beta_1 \ldots \beta_6$ are unadjusted estimates for confounder U. Finally, he calculated, for each study the adjusted estimate including in the model the confounder U (Gold Standard) and the adjusted estimate trough Bayes method. He repeated this simulation 100 times and he calculated the coverage probability of 95% interval estimates for each method. We performed the same simulations and we calculated the coverage probability for MCSA method (see table below). The table below shows the results of the MCSA and the Bayesian Methods:

Study-specific effects for the 6 studies in the meta-analysis

	θ_1	θ_2	θ_3	θ_4	θ_5	θ_6
Gold standard	95%	96%	96%	95%	95%	96%
Bayes	95%	96%	95%	95%	96%	95%
MCSA	95%	93%	95%	91%	89%	95%

The MCSA and Bayes seem very similar and so both methods could be used to adjustment for unmeasured confounder. Moreover, we made a macro in SAS that allows calculating estimates adjusted for the presence of the unmeasured confounder through MCSA method. To calculate the unbiased estimates through bayes methods we used the R program provided bu McCandless (available contacting the author).

3 RESULTS

3.1 OFFICE AND AMBULATORY BLOOD PRESSURE TO ASSESS THE CHANGE OF BLOOD PRESSURE: A META-ANALYSIS

Introduction

24 hours ambulatory monitoring (ABPM) and Office are the main settings in which to measure blood pressure (BP). Traditionally, management of hypertension has been guided by Office BP measurements, but after the introduction of the ABPM (assessment of BP values during regular everyday life), there has been growing interest for the later technique, and some, though at all, guidelines recommend the use of a time-restricted ABPM before starting treatment [20]. Despite the added information provided and avoidance or reduction of the "white coat effect" [21], the use of ABPM technique is still restricted because of some limitations, which include limited availability and cost; the provision of intermittent measurements during which the patient is immobile rather than 'ambulatory'; the possibility of inaccurate readings during activity and the difficulty to detect artefactual measurements. Randomized clinical studies aimed at evaluating the effects of antihypertensive drugs in reducing blood pressure have used indifferently these techniques. The choice of the technique to be used has relevant implications because greater BP changes (treatment-end minus baseline) when using Office rather than ABPM (Office changes [OC] larger than Ambulatory changes [AC]) were reported by a previous meta-analysis [22] based on 44 studies.

The primary aims of this study were to quantify the summary estimates of the difference between OC and AC collected in randomized clinical trials and to investigate the conditions and mechanisms influencing this difference.

Materials and methods

We carried out a MEDLINE search of the literature to identify randomized clinical trials (RCT) published up to 31st December 2016 reporting the change of blood pressure (BP) measured with both Office and 24-h techniques in patients treated with antihypertensive drugs. We considered only RCT to assure high standard of BP measurement quality. The keywords and/or corresponding MeSH terms to carry out the search are reported in the Appendix A. In addition, the reference lists of reviews and meta-analyses published on this issue were hand-checked to identify additional relevant publications [22]. The present study was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analyses) statement [23]. Studies were considered if (a) at least an arm was constituted by patients treated with any antihypertensive drug belonging to the following classes (angiotensin-converting-enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs); beta blockers (BBs), calcium-channel blockers (CCBs) and diuretics); (b) they reported the mean change of BP from randomization to treatment end with both Office and by 24-h measurements in the same patients; (c) they reported variability measures of the BP change (standard deviation, standard error or 95% confidence interval) or sufficient data to allow their calculation. If the data of interest were available only from a graph, a software for digitizing graphs was used to extract it (GetData Graph Digitizer Version 2.26.0.20). When data were published more than once, the most recent and complete publication was considered. Two readers (Soranna D. and Zambon A.) independently determined the eligibility of each article for inclusion. Discrepancies between readers were resolved in conference. For each included study/arm, we extracted data on first author's name, country, publication year, length of follow-up, sample size, mean age and proportion of male patients, class of antihypertensive drug or placebo, systolic and diastolic BP mean (SBP and DBP) at baseline measured with both techniques, mean change (treatment end minus baseline) and its standard error. The summary Δ (OC-AC) was the main measure of interest. Because the OC and AC are measured on the same patients the two changes are correlated and so we calculated the summary estimates Δ (OC-AC) using Linear mixed models (section 2.2) where

the value of the correlation coefficient (ρ), between the treatment-induced changes in Office BP and in ABPM, has been identified from the literature [24]. To take into account the random uncertainty about this parameter we built the 95% confidence interval (95% CI) by means the Fisher transformation method. The lower limit of this interval was considered to be more conservative. If the linear mixed model could not converge then we calculated the summary estimates building for each estimate Δ (OC-AC) and its standard error using the following formula: SE= square root $[(SD_{Office})^2 + (SD_{ABPM})^2 – (2*\rho* SD_{Office}* SD_{ABPM})]$/square root [number of patients]. We pooled the OC and AC estimates by using both the fixed and random effects models proposed by DerSimonian and Laird [12]. Between-study heterogeneity was tested by Cochrane's Q test and quantified with the I^2 statistics (the proportion of between-study variability caused by heterogeneity) [13]. When a significant heterogeneity was found, the results from the random-effects model were used. Concerning all BP change measures, between-study sources of heterogeneity were investigated by stratifying original estimates according to: type of antihypertensive treatment (monotherapy versus combination therapy), antihypertensive class (ACEIs, ARBs, BBs, CCBs, Diuretics), geographic area in which the study was performed (Europe, Asia, North America, South America or Australia), length of follow-up, average age of patients included in the trial and average Office BP at baseline. A Cochrane's Q test was computed for each subgroup difference. An influence analysis was carried out, for each BP change measure, by omitting one study at a time, to identify to what extent the results were influenced by a single study. Finally, publication bias was evaluated through funnel plot analysis and the Egger's test [25].

The relationship between baseline Office BP and Δ (OC-AC) was explored considering a flexible regression modelling approach based on first-order and second-order fractional polynomials:

$$y = x^p + x^q$$

where y represent the Δ (OC-AC), x represent the baseline BP Office and p and q are power selected in the set (-2, -1, -0.5, 0, 0.5, 1, 2 where x^0 denotes *logx*). This method allows expression of

the relationship between two continuous variables in a flexible way by selecting the best functional form from a predetermined set of linear and nonlinear relationships. The best functional form was then selected on the basis of the AIC (Akaike Information Criterion) preferring the most parsimonious among all those with an average AIC within 2 units of the lowest [26].

For all hypothesis tests, evidence was based on a p-value < 0.05, and the 95% CIs were therefore presented. The corresponding calculations and graphical visualizations of forest and funnel plots were respectively carried out using RevMan version 5.1 (Nordic Cochrane Center) and STATA Software Program version 9 (STATA, College Station, TX). Summary estimates of dependent measures and flexible modelling was carried out using Statistical Analysis System Software (version 9.4; SAS Institute, Cary, NC, USA).

Results

Figure 1 shows the flow diagram for the studies inclusion. On the basis of title and abstract, we identified 2,415 papers. We excluded 1,800 of them because they were not related to the study objective according to title and/or abstract. The remaining 615 articles were considered of interest, and their full text was retrieved for detailed evaluation. Of these, 218 studies were not randomized control trial or the patients were not treated with antihypertensive drugs; 332 studies did not report all the data required for both Office and 24-h values or they did not report complete data; 13 articles did not analyze original data. Finally, 52 articles [27-78] complied with the inclusion criteria and were considered for meta-analysis.

Characteristics of the studies

Overall, we analyzed data from 9,500 patients in 52 studies, with sample sizes varying from 13 to 1,523 patients with a median follow-up of 12 weeks and an inter-quantile range of 8 – 24, and providing 118 estimates of SBP changes and 115 DBP changes. More than the half of these articles (35) comprised patients from Europe, 6 from North America, 5 from South America, 5 from Asia and one from Australia. In all studies, the initial ABPM SBP and DBP means were markedly lower

than the initial Office SBP and DBP means (SBP 141.63 mmHg vs 156.91 mmHg; DBP 85.42 mmHg vs 94.40 mmHg). Tables 1- 3 show the main characteristics of the studies included.

Summary estimates of systolic blood pressure changes

Figure 2 shows summary estimates of all SBP change measures (OC, AC and Δ (OC-AC)) for different strata. In patients treated with any type of antihypertensive treatment the SBP change measured by ABPM was significantly smaller than that measured by Office (Δ (OC-AC): -6.54 mmHg; 95% IC: -7.54 to -5.55). In the 6 placebo treatment arms we estimated a summary Δ (OC-AC) of -3.22 mmHg (95% IC: -6.81 to 0.36), which, probably because of the small sample size, fell short of statistical significance. In the 6 placebo controlled studies, the summary Δ (OC-AC) controlled for the placebo-effect was reduced to -1.97 mmHg (95% CI -4.34 to 0.40).

The Δ (OC-AC) measured in patients treated with a combination therapy and the one measured in patients in monotherapy were similar (-6.22 mmHg; 95% CI -7.83 to -4.62 vs -6.74 mmHg; 95% CI -7.91 to -5.57; P-value = 0.61). The Δ (OC-AC)s relative to different antihypertensive treatment in monotherapy were different (P-value=0.04); the greatest Δ (OC-AC) was observed for ACEIs (-9.24 mmHg; 95% IC: -11.71 to -6.77) and the lowest one for CCBs (-3.31 mmHg; 95% IC: -4.54 to -2.08). Similar Δ (OC-AC) values were also estimated for duration of follow-up (P-value=0.99). Figure 3 shows no differences on Δ (OC-AC) were found in different geographic area (P-value=0.77), while there was an increment of Δ (OC-AC) with increased age (P-value=0.03), and at Δ (OC-AC) higher levels of SBP at baseline (> 160 mmHg) (P-value=0.03). Detailed data for each study included are summarized in Table 2.

In several strata a high between-study heterogeneity was found (I^2 statistic ≥ 80%). Influence analysis failed to identify specific influent study (data not show) for all three main endpoints (Δ (OC-AC), OC and AC).

The visualization of the funnel plot (Figure 4A, 4C) and the corresponding Egger's test suggested evidence of publication bias only for AC (p-value = 0.003); studies reporting a strong change were more likely published (Figure 4B).

Summary estimates of diastolic blood pressure changes

Figure 5 shows a Δ (OC-AC) of -3.31 mmHg (95% CI, -3.89 to -2.72) in patients treated with any antihypertensive drugs. In the 6 studies providing placebo data a Δ (OC-AC) of -1.54 mmHg (95% CI -2.99, -0.09) was found, when the Δ (OC-AC) was adjusted for the placebo-effect it was reduced to a no significant values. The Δ (OC-AC)s in patients with combination therapy and in monotherapy were not statistically different (P-value = 0.68). Similar differences among antihypertensive drug classes were showed in Figure 4 with the highest Δ (OC-AC) for ACEIs users (-4.90 mmHg (95% CI: 6.86 to -2.95). No differences found for the follow-up duration. Concerning i) geographic area, ii) age classes and iii) baseline DBP levels, Figure 6 shows that the Δ (OC-AC)s were not statistically significant different among strata. Influence analysis showed that no study influenced summary changes (data not show). There was no evidence of publication bias for Δ (OC-AC) and OC from the visualization of the funnel plot (Figure 7A, 7C) and Egger's test (P-value = 0.640, P-value=0.739 respectively) but only for AC (Egger test p-value = 0.014) (Supplement Figure 7B). Figure 8 shows the functional relationship between the Δ (OC-AC) and the BP Office at baseline (SBP in Panel A and DBP in Panel B) estimated by the better fractional polynomials (linear model). A significant relationship was observed only for SBP with an increment of 0.19 Δ (OC-AC) mmHg for an increment of 1 mmHg of SBP Office.

Discussion

We performed a meta-analysis of randomized clinical studies of antihypertensive treatments to quantify the summary differences of SBP and DBP changes measured with Office and ABPM.

Our analysis, based on 52 studies and 108 treatments in about 9,500 patients, showed an average Δ (OC-AC) for SBP of -6.54 mmHg (95% CI -7.54 to -5.55 mmHg) and for DBP of -3.31 mmHg (95% CI -3.89 to -2.71 mmHg. The changes in Office SBP (-18.54 mmHg 95% CI -19.70 to -17.38) and in ABPM changes (-11.90 mmHg 95% CI -12.77 to -11.03) were very similar with those showed in the previous meta-analysis [22] (Office: -19.00 mmHg 95% CI -21.78 to -16.23 and ABPM: -12.00 mmHg 95% CI -13.96 to -10.04) as to DBP, in our meta-analysis, we calculated the reduction equal to -10.69 mmHg (95% CI -11.37 to -10.01) with Office and -7.22 mmHg (95% CI -7.78 to -6.66) with ABPM; the previous meta-analysis displayed OC equal to -13.00 mmHg (-14.96 to -11.04) and AC equal to -8.00 mmHg (-9.96 to -6.04) [22]

Moreover, considering separately the changes in Office SBP and ABPM SBP the summary change in ABPM was about 36% lower than the summary change in the Office BP. As for DBP we calculated a reduction of 33%. Both these results were similar to those shown in the previous meta-analysis (36.5% for SBP and 36.8% for DBP) [22].

Most of the studies included in the present meta-analysis did not consider a placebo group so we do not know how much of the Office BP and ABPM responses to treatment resulted from the placebo effect, i.e., a BP reduction because of the feeling of being treated, rather than from the effect of the drugs. We have tried to obtain some information from the six studies that included a placebo group. These studies indicate that there was a small SBP and DBP decrease from baseline in the placebo group also with Ambulatory BP measurement, but the decrease with Office BP measurement was greater. This suggests that Δ (OC-AC) observed both with placebo and with treatment results from mechanisms different from a real placebo effect, and which is present only, or particularly, with Office BP.

Two mechanisms can be considered for explaining Δ (OC-AC). First, the presence of a white-coat effect. The usually higher values of Office than ABPM measurements are often explained as resulting from the white coat effect, because of the demonstration that the presence of a doctor measuring BP is associated with a significant BP increase [79]. Second, the regression to the mean phenomenon (whereby any extreme measurement tends to become closer to the mean value when re-measured) which should affect mainly the BP Office changes because the individuals included in the studies were enrolled having been defined as hypertensive on the Office rather than Ambulatory BP measurements and this make Office measurements more prone to regression to the mean than Ambulatory measurements. Indeed, our findings that a greater Δ (OC-AC) occurred with higher baseline Office SBP values and at an age older 60 years (also associated with higher SBP values) support the interpretation that regression to the mean accounts for at least a large part of the Δ (OC-AC) we have described. Regression to the mean is further suggested by the linear functional relationship we found between SBP Δ (OC-AC) and baseline SBP Office values. The increment we observed of 0.19 mmHg in Δ (OC-AC) for an increment of one mmHg in baseline BP Office is consistent with that reported in the PAMELA study [80]. This paper had some strengths. Specifically we included only: i) intervention randomized clinical trials on antihypertensive drugs to augment homogeneity among published estimates; ii) trials reporting changes of BP measured with both Office and ABPM techniques in the same patients, to reduce confounding effect in interpreting the Δ (OC-AC)s. Nevertheless, our results were affected by high heterogeneity of OC, AC and Δ (OC-AC) trial-estimates and caution is needed to interpret them. We have investigated some available sources of this heterogeneity but the I^2 index remained high in each strata. Probably, other sources, not measured or not reported, were responsible for this heterogeneity, for example: different precision in Office BP measurements, different operators (physician, nurse, health assistant) measuring Office BP, different instruments being use, patients with different characteristic being enrolled, different type of blindness etc.

In conclusion, our analyses call attention on the significant differences between the responses to BP-lowering treatment when these responses are assessed by BP measurements in the office or by 24-h ambulatory BP monitoring. The summary estimates based on the present meta-analysis show differences both for SBP and DBP, amount to several mmHg, which should be taken into account when evaluating the results of individual BP lowering studies in which either BP measuring methodologies has been used. We have also showed that, this difference seems, although a small placebo effect also occurs in ABPM, largely due to the regression to the mean phenomenon, though a white-coat component cannot be excluded.

Table 1. Chronological summary of literature on reduction blood pressure due to pharmacological antihypertensive treatment, and their main characteristics.

First author, publication year, country (project)	Sample Size	Follow-up (weeks)	Male gender (%)	Age *Mean*	Antihypertensive drug	Strata	BP type
Grandi, 1995, Italy [27]	18	24	50	45	Isradipine		SBP/DBP
	18	24	50	44	Perindopril		
Lacourciere, 1995, Canada [28]	21	32	57	69	Amlodipine + Hydrochlorotiazide		SBP/DBP
	21	32	62	57	Hydrochlorotiazide + Amlodipine		
Staessen, 1996, Europe and Israel (SYST-EUR) [29]	168	52	NA	NA	Antihypertensive treatments		SBP/DBP
	169	52	NA	NA	Placebo		
Fagard, 1997, Belgium [30]	48	24	NA	44	Antihypertensive treatments		SBP/DBP
Mancia, 1997, Italy (Veratran study) [31]	51	8	NA	NA	Placebo		SBP/DBP
	56	8	NA	NA	Verapamil		
	50	8	NA	NA	Trandolapril		
	77	8	NA	NA	Verapamil + Tranodlapril		
Vaisse, 1997, France [32]	30	8	65	53	Lisinopril	High ABP	SBP/DBP
	18	8	29	50	Lisinopril	Low ABP	
	30	8	45	56	Bisoprolol	High ABP	
	18	8	42	51	Bisoprolol	Low ABP	
Neutel, 1998, USA [33]	132	10	67	52	Tasosartan		SBP/DBP
	130	10	71	53	Placebo		
Asmar, 2000, France (CHAMP) [34]	115	8	63	55	Candersartan		SBP/DBP
	115	8	63	55	Losartan		
Myers, 2000, Europe and Canada [35]	65	8	62	55	Perindopril + Indapamide	p2 + i0.625	SBP/DBP
	65	8	54	54	Perindopril + Indapamide	p2 + i1.25	
	61	8	57	54	Perindopril + Indapamide	p4 + i1.25	
	64	8	63	57	Perindopril + Indapamide	p8 + i1.25	
	62	8	55	55	Perindopril +	p8 + i2.5	

					Indapamide		
	60	8	55	56	Indapamide	i1.25	
	61	8	52	56	Placebo		
Mancia, 2001, Italy (INSIGHT) [36]	41	208	61	62	Nifedipine		SBP/DBP
	37	208	54	64	Diuretici		
de la Sierra, 2004, Spain [37]	45	4	64	57	Enalaprin + Nitrendipine		SBP/DBP
	49	4	49	57	Losartan + Hydrochlorotiazide		
Eguchi, 2004, Japan[38]	38	18	37	66	Valsartan		SBP/DBP
	38	18	37	66	Amlodipine		
Staessen, 2004, Belgium and Ireland (THOP) [39]	203	52	47	54	Antihypertensive treatments	Group 1	SBP/DBP
	197	52	48	53	Antihypertensive treatments	Group 2	
Zanchetti, 2004, Italy (PHYLLIS) [40]	127	12	40.9	58.4	Hydrochlorotiazide		SBP/DBP
	127	12	37	58.2	Fosinopril		
	126	12	40.2	58.2	Hydrochlorotiazide+Pravastatin		
	126	12	42.9	58.7	Fosinopril+ Pravastatin		
Anichkov, 2005, Russia [41]	27	12	NA	54	Lisinopril		SBP/DBP
	24	12	NA	53	Rilmenidine		
Fogari, 2005, Italy (PROBE) [42]	62	12	49	58	Telmisartan + Hydrochlorotiazide		SBP/DBP
	62	12	47	59	Nifedipine		
Ernst, 2006, USA [43]	16	8	56	49	Hydrochlorotiazide		SBP/DBP
Asmar, 2007, Europe, (PICXEL) [44]	62	36	45	56	Enalaprin		SBP/DBP
	65	36	39	55	Perindropil + Indapamide		
Mancia, 2007, Europe (ELSA) [45]	1523	208	55	56	Antihypertensive treatments		SBP/DBP
Ribeiro,2007, South America [46]	94	12	42	52	Amlodipine		SBP/DBP
	92	12	46	55	Losartan		
Ferguson, 2008, Australia [47]	25	8	NA	62	Fosinopril + Hydrochlorotiazide		SBP/DBP
	25	8	NA	62	Amlodipine		
	25	8	NA	62	Indapamide		

Guerrero, 2008, Brazil [48]	39	12	26	58	Hydrochlorithiazide + Amiloride		SBP/DBP
	43	12	35	57	Hydrochlorithiazide + Enalapril		
Miranda, 2008, Brazil (ATAR) [49]	117	12	37	59	Amlodipine		SBP/DBP
	105	12	44	58	Amlodipine + Ramipril		
Suonsyrja, 2008, Finland (GENRES) [50]	208	4	100	51	Amlodipine		SBP/DBP
	208	4	100	51	Bisoprol		
	208	4	100	51	Hydrochlorotiazide		
	208	4	100	51	Losartan		
Ambrosioni, 2010, Italy (INSIST) [51]	47	12	60	67	Eprosartan + Hydrochlorithiazide		SBP
	45	12	58	67	Losartan + Hydrochlorithiazide		
Andreadis, 2010, Greece [52]	83	12	NA	55	ARB	low dose	SBP/DBP
	69	12	NA	56	CCB	low dose	
Parati, 2010, Italy [53]	28	12	19	55	Barnidipine+ Losartan		SBP/DBP
	25	12	12	54	Losartan		
Wright, 2010, Canada and USA (EVALUATE) [54]	223	10	60	60	Valsartan + Hydrochlorithiazide		SBP/DBP
	225	10	66	59	Amlodipine + Hydrochlorithiazide		
Giles, 2011, USA [55]	41	12	55	53	Amlodipine+ Valsartan+ Hydrochlorothiazide	Intensive treatment	SBP/DBP
	34	12	39	57	Amlodipine+ Valsartan+ Hydrochlorothiazide	Moderate treatment	
Kostka-Jeziorny, 2011, Poland [56]	35	8	60	45	Perindopril		SBP/DBP
	31	8	53	47	Hydrochlorithiazide		
Mancia, 2011, Italy and Spain (TALENT) [57]	74	24	61	59	Telmisartan		SBP/DBP
	164	24	70	58	Nifedipine + Telmisartan		
	89	24	72	59	Nifedipine		
Muiesan, 2011, Italy [58]	20	24	75	50	Barnidipine		SBP/DBP

Raij, 2011, USA (VITAE) [59]	61	16	51	58.5	Valsartan+ Hydrochlorithiazide		SBP/DBP
	50	16	40	56.1	Hydrochlorithiazide+A mlodipine		
Zamboli, 2011, Italy [60]	20	52	60	73.5	Antihypertensive treatment NO diuretics		SBP/DBP
	20	52	50	73.6	Antihypertensive treatment and Furosemide		
Krzenski, 2012, Poland [61]	34	12	75	45	Antihypertensive treatment		SBP/DBP
	41	12	64	46	Antihypertensive treatment		
Lee, 2012, Korea [62]	14	4	56.3	55.1	Firmasartan	20 mg daily	SBP/DBP
	14	4	66.7	50.6	Firmasartan	60 mg daily	
Mancia, 2012, Italy (ONTARGET) [63]	139	104	76	66	Telmisartan		SBP/DBP
	141	104	79	65	Telmisartan + Ramipril		
	142	104	72	66	Ramipril		
Omboni, 2012, Europe [64]	359	12	53	72	Ramipril		SBP/DBP
	356	12	54	72	Olmesartan		
Oigman, 2013, Brazil [65]	55	8	52.7	56.7	Ramipiril+ Hydrochlorotiazide	Fixed dose	SBP/DBP
	54	8	44.4	56.6	Ramipiril+ Hydrochlorotiazide	Reference formulation	
Oxlund, 2013, Denmark [66]	61	16	75	62.9	Spironolactone		SBP/DBP
	58	16	78	63.9	Placebo		
Tryambake, 2013, UK [67]	17	12	74	89	Antihypertensive treatment	Usual treatment	SBP/DBP
	20	12	75	89	Antihypertensive treatment	Intensive treatment	
Fogari, 2014, Italy (PROBE) [68]	54	24	52	65	Valsartan + Amlodipine + Canrenone		SBP/DBP
	55	24	55	65	Valsartan + Amlodipine + Hydrochlorotiazide		
Vaclavik, 2014, Czech Republic (ASPIRAN-EXT) [69]	74	8	67.6	60.4	Spironolactone		SBP/DBP
	76	8	63.2	59.7	Placebo		
Fonseca, 2015, Brazil	27	12	56	57	Perindopril		SBP/DBP

[70]	32	12	50	54	Hydrochlorotiazide		
Pareek, 2015, India [71]	20	12	50	47	Hydrochlorotiazide CR		SBP/DBP
	18	12	44	48	Hydrochlorotiazide		
	16	12	56	41	Chlorthalidone		
Rosa, 2015, Czech Republic (PRAGUE-15) [72]	54	240	63	59	Antihypertensive treatments		SBP/DBP
Kario, 2016, Japan [73]	12	42	71	74	Telmisartan + Amlodipine	Administration morning	SBP/DBP
	12	39	51	76	Telmisartan + Amlodipine	Administration bedtime	
Mazza, 2016, Italy [74]	24	31	NA	49	Nebivolol + Hydrochlorotiazide		SBP/DBP
	24	33	NA	47	Enalapril + Hydrochlorotiazide		
	24	34	NA	48	Olmesartan + Hydrochlorotiazide		
	24	35	NA	49	Olmesartan + Amlodipine		
	24	35	NA	48	Perinopril + Amlodipine		
Mizuno, 2016, Japan [75]	47	16	51	77	Amlodipine + Aliskiren		SBP/DBP
	51	16	31	77	Amlodipine		
Modesti, 2016, Europe [76]	107	18	55	73	Zofenopril + Hydrochlorotiazide		SBP
	109	18	56	72	Irbesartan + Hydrochlorotiazide		
Oliveras, 2016, Spain [77]	13	24	69	65	Spironolactone		SBP/DBP
Seravalle, 2016, Japan [78]	36	8	81	49	Enalapril + Felodipine		SBP/DBP
	36	8	81	49	Enalapril + Lercanidpine		

Table 2. Chronological summary of literature on reduction systolic blood pressure due to pharmacological antihypertensive treatment

First author, publication year, country (project)	Antihypertensive drug	Strata	Systolic Office		Systolic 24 h		O-A-D
			Baseline *Mean*	Change *Mean (SE)*	Baseline *Mean*	Change *Mean (SE)*	*Mean (SE)*
Grandi, 1995, Italy [27]	Isradipine		155.00	-20.00 (5.04)	143.00	-16.00 (4.04)	-4.00 (6.17)
	Perindopril		156.00	-20.00 (5.04)	144.00	-15.00 (3.78)	-5.00 (6.03)
Lacourciere, 1995, Canada [28]	Amlodipine + Hydrochlorotiazide		167.00	-19.00 (4.94)	157.00	-16.00 (4.16)	-3.00 (6.16)
	Hydrochlorotiazide + Amlodipine		167.00	-21.00 (2.27)	154.00	-19.00 (3.93)	-2.00 (4.36)
Staessen, 1996, Europe and Israel (SYST-EUR) [29]	Antihypertensive treatments		175.00	-22.70 (1.22)	148.00	-10.50 (0.99)	-12.20 (1.51)
	Placebo		175.00	-9.80 (1.22)	148.00	-2.10 (0.87)	-7.70 (1.44)
Fagard, 1997, Belgium [30]	Antihypertensive treatments		161.00	-22.00(2.31)	143.00	-17.00 (1.73)	-6.00 (2.87)
Mancia, 1997, Italy (Veratran study) [31]	Placebo		NA	-8.00 (2.99)	NA	-0.50 (1.26)	-7.50 (3.14)
	Verapamil		NA	-11.00 (4.12)	NA	-8.50 (1.20)	-2.50 (4.19)
	Trandolapril		NA	-20.20 (7.54)	NA	-10.60 (1.56)	-9.60 (7.56)
	Verapamil + Tranodlapril		NA	-18.90 (7.15)	NA	-14.20 (1.14)	-4.70 (7.14)
Vaisse, 1997, France [32]	Lisinopril	High ABP	167.70	-23.70 (2.57)	149.80	-18.40 (2.19)	-5.30 (3.23)
	Lisinopril	Low ABP	156.90	-20.70 (3.25)	128.50	-6.20 (1.86)	-14.50 (3.60)
	Bisoprolol	High ABP	164.20	-19.10 (3.12)	147.50	-14.90 (2.57)	-4.20 (3.86)
	Bisoprolol	Low ABP	153.00	-20.70 (2.85)	126.10	-6.60 (2.80)	-14.10 (3.82)
Neutel, 1998, USA [33]	Tasosartan		150.60	-12.20 (1.20)	147.30	-12.60 (0.90)	0.40 (1.43)
	Placebo		150.10	0.40 (1.20)	145.20	0.60 (0.90)	-0.20 (1.43)
Asmar, 2000, France (CHAMP) [34]	Candersartan		162.10	-20.00 (2.04)	NA	-13.40 (1.05)	-6.60 (2.21)
	Losartan		160.60	-15.00 (1.53)	NA	-9.30 (1.10)	-5.70 (1.80)
Myers, 2000, Europe and Canada [35]	Perindopril + Indapamide	p2 + i0.625	163.00	-14.00 (1.00)	151.00	-10.00 (1.00)	-4.00 (1.35)
	Perindopril + Indapamide	p2 + i1.25	159.00	-15.70 (1.00)	150.00	-15.00 (1.00)	-0.70 (1.35)
	Perindopril + Indapamide	p4 + i1.25	160.00	-18.00 (1.50)	150.00	-18.00 (1.00)	0.00 (1.73)
	Perindopril + Indapamide	p8 + i1.25	164.00	-20.60 (1.50)	152.00	-19.00 (2.00)	-1.60 (2.39)
	Perindopril + Indapamide	p8 + i2.5	162.00	-21.30 (1.50)	150.00	-19.00 (2.00)	-2.30 (2.39)

	Indapamide	i1.25	161.00	-13.00 (1.00)	148.00	-8.00 (1.00)	-5.00 (1.35)
	Placebo		164.00	-7.50 (1.00)	151.00	-3.50 (1.00)	-4.00 (1.35)
Mancia, 2001, Italy (INSIGHT) [36]	Nifedipine		167.00	-30.00 (2.03)	NA	-15.00 (1.56)	-15.00 (2.45)
	Diuretici		166.00	-30.00 (2.14)	NA	-16.00 (1.97)	-14.00 (2.77)
de la Sierra, 2004, Spain [37]	Enalaprin + Nitrendipine		161.90	-21.00 (1.84)	145.10	-17.00 (2.19)	-4.00 (2.73)
	Losartan + Hydrochlorotiazide		164.40	-19.40 (1.73)	145.40	-16.20 (1.73)	-3.20 (2.34)
Eguchi, 2004, Japan[38]	Valsartan		163.00	-13.00 (3.64)	148.00	-7.00 (1.96)	-12.00 (7.95)
	Amlodipine		164.00	-26.00 (7.28)	147.00	-14.00 (3.92)	-6.00 (3.97)
Staessen, 2004, Belgium and Ireland (THOP) [39]	Antihypertensive treatments	Group 1	160.80	-15.30 (1.10)	141.90	-9.90 (0.90)	-5.40 (1.36)
	Antihypertensive treatments	Group 2	159.10	-22.00 (1.10)	141.00	-14.80 (0.90)	-7.20 (1.36)
Zanchetti, 2004, Italy (PHYLLIS) [40]	Hydrochlorotiazide		161.30	-19.50 (1.50)	138.00	-9.80 (1.50)	-9.70 (2.02)
	Fosinopril		158.60	-18.60 (1.00)	134.90	-8.30 (1.00)	-10.30 (1.35)
	Hydrochlorotiazide+Pravastatin		159.20	-17.50 (1.30)	135.90	-8.00 (1.00)	-9.50 (1.57)
	Fosinopril+ Pravastatin		160.00	-18.00 (1.30)	136.50	-6.30 (1.00)	-11.70 (1.57)
Anichkov, 2005, Russia [41]	Lisinopril		171.00	-26.00 (3.00)	154.00	-11.00 (1.80)	-15.00 (3.36)
	Rilmenidine		170.00	-19.00 (3.00)	152.00	-11.90 (1.90)	-7.10 (3.40)
Fogari, 2005, Italy (PROBE) [42]	Telmisartan + Hydrochlorotiazide		164.00	-20.10 (5.80)	145.80	-15.90 (4.59)	-4.20 (7.07)
	Nifedipine		163.00	-15.20 (4.38)	147.40	-12.60 (3.63)	-2.60 (5.43)
Ernst, 2006, USA [43]	Hydrochlorotiazide		140.10	-10.80 (3.50)	NA	-7.40 (1.70)	-3.40 (3.75)
Asmar, 2007, Europe, (PICXEL) [44]	Enalaprin		166.30	-18.70 (2.18)	151.80	-9.10 (2.20)	-9.60 (2.96)
	Perindropil + Indapamide		166.70	-28.10 (2.05)	152.00	-15.20(2.02)	-12.90 (2.74)
Mancia, 2007, Europe (ELSA) [45]	Antihypertensive treatments		163.70	-21.95 (0.43)	NA	-8.05 (0.38)	-13.90 (0.55)
Ribeiro,2007, South America [46]	Amlodipine		156.50	-21.60 (1.30)	147.80	-18.00 (1.18)	-3.60 (1.67)
	Losartan		158.90	-13.70 (1.71)	149.50	-10.80 (1.27)	-2.90 (2.04)
Ferguson, 2008, Australia [47]	Fosinopril + Hydrochlorotiazide		165.00	-21.50 (1.50)	NA	-17.60 (1.60)	-3.90 (2.09)
	Amlodipine		165.00	-16.00 (2.00)	NA	-13.00 (1.40)	-3.00 (2.34)
	Indapamide		165.00	-23.10 (1.50)	NA	-11.30 (1.00)	-11.80 (1.73)
Guerrero, 2008, Brazil [48]	Hydrochlorithiazide + Amiloride		150.60	-16.40 (1.97)	132.80	-9.60 (1.70)	-6.80 (2.48)

	Hydrochlorithiazide + Enalapril		149.50	-22.20 (2.41)	133.30	-13.20 (1.40)	-9.00 (2.68)
Miranda, 2008, Brazil (ATAR) [49]	Amlodipine		160.65	-22.97 (1.30)	144.87	-15.31(1.12)	-7.66 (1.64)
	Amlodipine + Ramipril		162.61	-26.60 (1.34)	146.88	-20.21 (1.14)	-6.39 (1.68)
Suonsyrja, 2008, Finland (GENRES) [50]	Amlodipine		152.00	-7.70 (0.73)	135.00	-7.40 (0.50)	-0.30 (0.84)
	Bisoprol		152.00	-12.90 (0.74)	135.00	-11.10 (0.43)	-1.80 (0.82)
	Hydrochlorotiazide		152.00	-4.70 (0.77)	135.00	-4.90 (0.44)	0.20 (0.85)
	Losartan		152.00	-9.20 (0.82)	135.00	-9.10 (0.46)	-0.10 (0.90)
Ambrosioni, 2010, Italy (INSIST) [51]	Eprosartan + Hydrochlorithiazide		169.70	-24.80 (7.06)	145.90	-12.20 (3.47)	-12.60 (7.58)
	Losartan + Hydrochlorithiazide		168.10	-25.80 (7.32)	146.70	-15.40 (4.37)	-10.40 (8.18)
Andreadis, 2010, Greece [52]	ARB	low dose	148.77	-21.22 (5.19)	138.21	-5.63 (2.19)	-15.59 (5.45)
	CCB	low dose	152.73	-20.60 (4.98)	144.91	-7.65 (2.22)	-12.95 (5.27)
Parati, 2010, Italy [53]	Barnidipine+ Losartan		156.00	-18.00 (2.04)	NA	-11.00 (1.53)	-7.00 (2.44)
	Losartan		153.00	-13.00 (2.04)	NA	-12.50 (1.53)	-0.50 (2.44)
Wright, 2010, Canada and USA (EVALUATE) [54]	Valsartan + Hydrochlorithiazide		NA	-33.70 (1.30)	147.00	-21.10 (0.80)	-12.60 (1.46)
	Amlodipine + Hydrochlorithiazide		NA	-33.00 (1.30)	147.00	-18.10 (0.80)	-14.90 (1.46)
Giles, 2011, USA [55]	Amlodipine+ Valsartan+ Hydrochlorothiazide	Intensive treatment	163.20	-34.60 (2.50)	140.70	-21.50 (2.00)	-13.10 (3.06)
	Amlodipine+ Valsartan+ Hydrochlorothiazide	Moderate treatment	165.70	-26.70 (3.40)	143.50	-16.30 (2.00)	-10.40 (3.79)
Kostka-Jeziorny, 2011, Poland [56]	Perindopril		157.79	-23.49 (5.33)	143.94	-13.11 (2.98)	-10.38 (5.87)
	Hydrochlorithiazide		158.53	-23.81 (5.31)	145.03	-18.39(4.10)	-5.42 (6.41)
Mancia, 2011, Italy and Spain (TALENT) [57]	Telmisartan		151.70	-17.00 (1.50)	136.20	-13.90 (1.30)	-3.10 (1.89)
	Nifedipine + Telmisartan		151.10	-16.10 (1.10)	136.80	-11.70 (0.90)	-4.40 (1.36)
	Nifedipine		151.30	-15.10 (1.40)	137.20	-11.70 (1.20)	-3.40 (1.76)
Muiesan, 2011, Italy [58]	Barnidipine		147.00	-13.00 (6.19)	138.00	-9.00 (4.28)	-4.00 (7.20)
Raij, 2011, USA (VITAE) [59]	Valsartan+ Hydrochlorithiazide		NA	-29.60 (1.50)	NA	-20.60 (1.50)	-9.00 (2.02)
	Hydrochlorithiazide+A		NA	-25.70 (2.00)	NA	-14.50 (1.50)	-11.20 (2.39)

	mlodipine						
Zamboli, 2011, Italy [60]	Antihypertensive treatment NO diuretics		159.00	-22.00 (4.49)	126.00	-8.00 (3.82)	-14.00 (5.63)
	Antihypertensive treatment and Furosemide		161.00	-22.00 (4.49)	130.00	-8.00 (3.82)	-14.00 (5.63)
Krzenski, 2012, Poland [61]	Antihypertensive treatment		145.90	-10.70 (2.04)	141.40	-10.50 (2.04)	-0.20 (2.75)
	Antihypertensive treatment		150.60	-18.10 (2.00)	143.60	-16.70 (1.45)	-1.40 (2.36)
Lee, 2012, Korea [62]	Firmasartan	20 mg daily	NA	-21.30 (4.65)	NA	-14.70 (3.93)	-6.60 (5.81)
	Firmasartan	60 mg daily	NA	-21.90 (4.76)	NA	-14.10 (3.93)	-7.80 (5.89)
Mancia, 2012, Italy (ONTARGET) [63]	Telmisartan		138.30	-4.33 (1.29)	124.60	-2.09 (1.01)	-2.24 (1.56)
	Telmisartan + Ramipril		139.40	-9.28 (3.55)	125.70	-5.28 (1.32)	-4.00 (3.68)
	Ramipril		139.40	-4.39 (1.45)	126.70	-2.01 (0.97)	-2.38 (1.67)
Omboni, 2012, Europe [64]	Ramipril		156.40	-16.10 (0.66)	141.10	-8.70 (0.56)	-7.40 (0.83)
	Olmesartan		156.70	-18.80 (0.66)	141.40	-10.90 (0.56)	-7.90 (0.83)
Oigman, 2013, Brazil [65]	Ramipiril+ Hydrochlorotiazide	Fixed dose	158.50	-14.00 (1.96)	149.10	-16.10 (1.20)	2.10 (2.20)
	Ramipiril+ Hydrochlorotiazide	Reference formulation	159.70	-18.80 (1.78)	146.20	-15.60 (1.30)	-3.20 (2.11)
Oxlund, 2013, Denmark [66]	Spironolactone		144.00	-10.50 (2.04)	144.00	-9.70 (1.68)	-0.80 (2.53)
	Placebo		139.00	5.30 (2.35)	143.00	-0.80 (1.45)	6.10 (2.65)
Tryambake, 2013, UK [67]	Antihypertensive treatment	Usual treatment	155.00	-15.00 (3.40)	131.00	-2.00 (2.43)	-13.00 (3.99)
	Antihypertensive treatment	Intensive treatment	149.00	-26.00 (3.13)	128.00	-12.00 (1.57)	-14.00 (3.37)
Fogari, 2014, Italy (PROBE) [68]	Valsartan + Amlodipine + Canrenone		144.40	-13.30 (3.82)	137.90	-14.50 (4.16)	1.20 (5.39)
	Valsartan + Amlodipine + Hydrochlorotiazide		143.70	-13.40 (3.85)	138.20	-16.10 (4.63)	2.70 (5.75)
Vaclavik, 2014, Czech Republic (ASPIRAN-EXT) [69]	Spironolactone		154.90	-17.60 (1.80)	144.50	-12.60 (1.46)	-5.00 (2.22)
	Placebo		153.30	-7.70 (1.65)	141.20	-2.10 (1.51)	-5.60 (2.14)
Fonseca, 2015, Brazil [70]	Perindopril		150.00	-20.00 (5.40)	127.00	-3.00 (1.19)	-6.20 (3.47)
	Hydrochlorotiazide		149.00	-19.00 (5.23)	121.00	-12.00 (3.30)	-5.80 (6.84)
Pareek, 2015, India [71]	Hydrochlorotiazide CR		148.82	-15.43 (3.24)	NA	-10.27 (2.63)	-1.50 (3.11)

	Hydrochlorotiazide		149.87	-15.30 (2.80)	NA	-6.02 (2.94)	-2.50 (3.86)
	Chlorthalidone		147.38	-16.40 (3.85)	NA	-11.14 (3.10)	-1.30 (3.38)
Rosa, 2015, Czech Republic (PRAGUE-15) [72]	Antihypertensive treatments		155.00	-14.30 (2.76)	147.00	-8.10 (2.37)	-4.10 (4.40)
Kario, 2016, Japan [73]	Telmisartan + Amlodipine	Administration morning	150.80	-19.60 (2.81)	135.60	-11.60 (2.58)	-1.80 (5.12)
	Telmisartan + Amlodipine	Administration bedtime	151.30	-23.70 (2.57)	137.60	-12.80 (2.18)	-8.00 (3.64)
Mazza, 2016, Italy [74]	Nebivolol + Hydrochlorotiazide		149.20	-6.90 (1.89)	141.90	-5.40 (2.64)	-10.90 (3.22)
	Enalapril + Hydrochlorotiazide		150.00	-9.00 (2.49)	142.50	-6.50 (3.19)	-3.00 (3.06)
	Olmesartan + Hydrochlorotiazide		148.20	-6.90 (2.23)	141.40	-5.60 (2.75)	-2.50 (3.33)
	Olmesartan + Amlodipine		150.60	-10.80 (3.23)	142.10	-6.70 (3.30)	-5.16 (3.99)
	Perinopril + Amlodipine		150.20	-10.80 (3.00)	144.00	-9.00 (4.43)	-9.28 (3.87)
Mizuno, 2016, Japan [75]	Amlodipine + Aliskiren		147.60	-11.90 (2.98)	134.20	-7.00 (1.93)	-5.26 (4.72)
	Amlodipine		147.70	-8.90 (2.38)	133.80	-6.00 (1.36)	-9.50 (1.68)
Modesti, 2016, Europe [76]	Zofenopril + Hydrochlorotiazide		156.00	-20.20 (1.42)	137.50	-10.70 (1.05)	-10.80 (1.64)
	Irbesartan + Hydrochlorotiazide		156.70	-19.80 (1.38)	137.70	-9.00 (1.02)	-17.00 (5.42)
Oliveras, 2016, Spain [77]	Spironolactone		171.20	-29.40 (.77)	155.40	-23.60 (4.23)	-7.00 (5.93)
Seravalle, 2016, Japan [78]	Enalapril + Felodipine		153.40	-11.00 (2.50)	147.20	-8.00 (2.00)	-4.90 (3.40)
Grandi, 1995, Italy [27]	Enalapril + Lercanidpine		153.40	-12.30 (2.85)	147.20	-9.80 (2.00)	-2.90 (2.63)

Table 3. Chronological summary of literature on reduction diastolic blood pressure due to pharmacological antihypertensive treatment

First author, publication year, country (project)	Antihypertensive drug	Strata	Diastolic Office		Diastolic 24 h		
			Baseline *Mean*	Change *Mean (SE)*	Baseline *Mean*	Change *Mean (SE)*	O-A-D *Mean (SE)*
Grandi, 1995, Italy [27]	Isradipine		106.00	-14.00 (3.53)	98.00	-13.00 (3.28)	-1.00 (4.60)
	Perindopril		105.00	-14.00 (3.53)	96.00	-12.00 (3.03)	-2.00 (4,44)
Lacourciere, 1995, Canada [28]	Amlodipine + Hydrochlorotiazide		99.00	-14.00 (2.89)	89.00	-10.00 (2.60)	-4.00 (3.71)
	Hydrochlorotiazide +Amlodipine		101.00	-13.00(2.69)	89.00	-10.00 (2.60)	-3.00 (3.57)
Staessen, 1996, Europe and Israel (SYST-EUR) [29]	Antihypertensive treatments		85.00	-7.00 (0.66)	79.00	-4.50 (0.61)	-2.50 (0.86)
	Placebo		86.00	-1.60 (0.61)	80.00	-1.10 (0.51)	-0.50 (0.76)
Fagard, 1997, Belgium [30]	Antihypertensive treatments		104.00	-16.00 (1.73)	94.00	-11.00 (1.30)	-5.00 (2.07)
Mancia, 1997, Italy (Veratran study) [31]	Placebo		NA	-6.00 (2.24)	NA	-0.50 (0.84)	-5.50 (2.32)
	Verapamil		NA	-10.00 (3.75)	NA	-6.20 (0.80)	-3.80 (3.76)
	Trandolapril		NA	-12.70 (4.74)	NA	-6.50 (1.13)	-6.20 (4.77)
	Verapamil + Trandolapril		NA	-13.30 (5.03)	NA	-11.30 (0.91)	-2.00 (5.03)
Vaisse, 1997, France [32]	Lisinopril	High ABP	103.30	-12.60 (1.30)	93.70	-12.70 (1.50)	0.10 (1.89)
	Lisinopril	Low ABP	100.00	-13.70 (1.65)	81.40	-5.80 (1.13)	-7.90 (1.91)
	Bisoprolol	High ABP	102.30	-13.70 (1.52)	93.10	-11.70 (1.42)	-2.00 (1.98)
	Bisoprolol	Low ABP	98.30	-14.60 (1.27)	79.50	-6.10 (2.07)	-8.50 (2.33)
Neutel, 1998, USA [33]	Tasosartan		100.30	-9.40 (0.70)	91.50	-8.10 (0.60)	-1.30 (0.88)
	Placebo		100.30	-2.00 (0.70)	89.90	0.50 (0.60)	-2.50 (0.88)
Asmar, 2000, France (CHAMP) [34]	Candersartan		101.30	-11.00 (1.02)	NA	-8.70 (0.69)	-2.30 (1.18)
	Losartan		100.10	-11.00 (1.02)	NA	-6.90 (0.71)	-4.10 (1.19)
Myers, 2000, Europe and Canada [35]	Perindopril + Indapamide	p2 + i0.625	102.00	-9.30 (1.00)	95.00	-6.00 (1.00)	-3.30 (1.35)
	Perindopril + Indapamide	p2 + i1.25	101.00	-8.50 (1.00)	95.00	-9.00 (1.00)	0.50 (1.35)
	Perindopril + Indapamide	p4 + i1.25	101.00	-10.50 (1.00)	93.00	-9.80 (1.00)	-0.70 (1.35)
	Perindopril + Indapamide	p8 + i1.25	102.00	-12.00 (1.00)	94.00	-10.70 (1.00)	-1.30 (1.35)
	Perindopril + Indapamide	p8 + i2.5	101.00	-15.00 (1.00)	92.00	-12.00 (1.00)	-3.00 (1.35)
	Indapamide	i1.25	101.00	-8.70 (1.00)	92.00	-5.00 (1.00)	-3.70 (1.35)

	Placebo		102.00	-4.50 (1.00)	93.00	-2.50 (1.00)	-2.00 (1.35)
Mancia, 2001, Italy (INSIGHT) [36]	Nifedipine		100.00	-15.60 (1.17)	NA	-9.80 (1.03)	-5.80 (1.49)
	Diuretics		101.00	-17.60 (1.45)	NA	-9.00 (1.02)	-8.60 (1.69)
de la Sierra, 2004, Spain [37]	Enalaprin + Nitrendipine		100.20	-12.10 (1.17)	90.00	-11.70 (1.28)	-0.40 (1.65)
	Losartan + Hydrochlorotiazide		101.00	-10.90 (0.94)	90.50	-10.40 (0.79)	-0.50 (1.18)
Eguchi, 2004, Japan [38]	Amlodipine		93.00	-10.00 (2.80)	83.00	-5.00(1.40)	-5.00 (3.01)
	Valsartan		91.00	-7.00 (1.96)	86.00	-3.00(0.84)	-4.00 (2.06)
Staessen, 2004, Belgium and Ireland (THOP) [39]	Antihypertensive treatments	Group 1	101.80	-10.50 (0.60)	88.00	-7.10 (0.50)	-3.40 (0.75)
	Antihypertensive treatments	Group 2	101.50	-14.00 (0.60)	87.90	-10.00 (0.50)	-4.00 (0.75)
Zanchetti, 2004, Italy (PHYLLIS) [40]	Hydrochlorotiazide		98.5	-12.00 (1.00)	85.1	-7.00 (1.00)	-5.00 (1.35)
	Fosinopril		98.2	-13.00 (1.00)	83.9	-6.00 (1.00)	-7.00 (1.35)
	Hydrochlothiazide + Pravastatin		98.1	-12.70 (0.60)	83.5	-5.00 (1.30)	-7.70 (1.38)
	Fosinopril + Pravastatin		98.4	-13.50 (0.80)	83.5	-5.00 (1.00)	-8.50 (1.22)
Anichkov, 2005, Russia [41]	Lisinopril		100.00	-10.00 (2.00)	91.00	-6.70 (0.70)	-3.30 (2.06)
	Rilmenidine		97.00	-10.00 (2.00)	89.00	-11.00 (1.80)	1.00 (2.57)
Fogari, 2005, Italy (PROBE) [42]	Telmisartan + Hydrochlorotiazide		102.70	-16.70 (4.82)	88.80	-12.70 (3.67)	-4.00 (5.79)
	Nifedipine		102.80	-13.80 (3.98)	89.10	-9.10 (2.62)	-4.70 (4.56)
Ernst, 2006, USA [43]	Hydrochlorotiazide		91.10	-6.90 (2.90)	NA	-5.10 (1.30)	-1.80 (3.07)
Asmar, 2007, Europe, (PICXEL) [44]	Enalaprin		98.80	-8.90 (1.41)	91.00	-5.60 (1.23)	-3.30 (1.79)
	Perindropil + Indapamide		100.10	-12.30 (1.04)	91.90	-8.00 (1.15)	-4.30 (1.48)
Mancia, 2007, Europe (ELSA) [45]	Antihypertensive treatments		101.30	-16.50 (0.36)	NA	-6.90 (0.36)	-9.60 (0.48)
Ribeiro,2007, South America [46]	Amlodipine		102.20	-14.30 (0.79)	95.20	-10.60 (0.83)	-3.70 (1.09)
	Losasartan		102.50	-9.00 (0.92)	96.30	-8.00 (0.88)	-1.00 (1.21)
Ferguson, 2008, Australia [47]	Fosinopril + Hydrochlorotiazide		85	-9.40 (1.00)	NA	-7.00 (0.70)	-2.40 (1.17)
	Amlodipine		85	-5.00 (1.00)	NA	-5.00 (0.80)	0.00 (1.22)
	Indapamide		85	-8.20 (1.30)	NA	-4.00 (1.00)	-4.20 (1.57)
Guerrero, 2008, Brazil [48]	Hydrochlorithiazide + Amiloride		92.20	-5.80 (2.03)	82.40	-6.40 (1.86)	0.60 (2.63)

	Hydrochlorithiazide + Enalapril		91.00	-9.70 (1.43)	83.00	-8.10 (0.90)	-1.60 (1.62)
Miranda, 2008, Brazil (ATAR) [49]	Amlodipine		99.83	-14.48 (0.75)	88.60	-8.42 (0.70)	-6.06 (0.98)
	Amlodipine + Ramipril		101.03	-16.48 (0.78)	90.60	-11.61 (0.72)	-4.87 (1.01)
Suonsyrja, 2008, Finland (GENRES) [51]	Amlodipine		100.00	-6.30 (0.46)	93.00	-4.90 (0.28)	-1.40 (0.51)
	Bisoprolol		100.00	-9.70 (0.49)	93.00	-8.40 (0.29)	-1.30 (0.55)
	Hydrochlorotiazide		100.00	-2.60 (0.44)	93.00	-1.70 (0.28)	-0.90 (0.50)
	Losasartan		100.00	-7.20 (0.48)	93.00	-6.10 (0.33)	-1.10 (0.55)
Andreadis, 2010, Greece [52]	ARB	Low dose	94.88	-10.62 (2.60)	92.15	-3.58 (1.21)	-7.04 (2.76)
	CCB	Low dose	96.89	-11.39 (2.76)	89.69	-5.17 (1.50)	-6.22 (3.02)
Parati 2010, Italy [53]	Barnidipine		97.00	-11.50 (1.28)	NA	-4.25 (1.40)	-7.25 (1.81)
	Losartan		96.00	-9.50 (1.28)	NA	-5.00 (1.53)	-4.50 (1.90)
Wright, 2010, Canada and USA (EVALUATE) [54]	Valsartan + Hydrochlorithiazide		NA	-13.90 (0.70)	87.30	-12.50 (0.60)	-1.40 (0.88)
	Amlodipine + Hydrochlorithiazide		NA	-14.00 (0.70)	87.30	-9.90 (0.50)	-4.10 (0.82)
Giles 2011, USA [55]	Amlodipine + Valsartan + Hydrochlorotiazide	Intensive treatment	95.40	-16.30 (1.50)	87.40	-13.70 (1.50)	-2.60 (2.02)
	Amlodipine + Valsartan + Hydrochlorotiazide	Moderate treatment	93.70	-12.80 (2.00)	85.30	-10.20 (1.20)	-2.60 (2.24)
Kostka-Jeziorny, 2011, Poland [56]	Perindopril + Amlodipine		97.41	-13.83 (3.14)	87.86	-5.58 (2.05)	-8.25 (3.59)
	Hydrochlorithiazide + Amlodipine		96.09	-12.21 (2.72)	86.62	-6.27 (2.28)	-5.94 (3.39)
Mancia, 2011, Italy and Spain (TALENT) [57]	Telmisartan		92.00	-9.90 (1.00)	82.60	-8.00 (0.80)	-1.90 (1.22)
	Nifedipine + Telmisartan		90.90	-9.30 (0.70)	81.70	-7.10 (0.60)	-2.20 (0.88)
	Nifedipine		90.70	-8.30 (1.00)	81.30	-7.50 (0.70)	-0.80 (1.17)
Muiesan 2011, Italy [53]	Barnidipine		96.00	-11.00 (5.24)	91.00	-6.00 (2.86)	-5.00 (5.73)
Raij 2011, USA (VITAE) [58]	Valsartan + Hydrochlorotiazide		NA	-14.00 (1.00)	NA	-11.70 (1.00)	-2.30 (1.35)
	Hydrochlorotiazide + Valsartan		NA	-13.10 (1.00)	NA	-8.90 (1.00)	-4.20 (1.35)
Zamboli 2011, Italy [59]	Antihypertensive treatments no diuretics		81.00	-7.00 (3.34)	68.00	-6.00 (2.10)	-1.00 (3.78)
	Antihypertensive		80.00	-6.00 (2.87)	69.00	-4.00 (1.91)	-2.00 (3.30)

	treatments and Furosemide						
Krzenski, 2012, Poland [61]	Antihypertensive treatment		94.70	-8.90 (1.51)	88.00	-8.50 (1.42)	-0.40 (1.98)
	Antihypertensive treatment		96.50	-12.20 (1.12)	89.30	-10.90 (1.12)	-1.30 (1.52)
Lee 2012, Korea [62]	Firmasartan	20 mg daily	NA	-11.40 (1.82)	NA	-9.50 (2.70)	-1.90 (3.12)
	Firmasartan	60 mg daily	NA	-11.20 (2.83)	NA	-10.70 (2.70)	-0.50 (3.74)
Mancia, 2012, Italy (ONTARGET) [53]	Telmisartan		80.10	-4.30 (1.07)	72.30	-1.73 (0.57)	-2.57 (1.17)
	Telmisartan + Ramipril		80.60	-4.78 (1.19)	73.10	-3.62 (0.90)	-1.16 (1.43)
Omboni, 2012, Europe [64]	Ramipril		91.00	-8.50 (0.36)	81.00	-5.30 (0.33)	-3.20 (0.46)
	Olmesartan		91.80	-10.10 (0.36)	81.00	-6.50 (0.33)	-3.60 (0.46)
Oigman 2013, Brazil [65]	Ramipril + Hydrochlorothiazide	Fixed dose	96.60	-7.50 (1.39)	93.70	-8.80 (1.13)	1.30 (1.71)
	Ramipril + Hydrochlorothiazide	Reference formulation	97.60	-9.70 (1.10)	91.50	-8.50 (1.20)	-1.20 (1.55)
Oxlund, 2013, Denmark [66]	Spironolactone		79.00	-5.70 (1.05)	78.00	-4.20 (0.82)	-1.50 (1.27)
	Placebo		76	1.10 (1.07)	78.00	-0.30 (0.82)	1.40 (1.29)
Tryambake 2013, UK [67]	Antihypertensive treatments	Usual treatment	84	-5.00 (1.70)	71.00	-1.00 (1.46)	-4.00 (2.13)
	Antihypertensive treatments	Intensive treatment	87	-17.00 (2.01)	69.00	-6.00 (1.12)	-11.00 (2.21)
Fogari, 2014, Italy (PROBE) [68]	Valsartan + Amlodipine + Canrenone		88.90	-10.80 (3.10)	82.40	-9.50 (2.73)	-1.30 (3.94)
	Valsartan + Amlodipine + Hydrochlorotiazide		88.50	-15.60 (4.48)	83.70	-9.90 (2.84)	-5.70 (5.09)
Vaclavik, 2014, Czech Republic (ASPIRANT-EXT) [69]	Spironolactone		93.10	-7.40 (1.56)	83.20	-5.50 (0.88)	-1.90 (1.72)
	Placebo		91.10	-4.40 (1.04)	81.30	-2.00 (0.89)	-2.40 (1.31)
Fonseca, 2015, Brazil [70]	Perindopril		90.00	-10.00 (2.91)	79.00	-3.00 (1.20)	-2.80 (1.85)
	Hydrochlorotiazide		90.00	-10.00 (2.75)	78.00	-9.00 (2.79)	-2.50 (4.09)
Pareek, 2015, India [71]	Hydrochlorotiazide CR		92.03	-5.85 (1.89)	NA	-8.10 (2.19)	-2.40 (3.07)
	Hydrochlorotiazide		93.39	-8.16 (1.51)	NA	-4.17 (1.92)	-5.80 (3.31)

	Chlorthalidone		93.94	-9.50 (2.60)	NA	-7.78 (2.44)	-6.50 (3.02)
Rosa, 2015, Czech Republic (PRAGUE-15) [72]	Antihypertensive treatments		89.00	-7.30 (1.56)	84.00	-4.50 (1.15)	-8.90 (3.50)
Kario, 2016, Japan [73]	Telmisartan + Amlodipine	Administration morning	83.40	-10.10 (1.43)	74.80	-5.00 (1.27)	-7.90 (2.95)
	Telmisartan + Amlodipine	Administration bedtime	82.50	-10.70 (1.83)	77.00	-6.40 (1.03)	-5.10 (1.83)
Mazza, 2016, Italy [74]	Nebivolol + Hydrochlorotiazide		93.00	-6.70 (2.44)	82.40	-4.30 (2.11)	-4.30 (2.02)
	Enalapril + Hydrochlorotiazide		94.60	-8.60 (3.14)	83.00	-2.80 (1.37)	-2.10 (1.51)
	Olmesartan + Hydrochlorotiazide		92.50	-7.40 (3.03)	81.30	-0.90 (0.44)	-2.10 (1.73)
	Olmesartan + Amlodipine		96.30	-11.90 (3.30)	82.80	-3.00 (1.48)	2.25 (2.76)
	Perinopril + Amlodipine		94.00	-10.20 (2.83)	81.50	-2.30 (1.13)	-3.99 (2.34)
Mizuno, 2016, Japan [75]	Amlodipine + Aliskiren		73.90	-3.40 (1.06)	74.50	-4.60 (0.99)	-1.72 (3.40)
	Amlodipine		76.20	-5.80 (1.05)	74.40	-3.70 (0.76)	-7.00 (3.05)
Oliveras, 2016, Spain [77]	Spironolactone		90.20	-12.70 (3.70)	80.90	-10.20 (2.12)	-1.00 (3.74)
Seravalle, 2016, Japan [78]	Enalapril + Felodipine		102.90	-6.50 (1.30)	98.10	-4.40 (0.90)	1.20 (1.39)
	Enalapril + Lercanidpine		102.90	-7.20 (1.50)	98.10	-5.10 (1.00)	-2.10 (1.24)

Figure 1.

Flow chart of the selection of studies for inclusion in the meta-analysis.

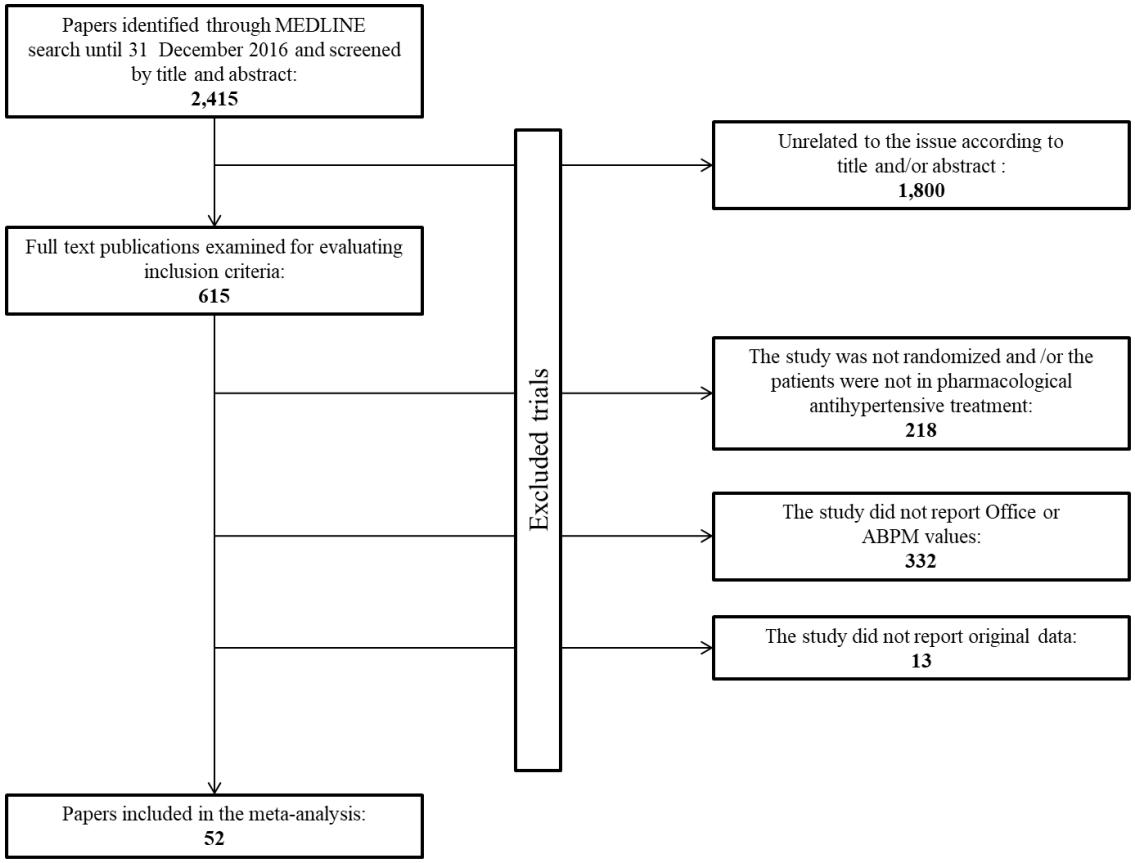


Figure 2

Forest plot of study-specific relative OC, AC and Δ(OC-AC) for Systolic blood pressure

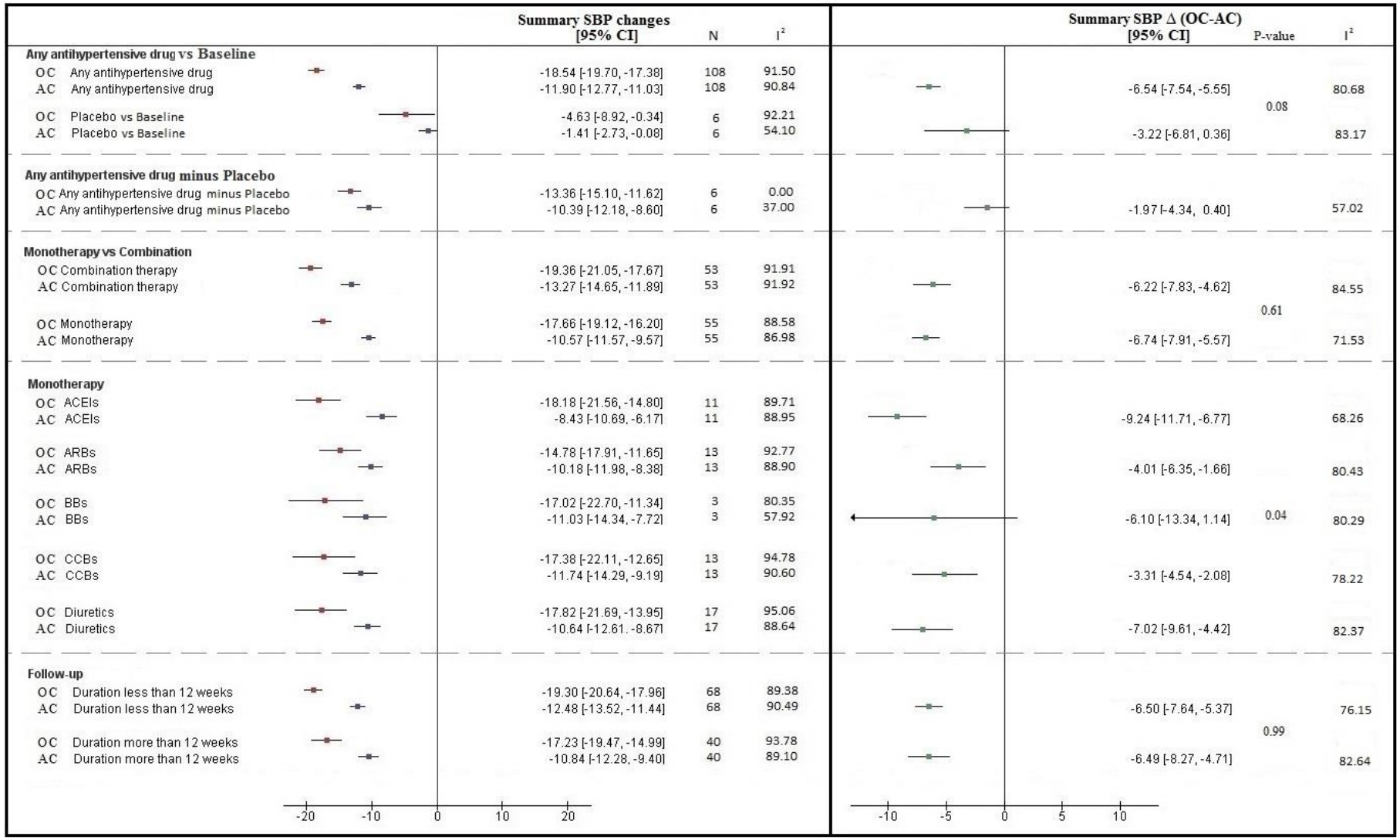


Figure 3

Forest plot of study-specific relative OC, AC and Δ(OC-AC) for Systolic blood pressure.

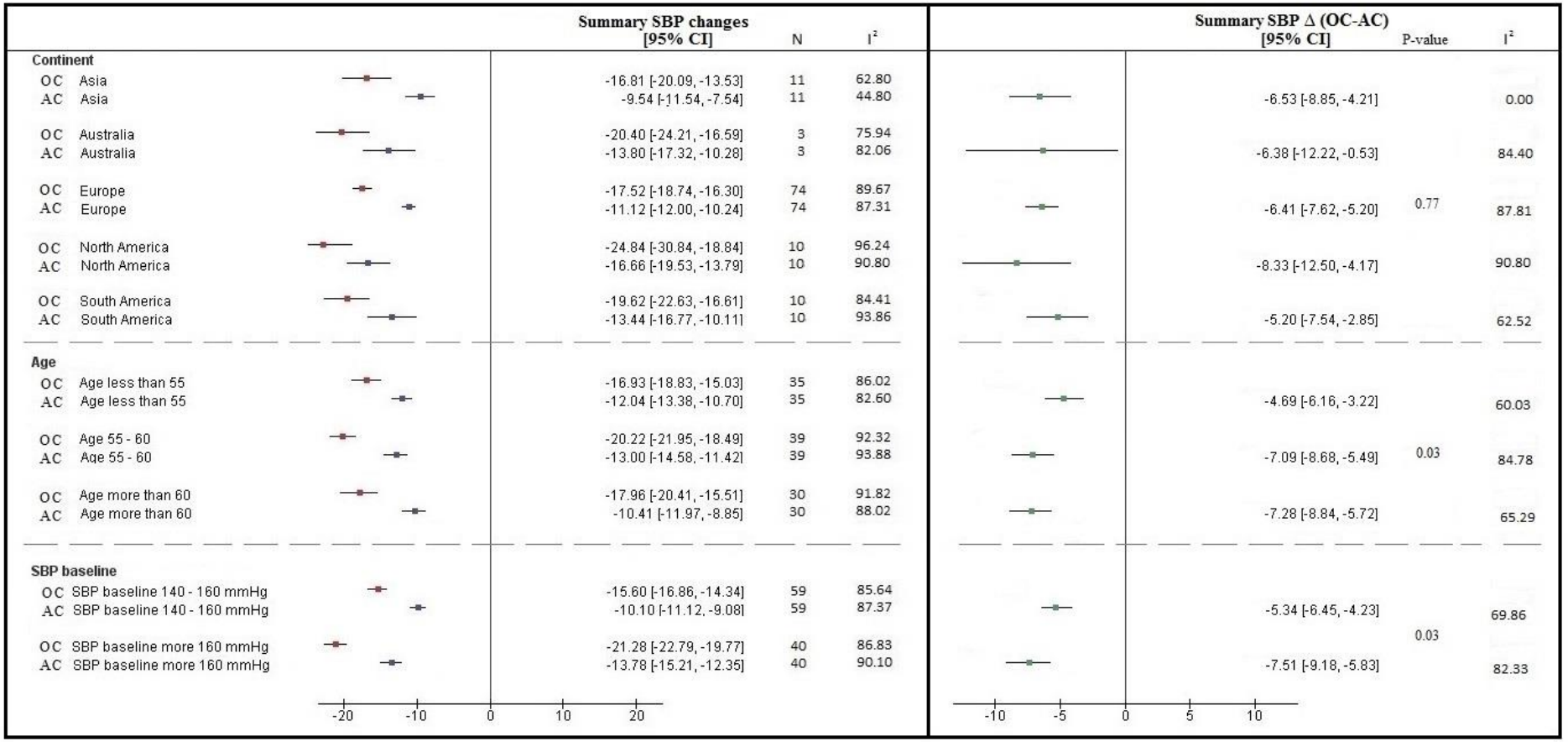


Figure 4 Funnel plot for publication bias in the studies investigating the changes of systolic blood pressure measured with Office (A), ABPM (B) and Δ(OC-AC) (C)

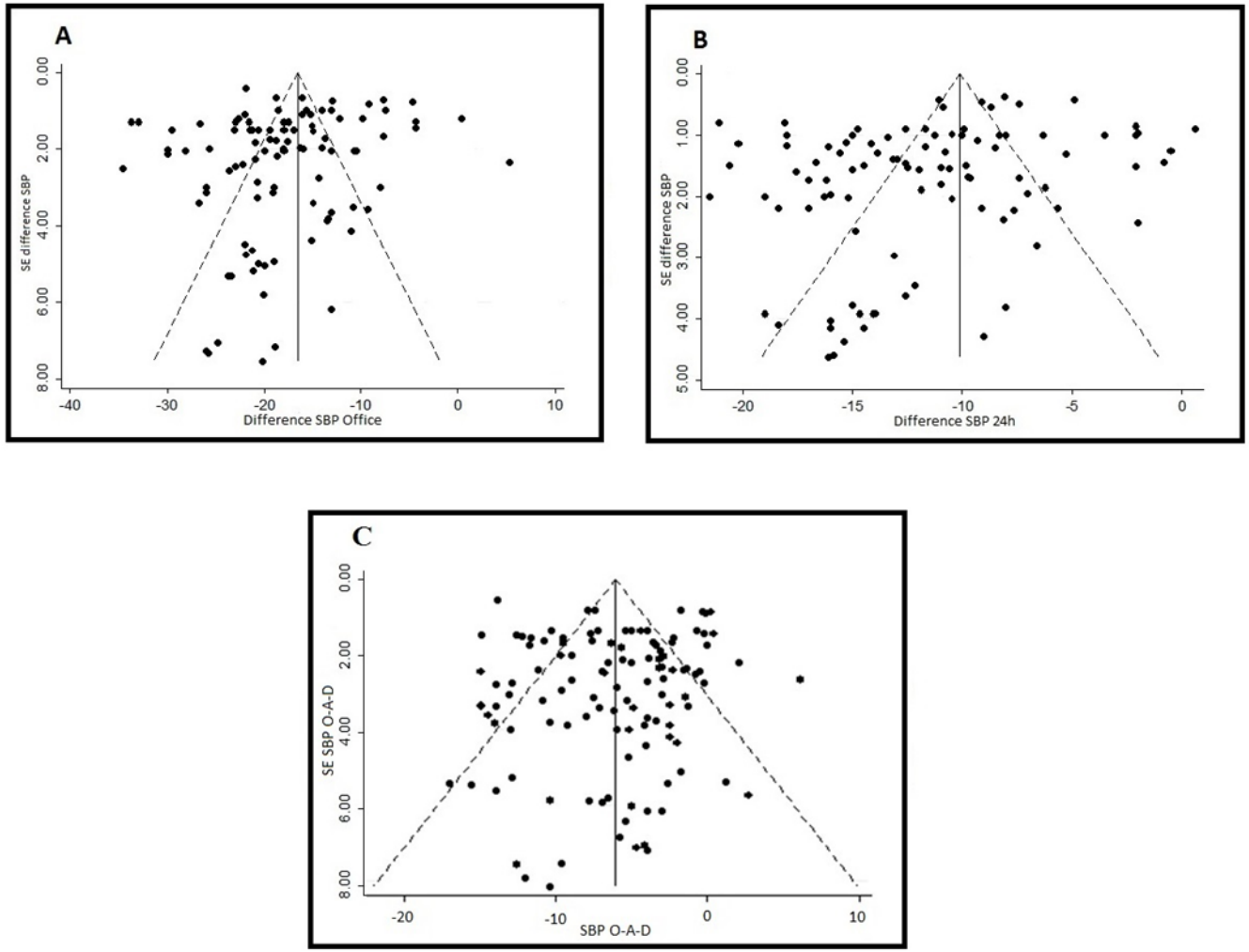


Figure 5

Forest plot of study-specific relative OC, AC and Δ(OC-AC) for Diastolic blood pressure.

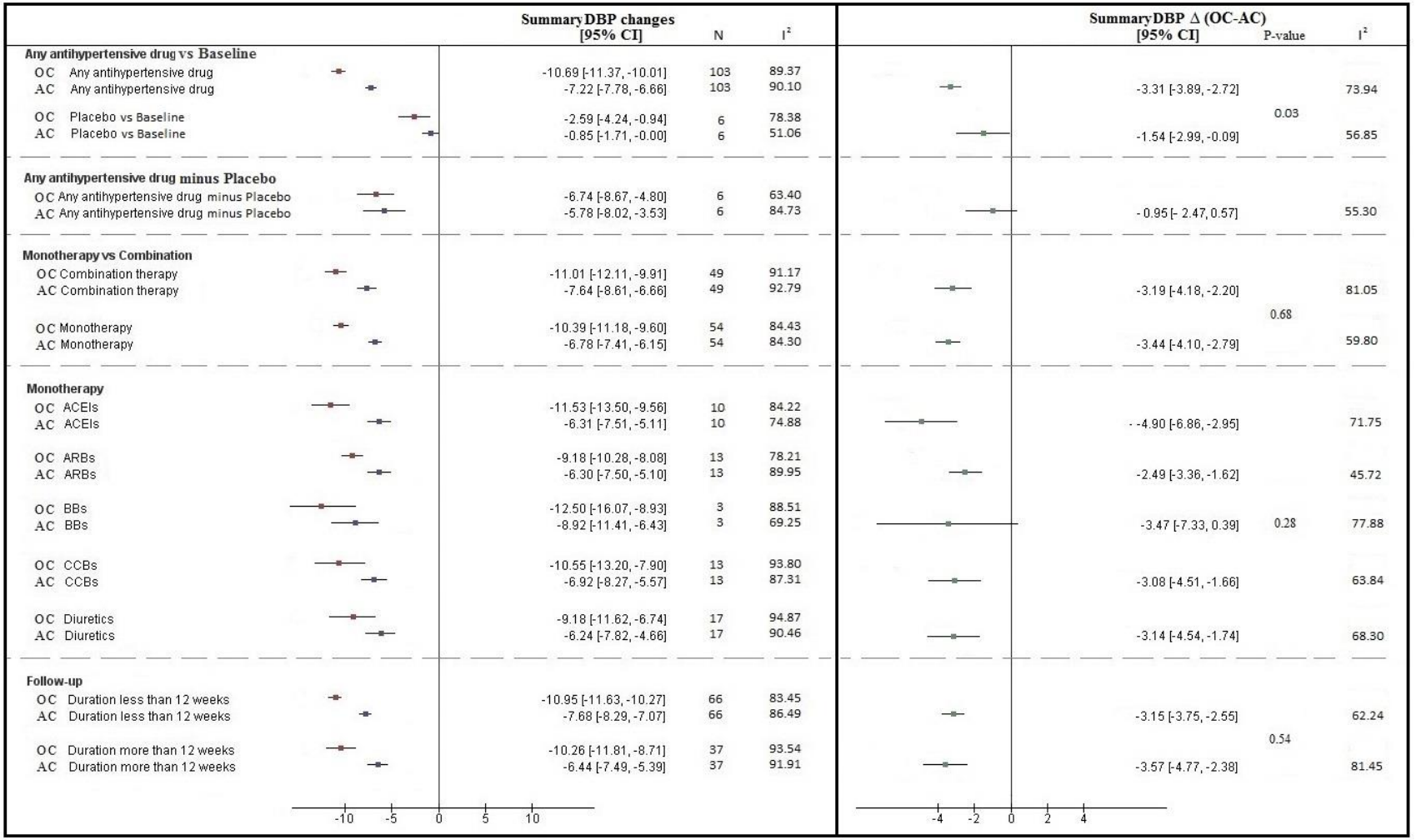


Figure 6

Forest plot of study-specific relative OC, AC and Δ(OC-AC) for Diastolic blood pressure.

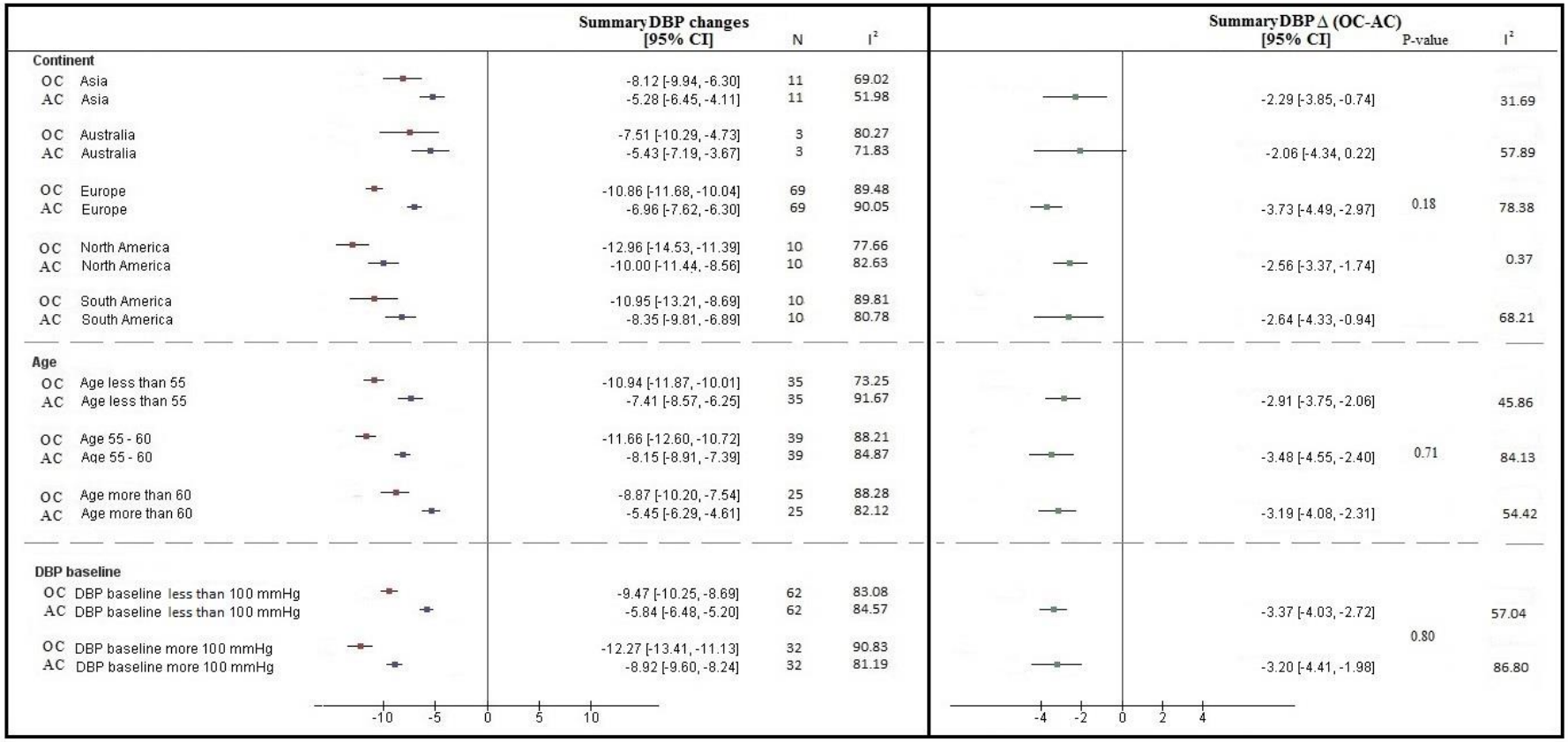


Figure 7

Funnel plot for publication bias in the studies investigating the changes of diatolic blood pressure measured with Office (A), ABPM (B) and Δ(OC-AC) (C)

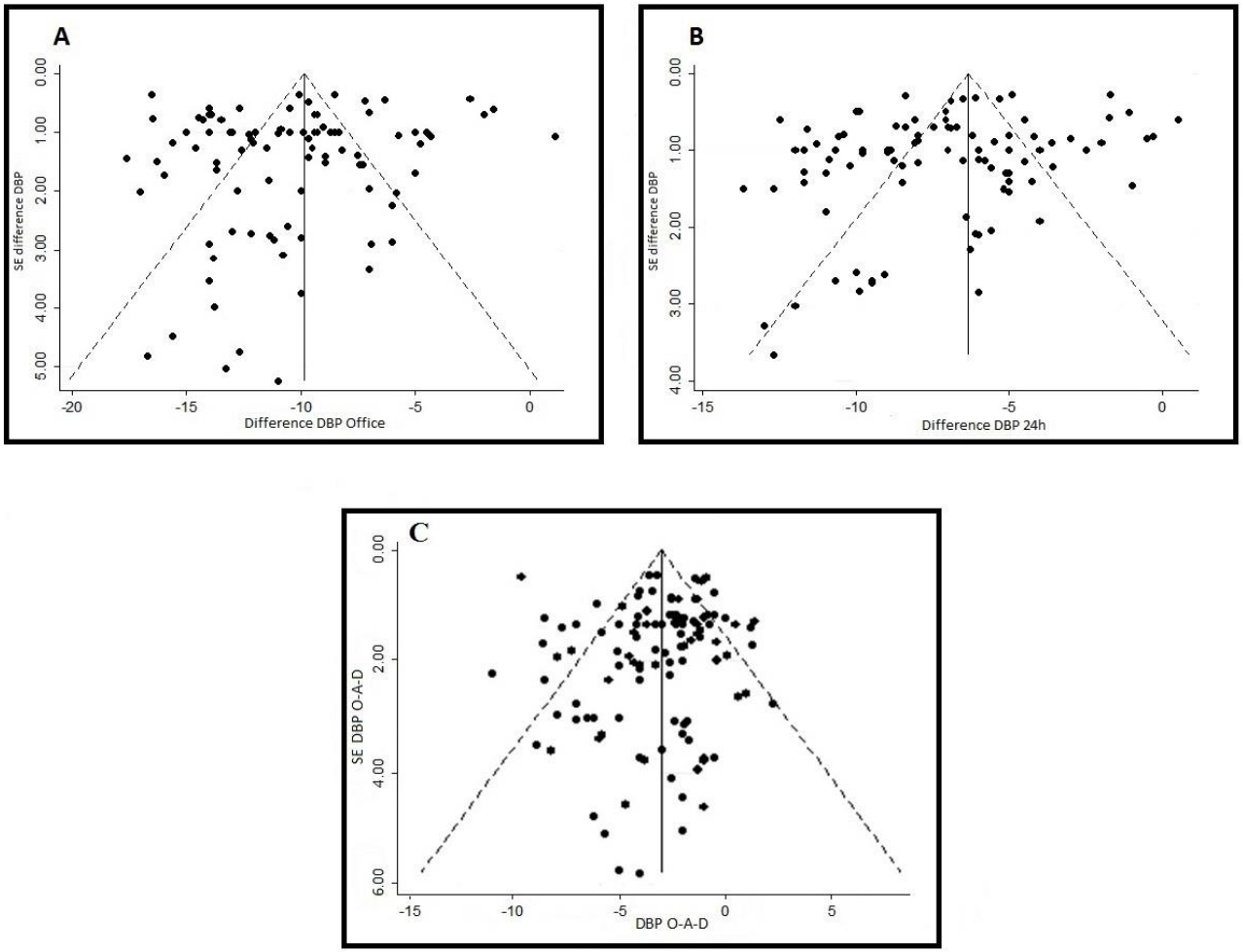
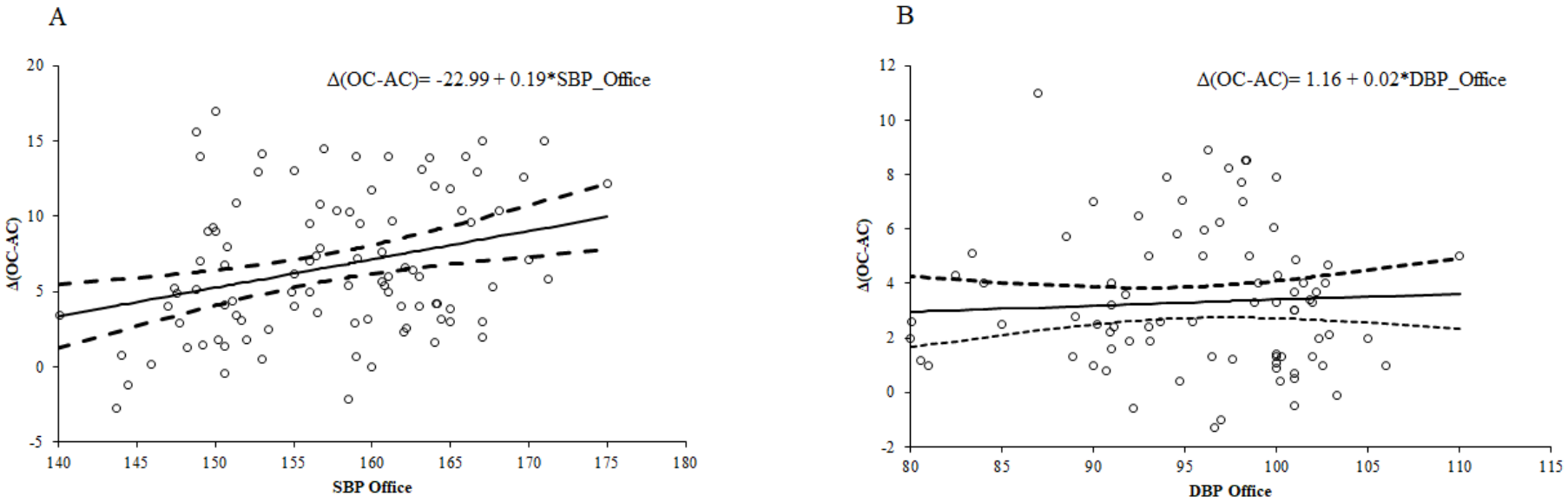


Figure 8

The functional relationship between the Δ (OC-AC) and the BP Office at baseline (SBP in Panel A and DBP in Panel B)



3.2 SAS MACRO FOR DEPENDENT MEASURES IN META-ANALYSIS

As explained in the section 2.2, if the researcher has to carry out a meta-analysis with dependent measure the linear mixed models could be implemented. The difficulty to use LMMs in a meta-analysis rests in the construction of the R matrix. The main statistical softwares allow the bivariate model but they do not build automatically the R matrix. Because the R matrix is not easy to be created in this thesis a macro SAS has been developed to calculate meta-analytic estimates both with independent and dependent estimates. The macro LMM_META was designed to estimate summary effect sizes and the corresponding 95% confidence intervals (95%CIs) for each scenario defined by the presence of heterogeneity (fixed and random effect) and correlation between estimates (dependent and independent estimates). The macro can accommodate any number of studies and any number of outcome variables. Several information need to be specified as input to run the macro such as the name of the SAS dataset containing the data, the type measure of the effect size (Mean difference = MD, Standardized Mean Difference = STD, Odds ratio= OR, Risk Relative = RR, Hazard Ratio = HR), names of the variables containing i) the effect sizes, ii) the variability measure of the effect sizes, iii) the first author's name of included studies, iv) publication year of included studies ecc. Clearly, the researcher can choose to carry out an analysis with fixed or random effect models and indicate if the estimates are independent or correlated. In any case the macro will build automatically the R matrix through the value of the correlation between estimates set by the researcher. Finally, the macro allows to draw the Forest Plot showing the study-specific and summary estimates and to carry out a meta-regression analysis useful to assess the effect of potential sources of heterogeneity. The macro LMM_META is illustrated in Appendix B.

SAS: Proc Mixed

SAS (Statistical Analysis System) is a software for advanced analytics. The SAS procedure to implement the linear mixed model is the "Proc Mixed". The researcher can specify the variables included as the random effects (statement *random*) and can insert the R matrix including the within study variance estimates and potential covariances (using the statements *repeated* and *parms*). In the *repeated* statement the researcher has to use the *subject* option. *Subject* option is necessary to define the blocks of R matrix while the *type* option is used to define their covariance structure. Moreover, the parms statement is needed to include the R matrix built with the variances and covariances of each included study.

Examples

To illustrate the four scenarios previously illustrated we used the meta-analysis data of the study "Office and ambulatory blood pressure to assess the change of blood pressure: a meta-analysis". The meta-analysis includes more than 50 RCTs and each study contains, for each arm, two blood pressure changes (treatment end minus baseline) measured on the same patients with two different techniques: Office and ABPM. The aim of the study was to quantify Office change (OC), ABPM change (AC) and the difference between the changes calculated with the two techniques Δ (OC-AC). For an easiest explanation of the process, only the estimates regarding the CCB, among all drug classes studied in the meta-analysis, will be considered. In scenarios 1 and 2 we will show the results of OC for systolic blood pressure.

Scenario 1. Indipendent estimates and fixed effects

The dataset "CCB all" has the following structure.

FIRST_AUTHOR	YEAR	ID_trial	ID_arm	Change	Change_variance	Technique
Grandi	1995	13	1	-20.00	25.44	01_office
Grandi	1995	13	1	-16.00	16.28	02_ABPM
Mancia	1997	28	2	-11.00	25.44	01_office

FIRST_AUTHOR	YEAR	ID_trial	ID_arm	Change	Change_variance	Technique
Mancia	1997	28	2	-15.00	14.31	02_ABPM
Mancia	2001	31	3	-30.00	24.36	01_office
Mancia	2001	31	3	-16.00	17.28	02_ABPM
Eguchi	2004	41	4	-26.00	5.17	01_office
Eguchi	2004	41	4	-19.00	15.43	02_ABPM

The variable ID_trial indicates the identification code for each study. The ID_arm indicates the identification code for each arm of each trial. This information is replicated because for each arm we have the information on both OC and AC. Finally, the variables change, change_variance and technique represent respectively the change in BP, the corresponding variance and the technique used to evaluate the change.

From this database we create the dataset "CCB Office". It includes only the OCs measurements. Now, if the researcher wants to make a fixed effects meta-analysis with independent estimates he/she should complete the macro statements in this way.

```
%LMM_META(db_input=CCB_office,
study=first_author,
year=year,
type_measure=MD,
num_measure=1,
outcome=Change,
variance_outcome=change_variance,
model=YES,
random=0,
output=matrix,
subject_repeat=id_trial,
group_repeat=id_trial,
type_matrix_R=un,
covcat=id_trial,
estimate_model=YES,
forest=YES,
Title_forest=Office change in CCB,
subtitle_forest=Mean Difference and 95% CI,
intercept=YES
);
```

And the SAS output is as follows:

(1) The options *model* and *estimate_model* are equal to YES and so the macro, initially, displays the results of the meta-analysis with fixed effects (statement *random* = 0) calculated both with inverse variance method (Overall IV fixed) and with LMM (Overall model fixed).

```
                      The SAS System

     STUDYNAME         estimate      lcl        ucl

 Overall IV fixed       -14.5692    -15.4947    -13.6437
 Overall model fixed    -14.5692    -15.5980    -13.5404
```

The two estimates are equal while the CIs are vary similar.

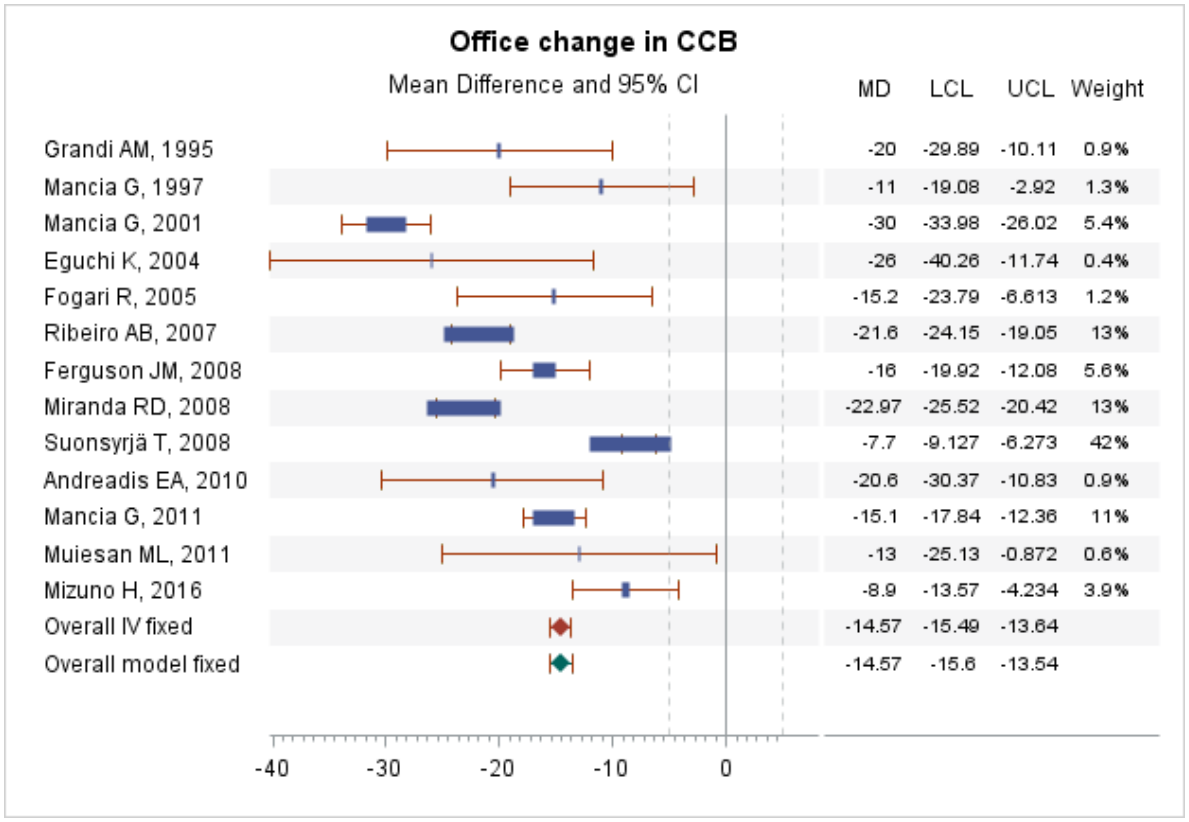
(2) If the researcher wants to calculate the summary estimate with the LMM, the macro, by default, displays a table reporting possible convergence problems

```
warning

The model converge
Estimated G matrix is positive definite
The Hessian matrix is positive definite
```

In our example we have not convergence problems and we have not problems in G and Hessian matrix.

(3) We have completed the statement forest=YES and so the macro builds the forest plot with the summary estimate calculated both with inverse variance and with LMM.



(4) Finally the macro, by default, evaluates the heterogeneity between studies, indeed we can observe the Q statistic and I^2.

```
variable                value

N estimates =           13.0000
Q =                    230.070
P value =               0.000
I square =             94.784
```

Scenario 2. Independent estimates and random effects

If the researcher is interested in the implementation of a random effect meta-analysis with independent estimates, he/she should complete the statement macro in this way (in our example, we considered the variable study as random effect).

```
%LMM_META(db_input=CCB_office,
study=first_author,
year=year,
type_measure=MD,
num_measure=1,
outcome=Change,
variance_outcome=change_variance
model=YES,
random=1,
random_value_1=0.5,
output=matrix,
eff_random_1=intercept,
subject_random_1=id_trial,
type_matrix_G=vc,
subject_repeat=id_trial,
group_repeat=id_trial,
type_matrix_R=un,
covcat=id_trial,
estimate_model=YES,
forest=YES,
Title_forest=Office change in CCB,
subtitle_forest=Mean Difference and 95% CI,
intercept=YES
);
```

Respect to Scenario 1 the researcher has to modify the statement *random* (which indicates the number of random effects) and he/she has to insert the statements *random_value_1, eff_random_1 and subject_random_1*. The type of matrix G is "variance components" by default but if the researcher wants to change this typology he/she should fill out the statement type_matrix_G.

The output of this scenario is as follows:

(1) Pooled estimates

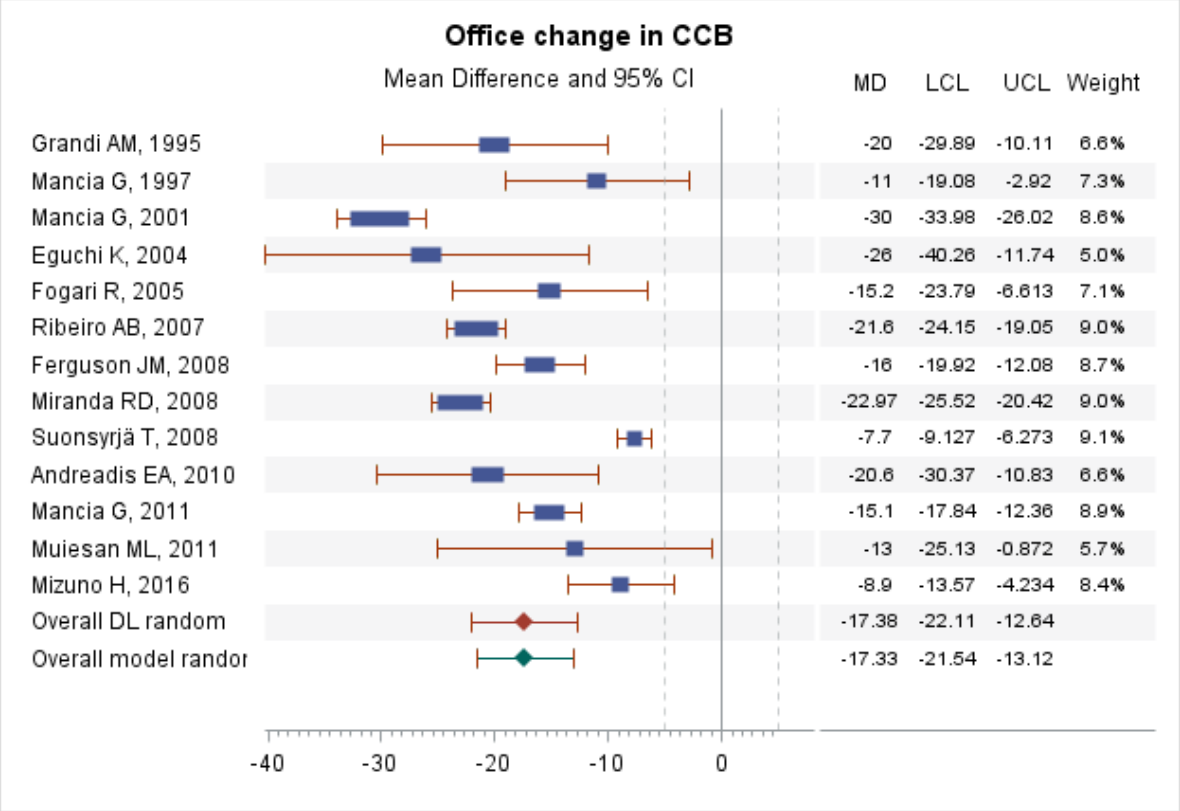
STUDYNAME	estimate	lcl	ucl
Overall DL random	-17.3760	-22.1099	-12.6421
Overall model random	-17.3320	-21.5406	-13.1230

(2) Convergence status

```
warning

 The model converge
 Estimated G matrix is positive definite
 The Hessian matrix is positive definite
```

(3) Forest Plot



(4) Heterogeneity statistics

variable	value
N estimates =	13.0000
Q =	230.070
P value =	0.000
I square =	94.784

Scenario 3. Dependent estimates and fixed effects

In scenarios 1 and 2, we showed scenarios on "classic" meta-analysis but our macro has the strength to carry out meta-analysis also with correlated data. To illustrate the scenarios 3 and 4 we use the database "CCB all". The aim of this analysis is to quantify the Δ (OC-AC). In order to calculate the summary estimate we found the value of the correlation coefficient (ρ), between the treatment-induced changes in Office BP and in ABPM, from the literature [24]. To take into account the random uncertainty about this parameter we built the 95% confidence interval (95% CI) by means the Fisher transformation method. The lower limit of this interval was considered to be more

conservative and its value was 0.09. For these situations the statement forest is equal to “NO”. In this example we complete the statement *intercept* equal to “YES” because we want to estimate the Δ (OC-AC). On the contrary, if we want to estimate the OC and AC considering the correlation between estimates the *intercept* statement have to be “NO”.

These are the macro statments for fixed effect meta-analysis:

```
%LMM_meta (db_input=CCB_all,
study=first_author,
year=year,
type_measure=MD,
measure_correlated=technique,
block_measure=id_arm,
num_measure=2,
outcome=change,
variance_outcome=change_variance,
model=YES,
corr21=0.09,
random=0,
output=matrix,
covariate= technique,
covcat= id_arm ID_trial technique,
subject_repeat=id_arm,
group_repeat=id_arm,
type_matrix_R=un,
estimate_model=YES,
forest=NO,
intercept=YES
);
```

As illustrated in Appendix B, we have to insert the statement *corr21* that indicates the correlation between OC and AC of the same study. We have to insert ID_arm in level_1 and technique in level_2 because, clearly, each arm includes both the OC and AC. Running this program gives the following output:

(1) Output results

STUDYNAME	estimate	lcl	ucl
Overall model fixed	-6.3087	-7.518	-5.0994

(2) Convergence status

```
 The model converge
 Estimated G matrix is positive definite
 The Hessian matrix is positive definite
```

Scenario 4. Dependent estimates and random effects

For random effect meta-analysis the macro statement are the following:

```
%LMM_meta (db_input=CCB_all,
study=first_author,
year=year,
type_measure=MD,
measure_correlated=technique,
block_measure=id_arm,
num_measure=2,
outcome=change,
variance_outcome=change_variance,
model=YES,
corr21=0.09,
random=1,
random_value_1=0.5,
output=matrix,
covariate=technique,
covcat=ID_arm ID_trial technique,
eff_random_1=intercept,
subject_random_1=ID_trial,
type_matrix_G=vc,
subject_repeat=id_arm,
group_repeat=id_arm,
type_matrix_R=un,
estimate_model=YES,
forest=NO,
intercept=YES
);
```

We considered the Study as random effects and the results are

(1) Outpu results

STUDYNAME	estimate	lcl	ucl
Overall model random	-5.1623	-7.9304	-2.3807

(2) Convergence status

```
warning

The model converge
Estimated G matrix is positive definite
The Hessian matrix is positive definite
```

(3) Heterogenity statistics

```
Isquare_R

6.13740
```

In this last scenario the macro calculates the I_R^2. The I_R^2 is the proportion of variation in the pooled estimates that is due to to heterogeneity between studies in a multivariate meta-analysis. As

explained by Jackson et al [81], the I_R^2 is calculated as $\frac{R^2-1}{R^2}$ where $R = \frac{V_R}{V_F}$. (VR and VF are the length of the confidence intervals for the pooled estimate from the random and fixed effect models). The previous explained method is used when the researcher wants to calculate the I_R^2 for each outcome separately, while if he/she wants to calculate the I_R^2 for all outcomes jointly the formula is: $\frac{|C_R|^{1/p}-|C_F|^{1/p}}{|C_R|^{1/p}}$. The matrix C_R is the covariance matrix obtained from the observed Fisher information matrix in random model, C_F is the same matrix but for the fixed model (p represents the number of included measures).

Meta regression

In a meta-analysis the researcher can observe high heterogeneity between estimates and it is therefore necessary to explore possible causes of heterogeneity. In a meta-analysis it is possible to implement meta-regression models including covariates at the study level that could explain the difference between the studies weighting each estimate for its variance. LMMs allow performing a meta-regression. To carry out a meta-regression with our macro is simple; the researcher has to input the covariates (i.e. the possible source of heterogeneity) in statement *covariate* and the macro will perform a meta-regression with random or fixed effects (depends by the choice of the researcher).

Conclusion

The results that we obtained with our macro were very similar to those obtained with R,STATA and Revman (for independents estimates) and with STATA (for dependent estimate). In Table is possible to compare the summary dependent estimates about Office and ABPM changes calculated considering jointly any antihypertensive drugs and obtained with SAS macro and with MVMETA command of STATA.

	SAS Change (95%CI)	STATA Change (95%CI)
Office	-18.92 (-20.11,-17.73)	-18.43 (-19.63,-17.24)
ABPM	-11.71 (-12.88,-10.54)	-11.87 (-12.74,-11.01)

In conclusion the macro allows i) to manipulate the data provided by the user in generic format to obtain the variance-covariance matrix to use in the mixed effect model; ii) modeling meta-analytical estimates by choosing between the fixed and random effects model for correlated and non-correlated data; iii) to build the forest plot of the chosen model; iv) to conduct a meta-regression analysis including fixed covariates in the LMM.

3.3 INCIDENT OF DEMENTIA ASSOCIATED WITH USE OF ACEIS: A META-ANALYSIS OF OBSERVATIONAL STUDIES

Introduction

The renin–angiotensin system (RAS) is a hormone system that regulates the blood volume and the systemic vascular resistance and mediates several physiological and pathological functions. Besides its presence at a peripheral level, its existence in the brain has been studied and confirmed recently by several works [82, 83]. The RAS controls fluid homeostasis in the body and plays a central role in blood pressure regulation by means of Angiotensin I (Ang I) to Angiotensin II (Ang II) peptide conversion pathway. Angiotensinogen peptide is cleaved by the hormone Renin to create Ang I which in turn is cleaved by the Ace Converting Enzyme (ACE) to produce the powerful vasoconstrictor Ang II. In response to low blood volumes, Ang II signalling is enhanced by the release of the steroid hormone aldosterone [84]. Currently this system has been implicated in several neurodegenerative disorders: the angiotensin peptide, and specifically Ang II, has been shown to induce cerebrovascular remodelling, promotion of vascular inflammation and oxidative stress resulting in dysregulation of cerebral blood flow [85, 86]. Pathological conditions related to this system include Alzheimer's disease (AD) and other forms of dementia [87]. The burden of dementia is increasing in the ageing populations worldwide. Increasing evidence shows the raised blood pressure is an important risk factor of dementia and cognitive impairment. Therefore, effective management of hypertension may translate into major health benefits through the preservation of cognitive function. Blood pressure, particularly systolic pressure, rises with age, leading to a high prevalence of hypertension in older people. Some trials have tried to study the association between antihypertensive treatment and incidence of dementia, however, few data available because cognitive measures are usually considered as secondary outcomes. In the last years some longitudinal observational studies have evaluated this association. A meta-analysis considering both RCTs and cohort studies showed the protective effect of any antihypertensive when only cohort studies are considered [88]. In another meta-analysis, considering the same

exposure, this protective effect was showed when RCT and observational studies were considered jointly [89]. Two meta-analysis conducted only on CCB [90] and diuretics [91] showed protective effects on the risk of dementia. To our knwlodge, no meta-analysis studied the effect of ACE on this outcome. Then, we decided to perform a meta-analysis to summarize the evidence regarding the effect of ACE in the prevention of incident dementia considering both observational and RCT. Regarding the pooled estimate on observational studies, we will adjust the summary estimate for an unmeasured confounder, namely chronic kidney disease.

Methods

We carried out a systematic MEDLINE search for observational studies and RCT published up to February 2017 investigating the association between use of ACE and risk of incident dementia. The search strategy used for the study identification is reported in Appendix C. All titles and abstracts were screened and the papers not compliant with the inclusion criteria were excluded. The specific inclusion criteria established following the tracks of Tully et al.'s work [91]. Study were included if (i) they were observational studies or RCT; (ii) they included adults without dementia at baseline; (iii) they considered as exposure ACE treatment, (iv) the comparator group consisted of subjects treated with placebo or any antihypertensive drug excluded ACE; (v) the diagnosis of incident dementia (outcome) was in accordance with standardized criteria (e.g. mini-mental state examination (MMSE) or diagnostic codes) and (vi) they reported crude or adjusted estimates of the association between exposure and outcome [hazard ratios (HRs) or relative risks (RRs) or odds ratios (ORs)] and the corresponding 95% confidence intervals (95% CIs) or data to calculate them. Incident dementia in any of the following categories: Alzheimer's disease, vascular dementia (VaD), mixed dementia, dementia unspecified, other dementia was considered as primary outcome. Several data were extracted from the studies: first author, year of publication, study design, study location, sample size, age, gender, dementia assessment, effect estimates and 95% CIs or information required to compute them and adjustment variables. When the studies reported multiple estimates with different levels of adjustment, only the most adjusted effect estimates were extracted.

For all analyses, the pooled RRs and 95% CIs were calculated. The HRs and ORs were considered to be approximations of the RRs. We used the Cochrane Q test [11] and the I^2 index [13] to assess the heterogeneity across all comparisons and to quantify the heterogeneity. If the heterogeneity was not significant, the RR from a fixed-effect model was reported. In presence of heterogeneity, the random-effect model was applied [12] and the potential sources of heterogeneity were explored. In the primary analysis, users of ACE were compared with no users. Moreover, we adjusted the estimates for chronic kidney disease (CKD) as dichotomous unmeasured confounder using McCandless method [18] and Monte Carlo Sensitivity Analysis (MCSA) (Section 2.3). CKD was chosen as unmeasured confounder because different studies showed that impaired kidney function is associated with the increased risk of cognitive impairment [92, 93] and recent studies observed that patients with CKD have an high risk of dementia[94-96]. In particular, Cheng et al. [96] developed a population-based cohort study using claims data of 1,000,000 insured residents covered in the universal health insurance of Taiwan. The study was carried out on about 100000 subjects whose about 37000 were with CKD newly diagnosed from years 2000 – 2006. The risk of dementia incidence was higher in the CKD patients (HR: 1.41 95% confidence interval (95%CI) 1.32 – 1.50). To apply the methods, we set the proportion of patients affected by CKD among ACE users to 4% and proportion of CKD among non-users equal to 2% [97]. An influence analysis was conducted by omitting one study at a time, to identify to what extent the results were influenced by a single study. Publication bias was assessed via visual inspection of the funnel plot and Egger test [25]. Results were considered statistically significant when two-tailed p-value was lower than <0.05. All analyses were performed with RevMan version 5.3 (Nordic Cochrane Center), STATA and SAS softwares (version 9.4; SAS Institute, Cary, NC, USA).

Results

Figure 9 shows the flow chart. The MEDLINE search identified 285 papers. On the basis on title and abstract 226 articles were excluded. 59 publications were included in the full-text evaluation , 13 articles were excluded because they were duplicated, 8 were not observational studies or RCTs,

17 had no relevant exposure or outcome and 12 papers had not the measure of interest. So 9 studies, including 2 RCTs [98-99] and 7 cohort studies [100-106] met the inclusion criteria. Because the trials were only two these were considered only for the discussion. The characteristics of the 7 included studies in the meta-analysis are shown in Table 4. The 7 observational studies comprised of 534.506 persons, of which 399.904 users of ACE drugs. All articles evaluated the incident dementia including both men and women, three papers had a mean age less than 75 [101, 104, 106] and four a mean age greater than 75 [100, 102, 103, 105]. We estimated the pooled effect obtained from 7 observational studies. In Figure 10 is shown the overall estimates of 7 suggesting that ACE drugs use appears to determine a non significant reduction of 9% of incident dementia (RR: 0.91; 95% CI: 0.76–1.08). Since a high heterogeneity was found (I^2=86%) and then the heterogeneity analysis was carried out stratifying by age and level of adjustments of association estimates. Stratifying for age (using 75 year as cut-off according to the World Health Organization criteria [107]), no difference between strata was observed in the association estimates (p-value=0.81 patients ≤ 75 years old (RR: 0.92; 95% CI: 0.70 –1.20) and patients > 75 years old (RR: 0.88; 95% CI,0.72–1.08), ,Figure 11). Analogously, stratifying for level of adjustments (< 5 adjustments versus ≥ 5 adjustments) we found that this characteristic was not a source of heterogenity (Figure 12, RR: 0.88 95% CI,0.69 –1.12 and RR: 0.93 95% CI,0.72 –1.19, respectively. p-value = 0.80) Influence analysis showed that no study influenced the summary estimate (data not show). There was not evidence of publication bias for studies investigating use of ACE and risk of dementia either from visualization of the funnel plot (Figure 13) and from the corresponding Egger's test (p-value = 0.576). Finally, adjusting for the CKD as unmeasured confounder, the summary estimate resulted 0.94 (95% CI: 0.62-1.47) with bayesian method and 0.92 (95% CI: 0.75-1.69) with MCSA method.

Discussion

The meta-analysis shows a non significant 9% reduction in dementia risk in patients treated with ACE drugs. The included studies were heterogenous but none of the sources of heterogeneity considered could explain it. Adjusting for unmeasured confounder the summary estimate was not changed.

This result is consistent with that reported by one of the RCTs identified in our MEDLINE search. The PROGRESS (Perindopril pROtection aGainst REcurrent Stroke Study) trial, a randomized, double-blind, placebo-controlled trial that enrolled 6105 men and women, with a mean age of 64 years, with previous stroke or transient ischemic attack reported a non significant reduced incidence of dementia (relative risk 0.88; p-value=0.2) in patients treated with perindopril and indapamide [98]. Conversely, our results are less consistent with those reported in the ONTARGET (Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial) trial, a double-blind, double-dummy, randomised controlled trial, based on 25620 participants with cardiovascular disease or diabetes (relative risk of 1.11; p-value=0.08). However, these comparisons should be take with caution because in these RCTs: i) dementia was a secondary outcome and therefore the study was insufficiently powered; ii) the effect was confounded by combined antihypertensive medication use to achieve acceptable blood pressure; iii) considered a composite outcome of dementia and cognitive impaiment.

Assurances with regard to the robustness of our findings arise from (i) a wide sample size obtained using a meta-analytic approach; (ii) the inclusion of only patients with hypertension because it is well known that high blood pressure is associated with cognitive deterioration and dementia [107]; iii) the inclusion of only studies that specifically evaluated the effect of ACE on the risk of dementia and not other form of cognitive impairment; (iv) the adjustmen of the summary estimate for potential unmeasured confounders. However, our results have limitations that mainly reflect the sources of bias of the observational studies included into the meta-analysis. First, we correct the summary estimates for a potential unmeasured confounders but we cannot exclude the possibility

that confounding by indication might explain our findings. Second, the strategy used for identification of the outcome varied from study to study and this could increase the heterogeneity. Third, the I^2 index was greater than 80%, representing a substantial heterogeneity. In conclusion, these results do not exclude the possibility that blood pressure management in general helps to preserve cognitive function. Clarifying this relationship remains of major importance. With the ageing of Western societies, we are facing an epidemic of dementia for which do not exist curative or preventive treatment. In this context, even a modest reduction in the risk would have important impact from a public health point of view. Moreover, even if high blood pressure is associated with a moderate reduction of the relative risk of dementia, the very high prevalence of the disease means that the risk of dementia attributable to high blood pressure may be elevated, and that an improved control of hypertension may be translated into a dramatic reduction in the number of cases of dementia [102].

Table 4 Chronological summary of literature on association between ACEI user and dementia, and their main characteristics.

Author	Sample size	Gender	Age mean	Investigated therapy	Reference therapy	Reported RR (95% CI)	Adjustment variables
Sink KM, 2009, USA [100]	1,054	MF	75	ACE	other HTN	1.01 (0.87 – 1.18)	age, sex, race, education, and income
Solfrizzi V et al., 2013, Italy [101]	873	MF	72	ACE	other HTN	0.39 (0.12 – 1.24)	age, gender, education, pack–years, type 2 diabetes, serum creatinine level, and apolipoprotein B to apolipoprotein A1 ratio at baseline as well as history of stroke, history of hypertension.
Yasar S et al., 2013, USA [102]	2,248	MF	78	ACE	BB	0.87 (0.53–1.42)	age, sex, years of education, income, smoking
Davies NM, 2014, UK [103]	97,496	MF	82	ACE	other HTN	0.80 (0.76 – 0.84)	age, gender, region, for number of GP consultations, systolic blood pressure, history of diabetes, stroke or coronary heart disease
Gohn KL et al, 2014, UK [104]	426,089	MF	< 75	ACE	ARB	1.09 (1.00 – 1.18)	Adjusted for age at first prescription, sex, body mass index, smoking, alcohol use, diabetes, hypertension, heart failure, statin use, socioeconomic status, calendar year, number of consultations in last 6 months and attained age
Chuang YF et al., 2015, USA [105]	1,992	MF	75	ACE	other HTN	1.06 (0.76 – 1.50)	age, sex, education, number of APOE ε4 alleles, and smoking, drinking habits at baseline, history of high cholesterol, diabetes, stroke, CABG, and MI
Kuan Y. C et al, 2016, Taiwan [106]	4,754	MF	65	ACEI	other HTN	0.74 (0.56 - 0.96)	Age, sex, comorbidities and medications

Figure 9

Flow chart of the selection of studies for inclusion in the meta-analysis.

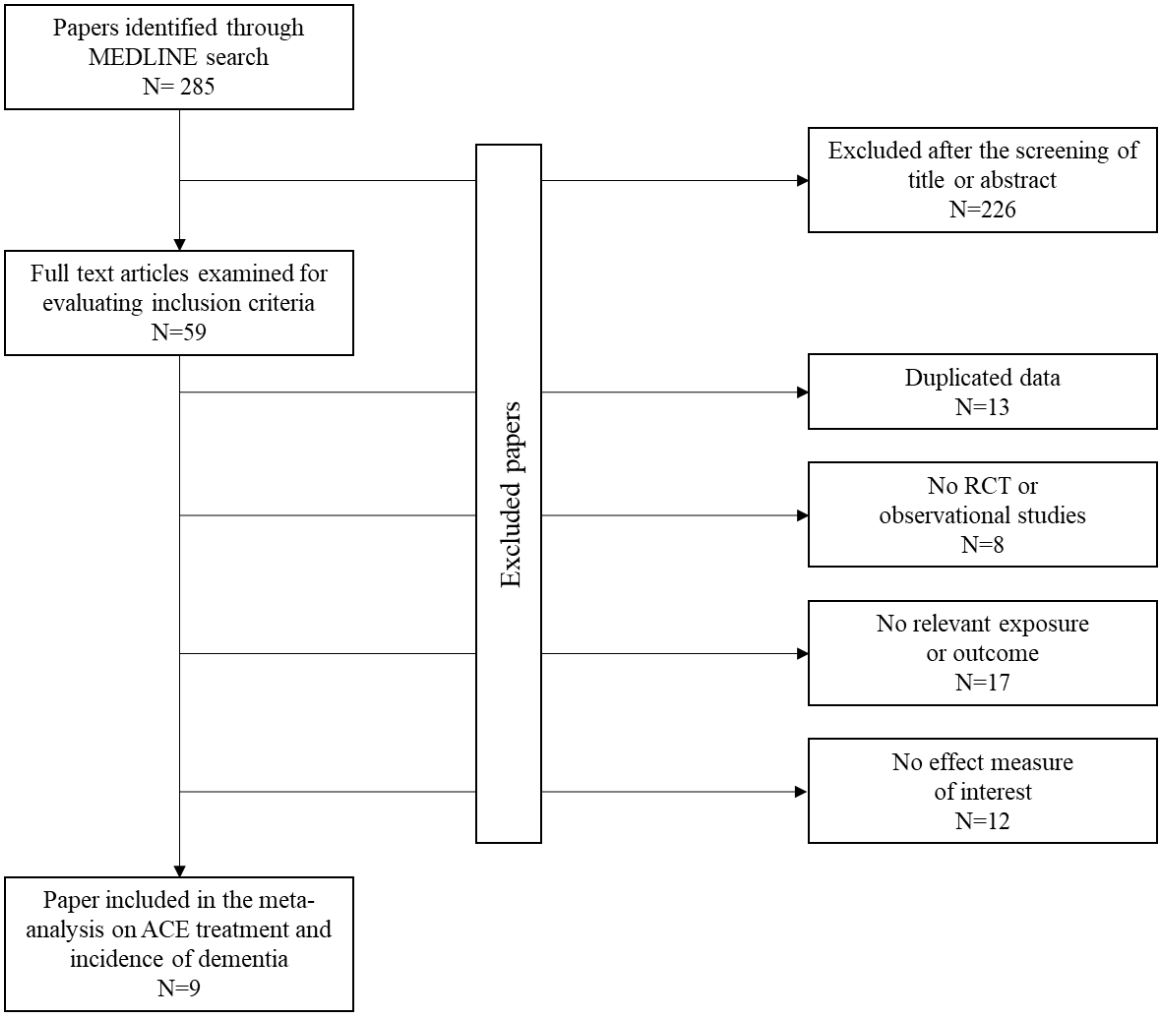


Figure 10

Study-specific and summary relative risk estimates for the association between ACE users and incidence of dementia in all included studies.

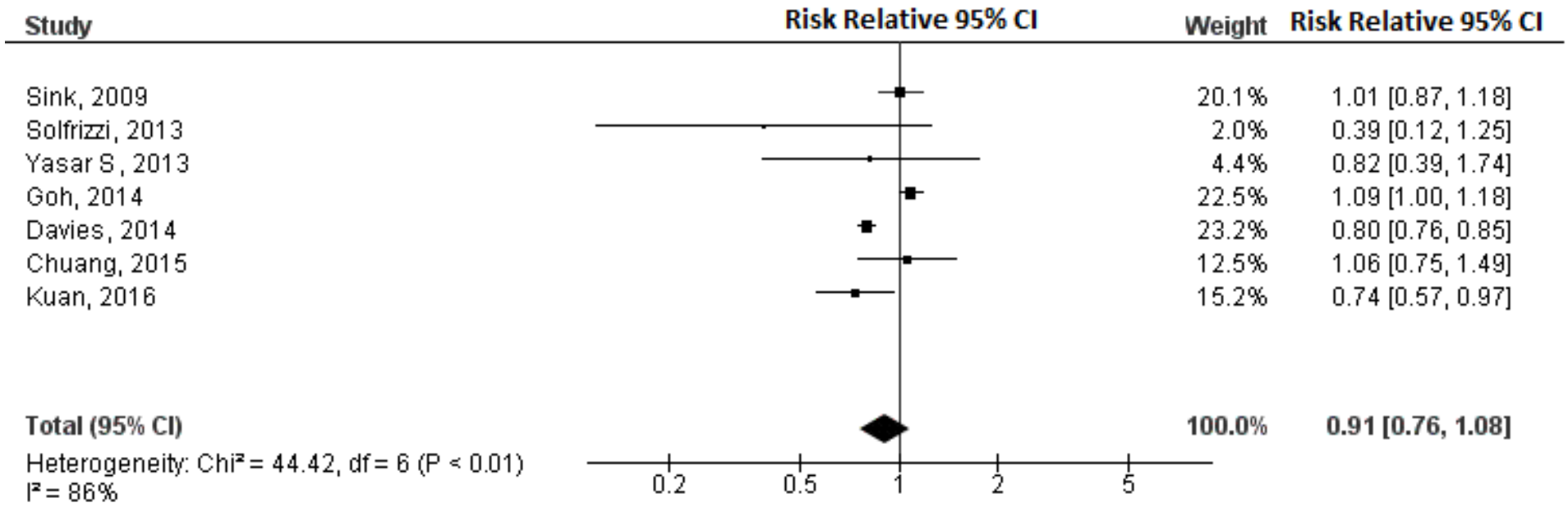


Figure 11

Study-specific and summary relative risk estimates for the association between ACEI users and incidence of dementia by age classes.

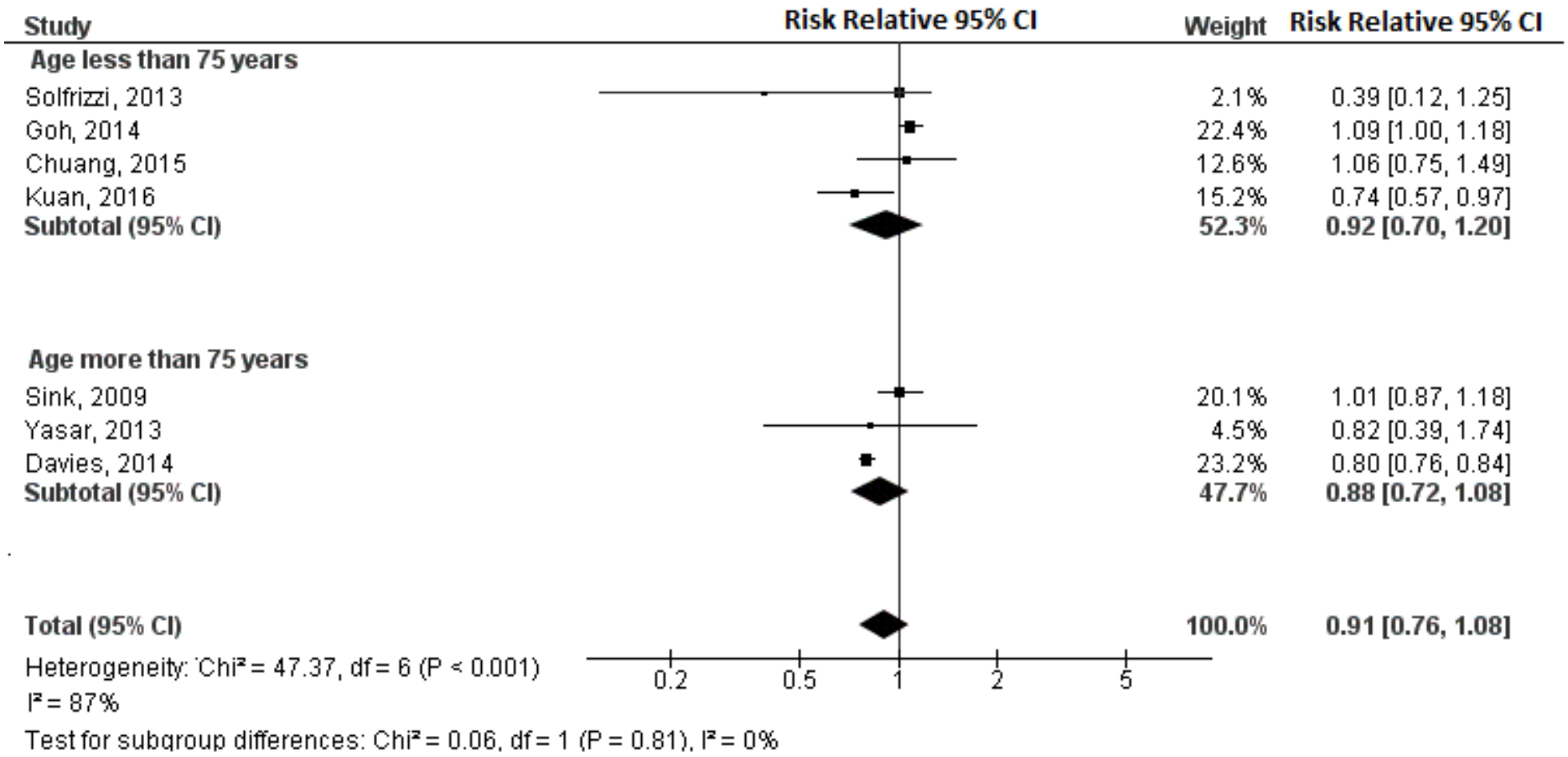


Figure 12

Study-specific and summary relative risk estimates for the association between ACE users and incidence of dementia by adjustment levels.

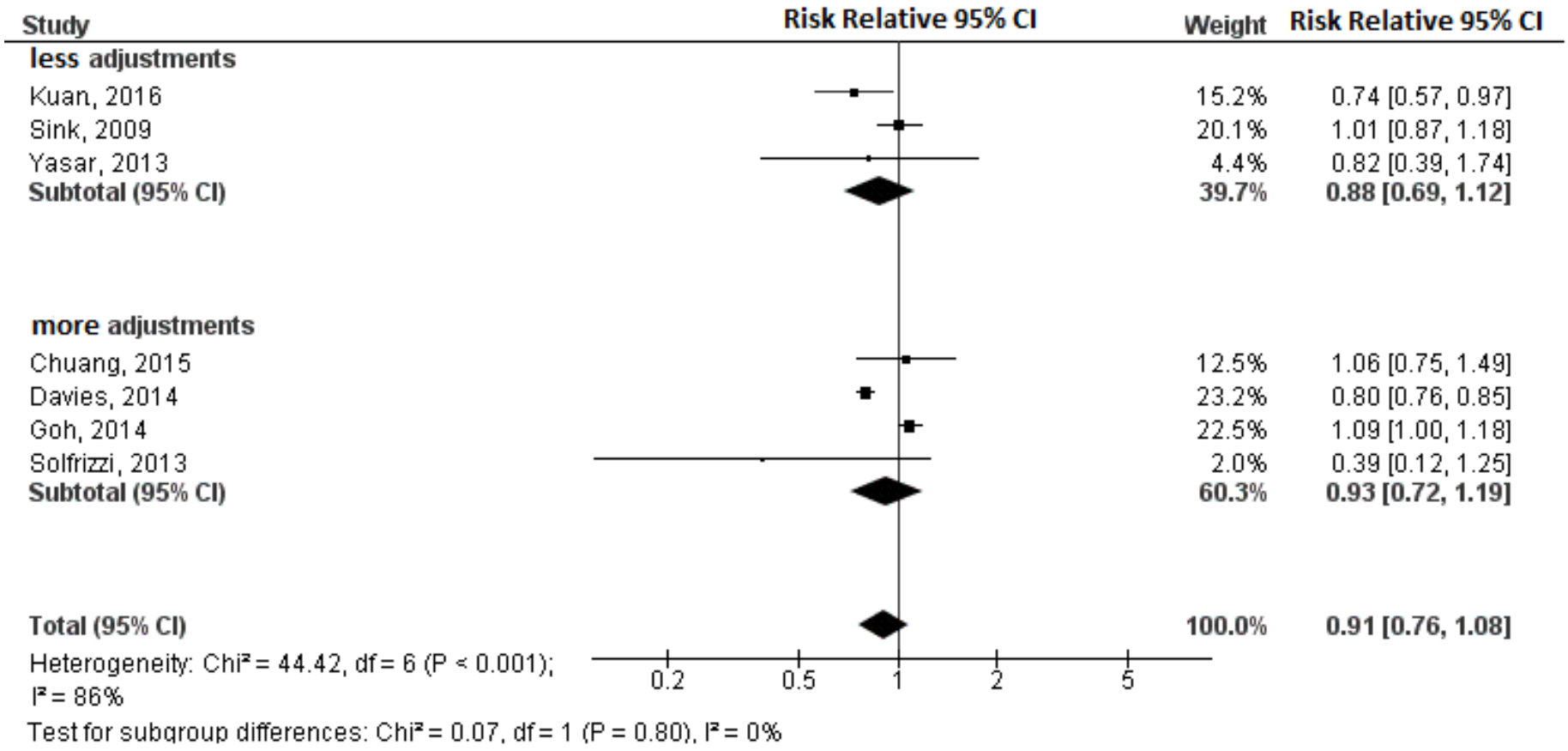
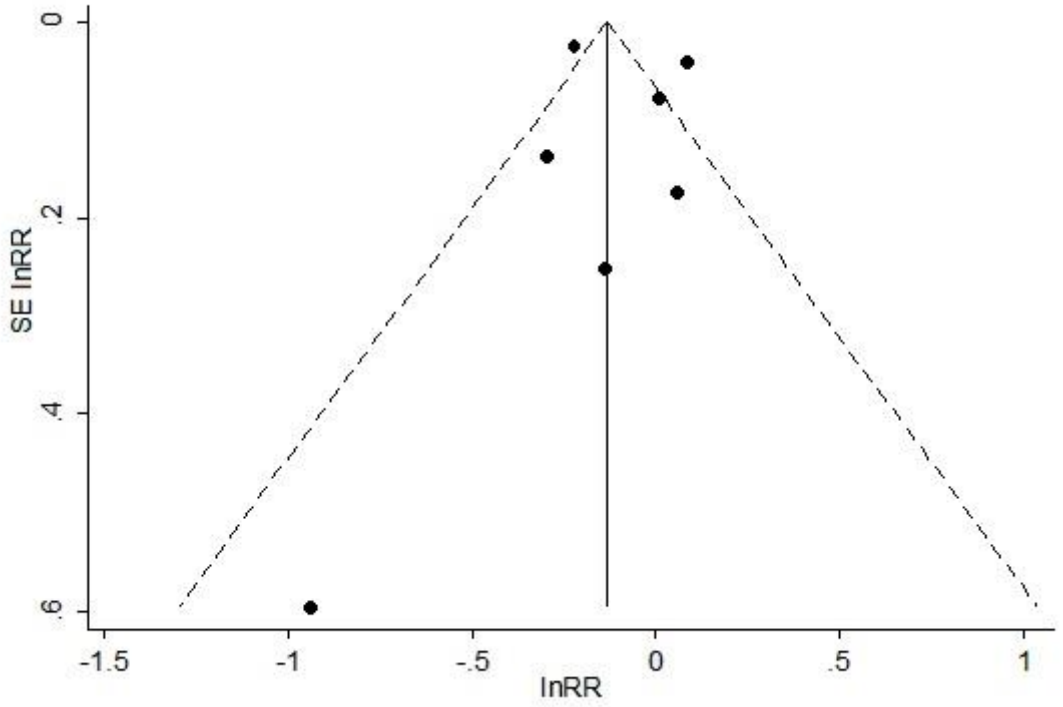


Figure 13

Funnel plot for publication bias in the study investigating use of ACE associated with incidence of dementia.



3.4 SAS MACRO FOR UNMEASURED CONFOUNDER IN META-ANALYSIS

As previous explained in section 2.2, if the researcher wants to apply the MCSA methods has to make different simulations, so we decided to build a SAS macro to simplify this process. The SAS macro is user friendly and the researcher has to insert only few statements (Appendix D). If we consider the data used in the meta-analysis ACE and dementia we have to build the database in this format:

First_author	RR	Inf	Sup	logy	tau
Sink	1.01	0.87	1.18	0.077749	0.00995
Solfrizzi	0.39	0.12	1.24	0.595759	-0.94161
Yasar	0.87	0.53	1.42	0.251412	-0.13926
Davies	0.8	0.76	0.84	0.025531	-0.22314

"logy" represents the logarithm of Risk Relative between ACE use and risk of dementia, "tau" is its standard error. Then we can complete the statements of the MCSA macro in this way:

```
%MCSA (data_input=DB, logRR=0.344, tauRR=0.032, logitp1=0.333, taup1=0.25,
logitp0=0.204, taup0=0.25, niter=100, outcome=logy, sd_outcome=tau);
```

Indeed, the logarithm of 1.41 is 0.344 and its standard error is 0.032. The prevalence of kidney injury in ACE users is 4% and so its logit is -3.178 while the prevalence of kidney injury in no ACE users is 2% and its logit is -3.892. We decided to set the standard deviations of the logits equal to 0.25. The output of the macro will be in the dataset "*output*" and finally we can input this dataset in the macro ***%LMM_meta*** to calculate the pooled estimate.

3.5 META ANALYSIS ON THE REGRESSION OF LEFT VENTRICULAR MASS IN PATIENTS TREATED WITH ANTIHYPERTENSIVE DRUGS

Introduction

Left ventricular hypertrophy (LVH) secondary to arterial hypertension is a complex cardiac phenotype resulting from the response of myocyte and non-myocyte components to mechanical and neuro-humoral stimuli [108]. LVH can be considered an important factor risk of cardiovascular (CV) events. To define LVH there are two measures: (i) left ventricular mass (LVM) and left ventricular mass index (LVMI). The left ventricular mass (LVM) is calculated as the difference between the epicardium delimited volume and the left ventricular chamber volume multiplied by an estimate of myocardial density while the LVMI is calculated as the ratio between the LVM and the body surface. Brown et al. showed that subjects with LVH were twice as likely to die of coronary hearth disease (CHD) (relative risk (RR) 2.0; 95% confidence interval (95% CI): 1.2 - 3.5) and diseases of the heart (relative risk (RR) 1.9; 95% CI 1.1, 3.0) respect to subjects without LVH. [109]. Also Desai et al [110] showed that the increase of LV mass (LVM) were associated with stroke and heart failure events (Hazard Ratio (HR) 1.20; 95% CI 1.00 – 1.40; HR 1.40; 95% CI 1.20 – 1.50, respectively). Because the hearth is a muscle, the ventricular hypertrophy can be considered a phenomenon almost reversible but to defeat the disease it is essential to intervene promptly, despite the symptoms manifest when the disease is already in advanced stage. The presence of LVH is determined using echocardiography (ECHO) or echocardiogram (ECG) to classify hypertensive patients as at high or very high risk for cardiovascular diseases (CVD). ECHO and ECG LVH have been found to carry different prognostic information [111]. Current guidelines have accepted LVH as an important risk factor for CVD morbidity and mortality and recommend that a 12-lead ECG should be the first-line method for the diagnosis of LVH in all hypertensive patients [111, 112]. If the hypertension is not treated the patient can have clinical events on more organs (heart, brain, kidney ecc..). Regard to the heart, the hypertension results one of the main causes leading to LVH. The increase of BP values can cause a burden of the left ventricular at each heart cycle. Because of high blood pressure, the left ventricular has to perform stronger contractions and then to increase

the ventricular tension. This phenomenon takes an increase in oxygen consumption because the heart has some difficulties in pumping blood to the rest of the body.

In the clinical practice, several drugs were administered in order to reduce BP values. The antihypertensive drugs can be divided into classes based on the main site of action or the mechanism on which they carry out their action. The principal classes are diuretics, beta-blockers (BBs), calcium antagonists (CCBs), angiontesin converting enzyme inhibitors (ACE) and angiotensin receptor blocker (ARB). In literature there are meta-analysis base on RCTs that show that the antihypertensive treatment reduces the LVM compared to placebo in hypertensive patients [113, 114, 115]. Moreover other meta-analysis, ever based on RCTs, studied the comparison between the reductions of LVM between each antihypertensive class. From this study seem that ARBs have a stronger effect on reduction of the LVM compared to other antihypertensive classes while the Beta-Blockers seem to reduce less the LVM [116]. In light of the increasing prevalence of patients under antihypertensive treatment, efforts aimed at elucidating the association between use of these drugs and regression of LVH have major implications for public health. In particular, because the regression of LVH is considered a surrogate endpoints of cardiovascular events. Indeed, it is demonstrated that the regression of LVM was associated with significant reductions in CV risk [117]. With these premises and in order to quantify the regression of LVM, in real world practice, both considering jointly all antihypertensive drugs and each class-specific separately, we performed a meta-analysis of observational studies.

Methods

We carried out a MEDLINE search for studies published up to March 2017 investigating the reduction of LVH in patients treated with antihypertensive drugs. The keyword and/or corresponding Mesh terms used in this search are in appendix E. Studies were included if they (i) were observational studies (cohort studies), (ii) had hypertensive patients treated with antihypertensive drugs, (iii) reported the mean change of LVM or LVMI from start to end of study

on the same patients; (c) reported variability measures of the mean change of LVM or LVMI (left ventricular mass index) (standard deviation, standard error or 95% confidence interval) or sufficient data to allow their calculation. For each study, we extracted details on study design, country, publication year, follow-up duration, number of patients, male percentage, age mean, baseline systolic and diastolic blood pressure, antihypertensive class, type outcome (LVM or LVMI) and type of ultrasound test (Echocardiography or Echocardiogram). The estimate of interest was the mean change of LVM or LVMI (Δ LVM and Δ LVMI) analysed separately. Heterogeneity between study-specific estimates was tested using the Q statistic [11] and quantified with I^2 [13]. We calculated the summary estimates using both fixed-effects model and the random effect models proposed by Dersimonian and Laird [12]. In presence of heterogeneity (p-value of Q statistic < 0.05), we showed the results from the random-effect models. Several subanalyses were carried out by stratifying original estimates according to: type of antihypertensive class, systolic blood pressure at baseline, LVM/LVMI at baseline, type of ultrasound test and length of follow-up. A Cochrane's Q test was computed for each subgroup difference. Finally publication bias was evaluated both by funnel plot and Egger test [25]. For all hypothesis tests, evidence was based on a p-value < 0.05, and the 95% CIs were therefore presented. The corresponding calculations and graphical visualizations of forest and funnel plots were respectively carried out using RevMan version 5.1 (Nordic Cochrane Center) and STATA Software Program version 9 (STATA, College Station, TX).

Results

On the basis of title and abstract we identified 2184 articles. Of these, 1977 studies did not include patients with antihypertensive drug or they were not observational studies; 197 papers had not the necessary data for the analysis. Finally 10 articles [118-127] were included in the meta-analysis. The flow chart is reported in Figure 14. The characteristics of the 10 included studies in the meta-analysis are shown in Table 5. A total of 2017 subjects were included, the average age ranged from 45.0 to 70.0 years and the percentage of men from 26.0% to 70.0%. All patients had hypertension by definition. 6 articles measured the change with LVMI and 4 articles measured the change both

with LVMI and LVM. 4 articles reported the change for ARB users, 2 papers for BB users and in 3 studies for CCB users. The difference in LVMI and LVM, between start and end of the study, were considered the outcomes of interest (Δ LVMI and Δ LVM). Considering all antihypertensive classes together the Δ LVMI was 10.53 g/m^2 (95% CI: 7.49 – 13.57, I^2=84%) while for ARB users 12.48 g/m^2 (95% CI: 6.89 – 18.07). For BB users the summary estimates was 10.62 g/m^2 (95% CI: 8.74 – 12.49), and for CCB users was 10.50 g/m^2 (95% CI: 1.21 – 19.79) (Figure 15). The difference between three estimates were not statistically significant (Q statistic p-value=0.80). The subanalyses showed that the Δ LVMI measured with echocardiography was higher than Δ LVMI measured with echocardiogram (Δ LVMI: 9.35 g/m^2 95% CI: 5.44 – 13.26 and Δ LVMI: 5.39 g/m^2 95% CI 3.91 – 6.86 respectively) but the difference was not statistically significant (p-value = 0.30). The Δ LVMI increased in studies with longer follow-up (FU) (7.66 g/m^2 (FU less than 6 months) vs 11.69 g/m^2 (FU more than 6 months), p-value=0.25). Concerning to patient charactheristics, the subjects with lower values of SBP at baseline (SBP < 160 mmHg) had a decrement LVMI equal to 11.22 g/m^2 (95% CI 7.99 – 14.45) while patients with higher value of SBP at baseline (SBP ≥ 160 mmHg) had Δ LVMI of 9.66 g/m^2 (95% CI 5.41 – 13.91) but also in this case the difference was not statistically significant (p-value = 0.57). There is no difference (p-value = 0.81) between patients with high values of LVMI at baseline (≥ 145 g/m^2) respect to patients with low values of LVMI (< 145 g/m^2) (10.25 g/m^2 95% CI 6.65 – 13.86 vs 11.02 g/m^2 95% CI 5.73 – 16.31). There was evidence of publication bias from both visualization of the funnel plot (Figure 16) and using the Egger test (p-value=0.017).

Finally we carried out the meta-analysis on the change of LVM. The Δ LVM was 19.46 g (95% CI: 5.90 – 33.30). In this case we did not perform the subanalysis because the low number of estimates.

Discussion

The focus of our meta-analysis based on observational studies was to quantify the summary difference of LVMI and LVM changes in hypertensive patients treated with antihypertensive drugs. Firstly, we wanted to quantify these changes in different classes of antihypertensive drugs and later in several study and patient characteristics. At the beginning, our analysis is focused on left ventricular mass index. Our results showed a Δ LVMI of 10.53 g/m^2 (95% CI: 7.49 to 13.57) and so this data confirms an effect of antihypertensive drugs in the reduction of left ventricular mass index. Concerning to the different antihypertensive classes, we showed that the reductions of BB, CCB and ARB are very similar. Indeed, in ARB users we observe a Δ LVMI of 12.48 g/m2 (95% CI: 6.89 to 18.07), in BB users of 10.62 g/m2 (95% CI: 8.74 to 12.99) and in CCB of 10.62 g/m2 (95% CI: 1.21 to 19.79). Also the percentage decrease is very similar in the three antihypertensive classes (9% (95% CI: 4% to 14%) in CCB and 8.5% (95% CI: 5% to 12%) in ARB and 8% (95% CI: 6% to 10%) BB).

These results agree with those reported in literature but suggest less pronounced percentage reduction and none differences between classes of antihypertensive drugs. Infact, the review by Ferreira Filho C. et al reported for BB and diuretics a 5-8% reductions of LVH, while the use of ACE and ARB resulted in a 13% reduction [128]. The meta-analysis conducted by Klingbeil et al. and based on 8 RCTs including 3767 patients, showed percentage reduction in LVMI of 13% with ARB (95% CI: 8% to 18%), 11% with CCBs (95% CI: 9% to 13%) and 6% with BB (95% CI: 3% to 8%)) [114]. The meta-analysis conducted by Dahlof et al [113] was based on 109 studies comprised 2357 patients with an average age of 49 years (range 30 to 71). Dalhof showed that the overall left ventricular mass (LVM) was reduced by 11.9% (95% CI: 10.1 to 13.7). Finally, the network meta-analysis conducted by Fagard [116] et al showed that in pairwise comparisons of the 5 drug classes (ACE, ARB, BB, CCB and diuretics), the only significant difference between drug classes is a lesser regression in LVM by BB respect to ARB. Several strengths characterized our

meta-analysis. First, it included studies based on large sample size allowing to obtain precise estimates; second, it included studies with only hypertensive patients because it is well known that hypertension is associated with LVH. Our meta-analysis can be affected by some limitations. First, patients treated with different antihypertensive classes are likely to have different clinical characteristics especially regarding comorbidity and severity of hypertension. So, we cannot exclude that the results are affected by confounding by indication, even if (i) the observed effects are similar between groups while in presence of confounding by indication we expected more pronounced differencences among groups; (ii) baseline blood pressure mean (both systolic and diastolic) in the 3 antihypertensive classes considered in our meta-analysis were very similar. Second, the included studies were heterogeneous particularly respect to the length of follow-up and to the instruments used to measure the LVH. Finally, we limited the analysis only to 3 antihypertensive class drugs for LVMI and to the antihypertensive drugs as a unique class for LVM because of the low number of available studies. Third, we found evidence for selective inclusion of studies reporting higher reduction of LVMI/LVM. The publication bias could be related to the use of MEDLINE (via Pubmed) as the only source for literature research, as well as to the exclusion of (i) “grey literature” (for example, Ph.D. theses and conference abstracts), (ii) other bibliographic databases (for example Embase) and (iii) of studies published in a language different from English. In conclusion, in the clinical practice, antihypertensive drug classes seem have no different effect on left ventricular mass index even if there are actually few observational studies available. Probably, in the next years, several meta-analyses more precise will be able to carry out. Evidences about the effect of antihypertensive drugs on the LVH damage can have an important impact because the decrease in ventricular mass during treatment is a favorable prognostic marker for subsequent morbid events.

Table 5 Chronological summary of literature on regression of left ventricular mass due to pharmacological antihypertensive treatment, and their main characteristics.

First author, publication year, country	Follow-up (weeks)	Number patients	% male	Age mean	Baseline SBP *mean±SD*	Baseline DBP *mean±SD*	Antihypertensive class	Type outcome	Ultrasound test	Pre LVM *mean±SD*	Post LVM *mean±SD*	Δ LVM *mean±SE*
Gu et al, 2016, China [118]	375	803	35.9	68.2	162.30±8.1	91.90±5.6	β-blocker	LVMI	Echocardiography	149.20±15.50		9.70±0.29
		695	33.8	67.7	161.60±9.2	91.60±6.8	Any (excepted β-blocker)	LVMI	Echocardiography	148.60±17.00		2.50±0.22
Zeng et al, 2016, China [119]	8	100	51.0	52.7	145.70±10.9	85.10±8.6	CCB + statins	LVMI	Echocardiography	121.65±35.19	108.07±36.92	13.58±0.87
Degirmenci et al, 2014, Norway [120]	52	28	53.6	56.7	151.40±8.5	92.00±7.0	ARB	LVMI	Echocardiography	144.20±151.00		6.90±1.63
		25	50.0	55.6	155.60±6.7	93.60±7.2	β-blocker	LVMI	Echocardiography	137.30±12.30		10.90±1.33
		32	40.0	57.7	153.40±7.5	90.80±5.3	β-blocker	LVMI	Echocardiography	139.50±12.40		14.00±2.30
Barrios et al, 2007, Spain [121]	26	97	30.9	68.9	160.40±12.0	90.40±9.0	ARB	LVMI	Echocardiogram	150.00±32.00	133.00±33.00	17.00±5.01
		30	100.0	65.0	157.50±10.0	91.60±8.0	ARB	LVMI	Echocardiogram	165.00±32.00	149.00±44.00	16.00±4.37
		67	0.0	70.5	162.60±12.0	90.30±9.0	ARB	LVMI	Echocardiogram	144.00±31.00	126.00±23.00	18.00±5.23
Hirono et al, 2002, China [122]	52	40	62.0	58.0	171.00±22.0	94.00±10.0	CCB	LVMI	Echocardiogram	139.00±9.00	121.00±18.00	18.00±6.11
Mutlu et al, 2002, Turkey [123]	26	30		51.2	162.60±20.1	100.30±3.9	ARB	LVM	Echocardiography	226.10±43.90	203.30±33.10	22.80±9.99
Klingbeil et al, 2000, Germany [124]	26	20	70.0	45.0	139.00±3.2	87.00±1.9	ARB	LVMI	Echocardiography	162.00±51.88	144.00±37.57	18.00±4.64
		20	70.0	45.0	139.00±3.2	87.00±1.9	ARB	LVM	Echocardiography	294.00±98.39	262.00±71.55	32.00±11.19
Ayoub et al, 1999, Brasil [125]	13	19	26.3	56.1			ARB	LVMI	Echocardiography	141.00±21.79	139.00±26.15	2.00±5.11
Aepfelbacher et al, 1997, Louisiana [126]	12	14	50.0	57.0	167.00±30.0	101.00±9.0	ACE	LVM	Echocardiography	275.00±104.00	250.00±95.00	25.00±7.82
		14	50.0	57.0	167.00±30.0	101.00±9.0	ACE	LVMI	Echocardiography	144.00±46.00	131.00±43.00	13.00±3.73
De Simone et al, 1987, Italy [127]	8	14	64.3	46.6	165.00±12.0	108.00±8.0	CCB	LVM	Echocardiogram	195.00±48.00	188.00±49.00	7.00±2.08
		14	64.3	46.6	165.00±12.0	108.00±8.0	CCB	LVMI	Echocardiogram	104.00±22.00	101.00±22.00	4.00±0.80

Figure 14

Flow chart of the selection of studies for inclusion in the meta-analysis.

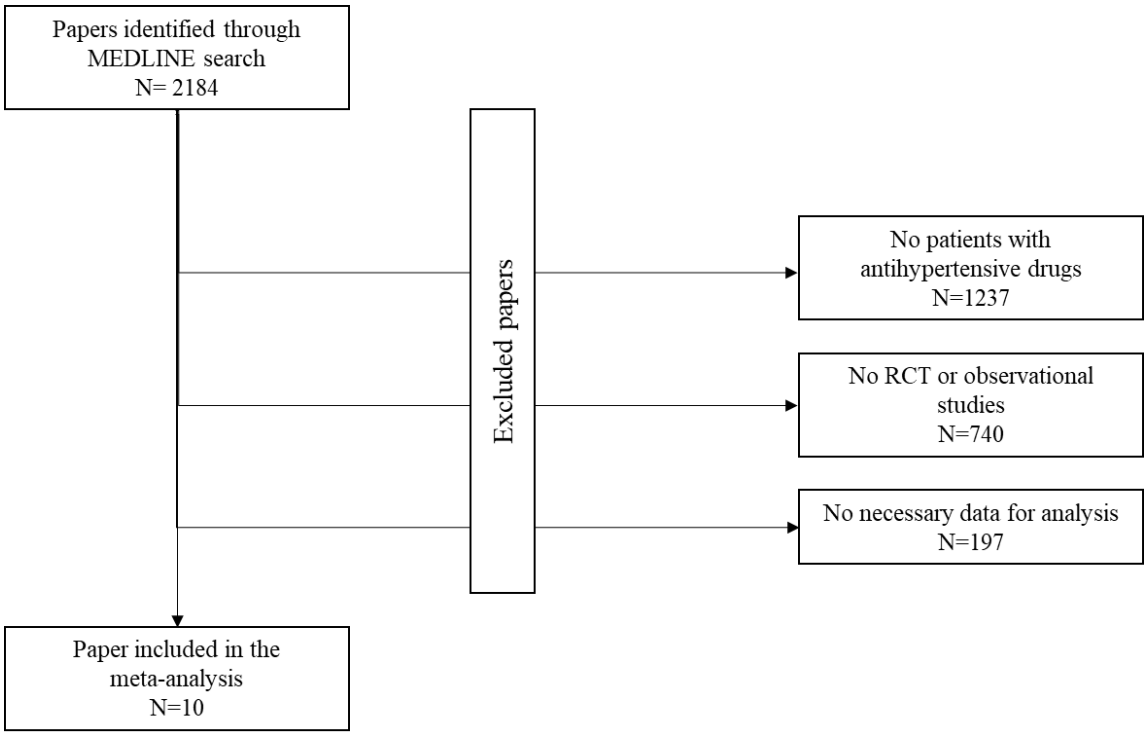


Figure 15

Study-specific and summary reduction estimates of LVMI estimates in all antihypertensive classes and in specific treatments (ARB, BB and CCB).

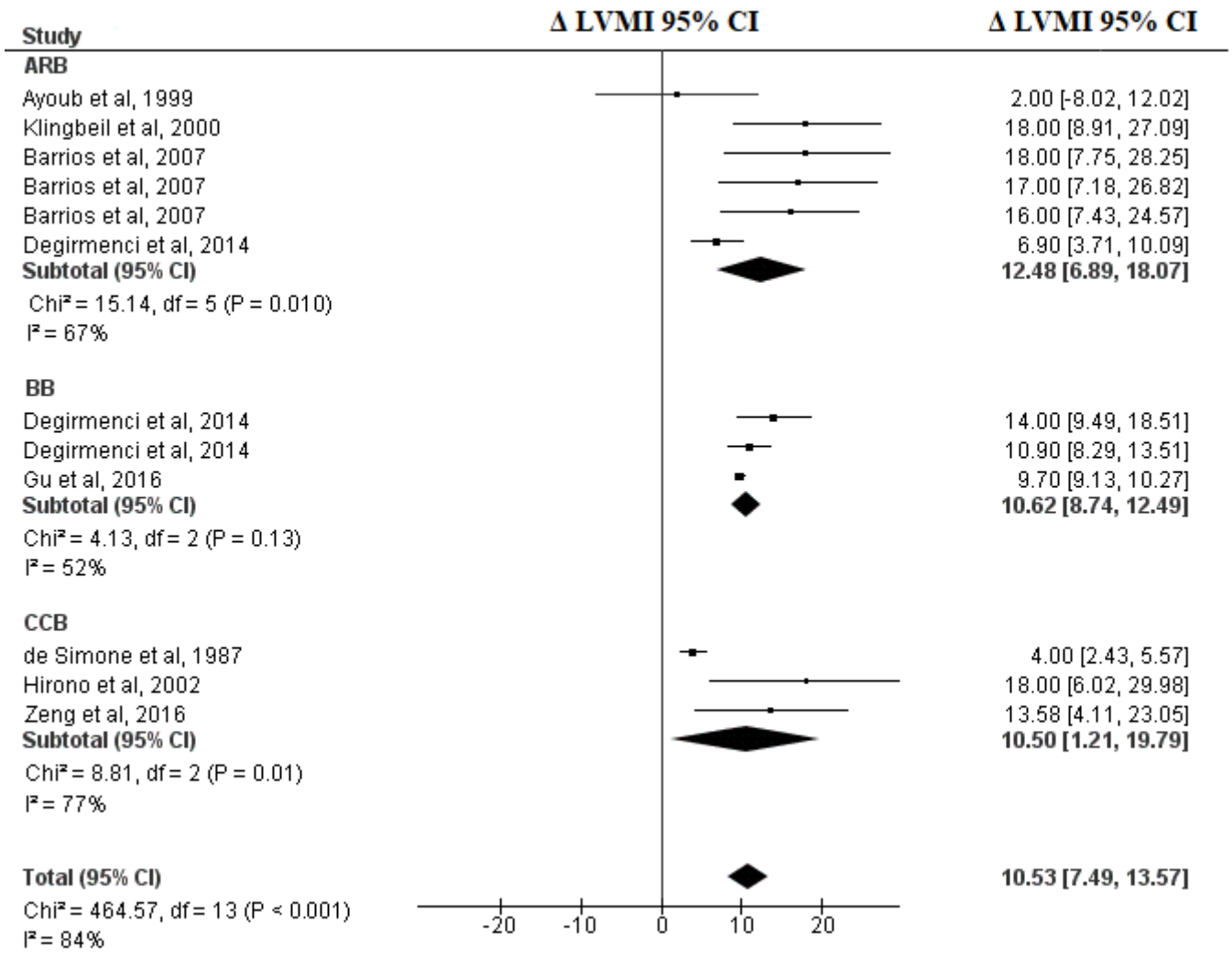
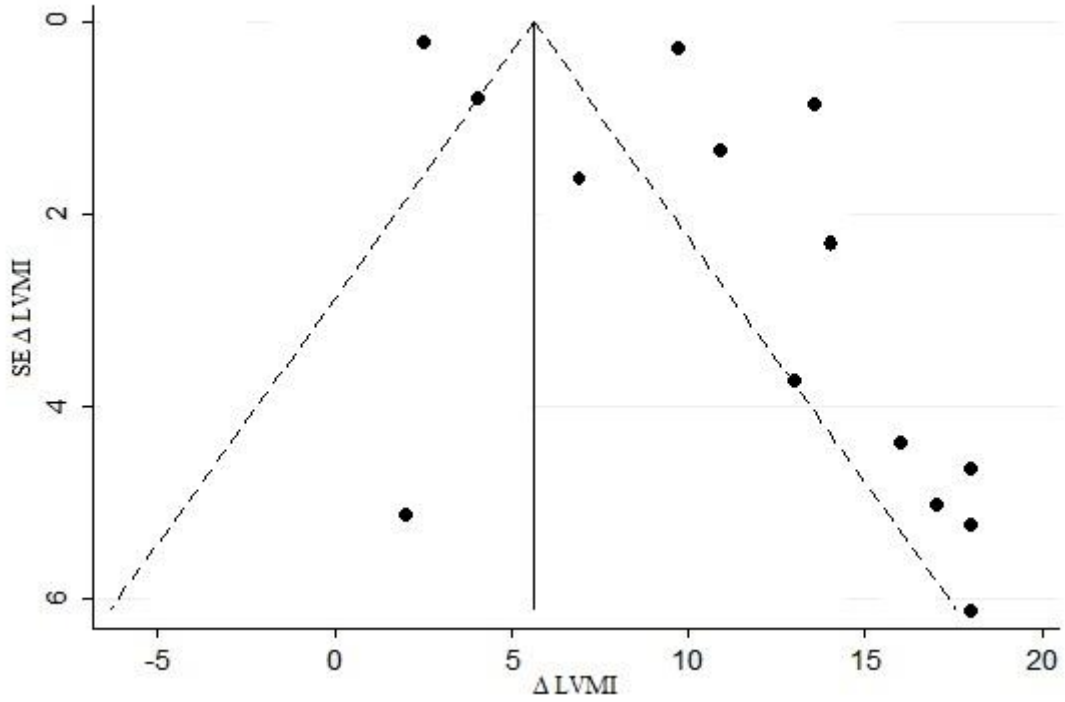


Figure 16

Funnel plot for publication bias in the study investigating the reduction of LVMI.



4 AUTOMATION OF META-ANALYTICAL SEARCH PROCESS

As introduced in Chapter 1, a meta-analysis collects and analyses the results from multiple studies that are all focused on the same research question. Meta-analytic studies can estimate the overall effect across the studies, lead to changes in clinical guidelines, or spur new directions for research. The first step in the meta-analysis consists in the identification of a search strategy to implement in the literature search engines (i.e. PubMed) aimed to identify all relevant studies related to the research question and then to clarify the inclusion and exclusion criteria. This process is usually performed manually. In this way, the researcher will find several potential articles to include in the meta-analysis and this is the base for the start of the review process of each paper. Initially the screening by title and abstract is carried out to exclude those articles that are clearly unrelated to the topic of interest and afterward a review of the full text of the remaining papers is performed to evaluate if the study met the inclusion criteria leading to final selection of the articles to be included. Finally, the data extraction of the final set of studies is realized. This is an extremely time consuming process. The sheer scope of the manual effort involved causes two fundamental challenges in widely applying meta-analysis to medicine in general. First, many topics are left unexplored, either due to the lack of researcher interest or lack of time to produce the review. That is, the sheer scope of time required may outweigh the interest in every possible disease and outcome. Second, meta-analyses are often not updated to reflect the latest results and studies. Rather than being a dynamic report that changes with the results, they reflect only a snapshot in time, up to the point when the review was produced. [127]. While daunting to produce, meta-analyses are nonetheless extremely important. Therefore, methods that address the automatization of the meta-analysis, as much as possible, process are very useful. This should greatly reduce human bias; increase the dissemination of evidence, especially for diseases and interventions with less focused attention.

In fact, the key tasks in the meta-study correspond to different techniques in machine learning (ML). The task of searching the literature by disease, intervention and primary outcome could be essentially a “clustering” task that should learn to automatically group the papers together based on the same sets of features [128]. Alternatively, it is possible to approach the problem considering that the data available in the main bibliographic database used for the search of the articles to include in the meta-analysis (Pubed central) can be imported in a graph database (a NoSQL database that is a DB without a priori domain or structure), and then it is possible to investigate the different type of relationship that link the different entities in the context of the scientific publication (e.g.. authors, journals, affiliations). In fact, they build up a social network, that identify a group of individuals connected by different links; therefore, the use of graphs to study networks of subjects is particularly suitable in this context where each entity is a knot and the relationship an arch. It is therefore natural to choose the graph database to model the information available in PubMed on the social network of the scientific publication. The process was divided in two fundamental phases:

Data structuring

The information of interest are contained in the scientific articles written in natural language, downloaded from PubMed Central database. To allow data upload on a generic database, it is necessary to extract the information of interest and to save them in tables. It is fundamental to verify the quality and consistency of the data and, eventually, to remove inconsistencies and redundancy in the database using record linkage techniques. Moreover, for each article, three keyword are extracted using the RAKE algorithm to obtain a first classification of the documents through a limited number of keywords.

Construction of the Graph Database

This phase aimed to upload the information extracted from the articles on an oriented graph structure, where each knot corresponds to an entity in the real world. In the conduction of this

activity, several computational and efficiency problems came to light due to the extent of the data analysed: for example, the construction of the relationship between knots within the graph resulted really complicated. Indeed, even after many days subsequent the run of the Cypher scripts, the relationships were not created, making impossible the simultaneous check the accuracy of the operations. Moreover, increasing the dimension of the database, the queries were processed slowlier creating a vicious circle. My contribution in the development of the prototype consisted in the project planning and especially in the share of the pubmed data structure and the intrinsic problems related to the handmade literature search. The technical development of the prototype was made by dr. Mirko Cesarini and an activity aimed to verify the learning ability of the algorithm was carried out exploiting the data collected for the meta-analysis on ACE use and incidence of dementia. Currently the first version of prototype of the bibliographic research tool can retrieve only a part of the entire set of suitable articles for the meta-analysis. Further efforts are needed to improve the search algorithm.

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6 APPENDICES

Appendix A

("Office"[All Fields] OR "clinic"[All Fields] OR "Office"[Mesh] OR "clinic"[Mesh]) AND ("ambulatory"[All Fields] OR "24-h"[All Fields] OR "24 hours"[All Fields] OR "ambulatory"[Mesh] OR "24-h"[Mesh] OR "24 hours"[Mesh]) AND ("antihypertensive"[All Fields] OR "antihypertensive agents"[MeSH Terms] OR "angiotensin II type 1 receptor blockers"[MeSH Terms] OR "receptors, angiotensin"[MeSH Terms] OR "losartan"[Text Word] OR "valsartan"[Text Word] OR "irbesartan"[Text Word] OR "candesartan"[Text Word] OR "telmisartan"[Text Word] OR "olmesartan"[Text Word] OR “eprosartan”[Text Word] OR "tasosartan"[Text Word] OR "azilsartan"[Text Word] OR "angiotensin converting enzyme inhibitors"[MeSH Terms] OR "captopril"[Text Word] OR "enalapril"[Text Word] OR "quinapril"[Text Word] OR "fosinopril"[Text Word] OR "lisinopril"[Text Word] OR “delapril” [Text Word] OR "cilazapril"[Text Word] OR "ramipril"[Text Word] OR "perindopril"[Text Word] OR "zofenopril"[Text Word] OR "benazepril"[Text Word] OR "trandolapril"[Text Word] OR "spirapril"[Text Word] OR "temocapril"[Text Word] OR "imidapril"[Text Word] OR "moexipril"[Text Word] OR "adrenergic beta antagonists"[MeSH Terms] OR "atenolol"[Text Word] OR "propranolol"[Text Word] OR "pindolol"[Text Word] OR "oxprenolol"[Text Word] OR "carvedilol"[Text Word] OR "carteolol"[Text Word] OR "metoprolol"[Text Word] OR "nebivolol"[Text Word] OR "labetalol"[Text Word] OR “bisoprolol”[Text Word] OR "bucindolol"[Text Word] OR "nadolol"[Text Word] OR "alprenolol"[Text Word] OR "calcium channel blockers"[MeSH Terms] OR "amlodipine"[Text Word] OR "felodipine"[Text Word] OR "lacidipine"[Text Word] OR "lercanidipine"[Text Word] OR "mepirodipine"[Text Word] OR "nifedipine"[Text Word] OR "nisoldipine"[Text Word] OR "nitrendipine"[Text Word] OR "verapamil"[Text Word] OR "diltiazem"[Text Word] OR "diuretics"[MeSH Terms] OR "hydrochlorothiazide"[Text Word] OR "chlorothiazide"[Text Word] OR “chlorthalidone”[Text Word] OR "spironolactone"[Text Word] OR "eplerenone"[Text Word] OR "amiloride"[Text Word] OR "triamterene"[Text Word] OR "indapamide"[Text Word] OR "bendroflumethiazide"[Text Word] OR "methyclothiazide"[Text Word] OR "furosemide"[Text Word] OR "placebos"[MeSH Terms] OR "reserpine"[Text Word] OR "tetrazoles"[MeSH Terms] OR "methyldopa"[Text Word] OR “doxazosin”[Text Word] OR “prazosin”[Text Word] OR “alpha adrenergic antagonists”[Text Word] OR “alpha adrenergic blockers” [Text Word])

Appendix B

%LMM_META(

db_input=,

Input database. The database has to include the variable study(name or code for each estimate), year (year of the study), estimate (measure of interest for example difference mean, Risk relative or Odds Ratio). The variance of measure of interest has to be included only in case of mean or standardized mean difference. In case of Odds Ratio, Relative Risk or Hazard Ratio the researcher has to include the variables "LCL" (Lower Confidence Limit) and "UCL" (Upper Confidence Limit"). Moreover for dependent estimates, the database has to include a variable that identifies the correlated measures (in the scenarios 3 and 4 the variable "technique").

study=,

variable that indicates the name of study

year=,

variable that indicates the publication year of study

type_measure=,

type of the outcome. OR (Odds Ratio), RR (Risk Relative), HR (Hazard Ratio), MD (Mean difference), SMD (Standardized Mean Difference)

measure_correlated=,

variable that indicates the correlated measures (in the example BP the variable "technique")

block_measure=,

variable that indicates the blocks for measure correlated (in the example BP the variable "ID_arm")

num_measure=,

indicates the maximum number of correlated estimates inside a block

outcome=,

variable of interest

variance_outcome=

variance of variable of interest. This statement has to be complete only if type measure is MD or SMD

model=YES,

if YES, the macro performs LMM

corr21=,
corr31=, corr32=,
corr41=, corr42=, corr43=,
corr51=, corr52=, corr53=, corr54=,

corr21= correlation between measure 2 and measure 1; corr31= correlation between measure 3 and measure 1 ecc…Caution !! If the variable technique is categorical the first measure is the first in alphabetical order

random=,

number of random effects (if the researcher wants to perform fixed effects model he/she has to insert the number 0)

random_value_1=0.5, random_value_2=, random_value_3=, random_value_4=,

In this statement the researcher has to the define a value (for each random effects) for the first covariance parameter, that is, the between-study variance, to get the profile likelihood function for the between-study variance to get its likelihood ratio based 95% CI. By default the value of the FIRST random effect is 0.5.

output=,

name of variance-covariance matrix

eff_random_1=, eff_random_2=, eff_random_3=,
variables considered as random effects (usually the researcher has only one random effects (the variable study) and in this field he/she should specify "intercept")

subject_random_1=, subject_random_2=, subject_random_3=,
identifies the subject in the model. The SUBJECT= option produces a block-diagonal structure in **G** with identical blocks (if the random effect is only one and it is the variable "study" then the researcher should complete "subject_random_1=study")

type_matrix_G=vc,
specifies the covariance structure of G. By default is variance components (vc)

subject_repeat=,
defines the blocks of R matrix

group_repeat=,
defines an effect specifying heterogeneity in the covariance structure of R. All observations having the same level of the GROUP effect have the same covariance parameters. Each new level of the GROUP effect produces a new set of covariance parameters with the same structure as the original group.

type_matrix_R=,
specifies the R-side covariance structure

covariate=,
includes all covariates (both continuous and categorical) to include in LMM

covcat=,
includes all categorical variables, both the covariates in LMM and the variables in statements eff_random_1, subject_random_1, subject_repeat and group repeat.

estimate_model=YES,
if YES, the macro prints the summary estimates

forest=YES
if YES, the macro builds the forest plot

Title_forest=,
Forest Plot title

subtitle_forest=,
Forest plot subtitle

intercept=
if YES the macro includes the intercept in the LMM. On the contrary if NO the macro does not include it in LMM.

);

```
************************************************************
MACRO FOR META-ANALYSIS WITH INDIPENDENT AND DEPENDENT DATA

VERSION: 1.0
RELEASE DATE: 16/10/2017

AUTHOR(S): DS AZ
*******************************;

************************************************************;

%macro LMM_META(db_input=,
study=,
year=,
type_measure=,
measure_correlated=,
block_measure=,
num_measure=,
outcome=,
variance_outcome=,
model=YES,
corr21=,
corr31=, corr32=,
corr41=, corr42=, corr43=,
corr51=, corr52=, corr53=, corr54=,
random=,
random_value_1=, random_value_2=, random_value_3=,
output=,
eff_random_1=, eff_random_2=, eff_random_3=,
subject_random_1=, subject_random_2=, subject_random_3=,
type_matrix_G=vc,
subject_repeat=,
group_repeat=,
type_matrix_R=,
covariate=,
covcat=,
estimate_model=YES,
forest=YES,
Title_forest=,
subtitle_forest=,
intercept=
);
options mprint;
%if "&type_measure"="OR" or "&type_measure"="HR" or "&type_measure"="RR" %then
%do;

data db;
set &db_input;
measure=log(&outcome);
se=(log(ucl)-log(lcl))/3.92;
variance=se**2;
run;

proc sort data=db;by &year &study;run;
%end;

%else %if "&type_measure"="MD" or "&type_measure"="SMD"  %then %do;

data db;
set &db_input;
measure=&outcome;
variance=&variance_outcome;
run;
```

```
%end;


/*****************************/
/* Database for fixed effects */
/*****************************/


/*%if &random=0 %then %do;*/

      data DB_fixed;
      SET db;
      label estimate="&type_measure";
      label lcl="LCL";
      label ucl="UCL";
      label w_t="WT";
      inv_var=1/variance;
      w_t=inv_var*measure;
      %if %length(&year) ne 0 %then %do;
      STUDYNAME=trim(left(&STUDY))|| ", " || trim(left(&YEAR));
      %end;
      %else %if %length(&year)=0 %then %do;
      STUDYNAME=trim(left(&STUDY));
      %end;
      GROUPID=1;
      if "&type_measure"="OR" or "&type_measure"="HR" or "&type_measure"="RR"
then do;
      estimate=exp(measure);
      lcl=exp(measure-(1.96*sqrt(variance)));
      ucl=exp(measure+(1.96*sqrt(variance)));
      end;
      else if "&type_measure"="MD" or "&type_measure"="SMD"  then do;
      estimate=measure;
      lcl=measure-1.96*sqrt(variance);
      ucl=measure+1.96*sqrt(variance);
      end;
      run;

/*********************************************************************/
/* To calculate summary estimate for fixed effects (inverse variance) */
/*********************************************************************/

/* To calculate Q E I2*/

      proc sql noprint;
      select sum(inv_var) into:var_tot
      from DB_fixed
      ;
      quit;

      proc sql noprint;
      select sum(w_t) into:sum_w_t
      from DB_fixed
      ;
      quit;

      %let est_DL=%sysevalf(&sum_w_t/&var_tot);
      %put &est_DL;
      %let cc=%sysevalf(1.96*((1/&var_tot**0.5)));
      %put &cc;
      %let lcl_dl=%sysevalf(&est_DL-&cc);
      %put &lcl_dl;
```

```
%let ucl_dl=%sysevalf(&est_DL+&cc);
%put &ucl_dl;

DATA DB_fixed;
SET DB_fixed;
format weight percent6.1;
weight=inv_var/&var_tot;
run;


data DB_fixed;
set DB_fixed;
estimate2=&est_DL;
Qi=inv_var*((measure-estimate2)**2);
run;

proc sql noprint;
select sum(Qi) into:Q
from DB_fixed
where groupid=1
;
quit;

proc sql noprint;
select count(groupid) into:k
from DB_fixed
where groupid=1
;
quit;

data DB_fixed;
set DB_fixed;
p_value=1-(probchi(&Q,%eval(&k-1)));
I_square=((&Q-(&k-1))/&Q)*100;
if _N_=1 then do;
      call symputx('p_value', p_value);
      call symputx('I_square', I_square);
end;
run;

%put &Q;
%put &k;
%put &p_value;
%put &I_square;

DATA DB_fixed;
SET DB_fixed end=eof;
output;
if eof then do;
      studyname="Overall IV fixed";
      groupID=2;
            if "&type_measure"="OR" or "&type_measure"="HR" or
"&type_measure"="RR" then do;
            estimate=exp(&est_dl);
            lcl=exp(&lcl_dl);
            ucl=exp(&ucl_dl);
            end;
            else if "&type_measure"="MD" or "&type_measure"="SMD"  then
do;
            estimate=&est_dl;
            lcl=&lcl_dl;
            ucl=&ucl_dl;
            end;
```

```
            output;
      end;
      run;

/*%end;*/


/*****************************************************************/
/*** End summary estimate for fixed effects (inverse variance) ***/
/*****************************************************************/




/*******************************/
/*  Database for random effects */
/*******************************/


%if &random>0 %then %do;

      data DB_random;
      SET db;
      label estimate="&type_measure";
      label lcl="LCL";
      label ucl="UCL";
      label w_t="WT";
      inv_var=1/variance;
      w_t=inv_var*measure;
      %if %length(&year) ne 0 %then %do;
      STUDYNAME=trim(left(&STUDY))|| ", " || trim(left(&YEAR));
      %end;
      %else %if %length(&year)=0 %then %do;
      STUDYNAME=trim(left(&STUDY));
      %end;
      GROUPID=1;
      if "&type_measure"="OR" or "&type_measure"="HR" or "&type_measure"="RR"
then do;
      estimate=exp(measure);
      lcl=exp(measure-(1.96*sqrt(variance)));
      ucl=exp(measure+(1.96*sqrt(variance)));
      end;
      else if "&type_measure"="MD" or "&type_measure"="SMD"  then do;
      estimate=measure;
      lcl=measure-1.96*sqrt(variance);
      ucl=measure+1.96*sqrt(variance);
      end;
      run;

/*****************************************************************/
/* Summary estimate for random effects (Der Simonian and Laird)  */
/*****************************************************************/

      proc sql noprint;
      select sum(inv_var) into:var_tot
      from DB_random
      ;
      quit;

      proc sql noprint;
      select sum(w_t) into:sum_w_t
      from DB_random
      ;
```

```
quit;

%let est_DL=%sysevalf(&sum_w_t/&var_tot);
%put &est_DL;
%let cc=%sysevalf(1.96*((1/&var_tot**0.5)));
%put &cc;
%let lcl_dl=%sysevalf(&est_DL-&cc);
%put &lcl_dl;
%let ucl_dl=%sysevalf(&est_DL+&cc);
%put &ucl_dl;

/* Q and I square */

data DB_random;
set DB_random;
estimate2=&est_DL;
Qi=inv_var*((measure-estimate2)**2);
run;

proc sql noprint;
select sum(Qi) into:Q
from DB_random
where groupid=1
;
quit;

proc sql noprint;
select count(groupid) into:k
from DB_random
where groupid=1
;
quit;

data DB_random;
set DB_random;
p_value=1-(probchi(&Q,%eval(&k-1)));
I_square=((&Q-(&k-1))/&Q)*100;
if _N_=1 then do;
      call symputx('p_value', p_value);
      call symputx('I_square', I_square);
end;
run;

%put &Q;
%put &k;
%put &p_value;
%put &I_square;


      data DB_random;
      set DB_random;
      inv_var_2=inv_var**2;
      run;

      proc sql noprint;
      select sum(inv_var) into:var_tot
      from DB_random
      where groupid =1
      ;
      quit;

      proc sql noprint;
      select sum(inv_var_2) into:var_tot_2
```

```
from DB_random
where groupid =1
;
quit;

data DB_random;
set DB_random;
t=(&Q-(&k-1))/(&var_tot-(&var_tot_2/&var_tot));
tau2=max(0,t);
new_wi=1/(variance+tau2);
new_wi_t=new_wi*measure;
run;

proc sql noprint;
select sum(new_wi) into:new_var_tot_2
from DB_random
where groupid =1
;
quit;

proc sql noprint;
select sum(new_wi_t) into:new_sum_wi_t
from DB_random
where groupid =1
;
quit;

%let est_DL_random=%sysevalf(&new_sum_wi_t/&new_var_tot_2);
%put &est_DL_random;
%let cc_random=%sysevalf(1.96*((1/&new_var_tot_2**0.5)));
%put &cc_random;
%let lcl_dl_random=%sysevalf(&est_DL_random-&cc_random);
%put &lcl_dl_random;
%let ucl_dl_random=%sysevalf(&est_DL_random+&cc_random);
%put &ucl_dl_random;

DATA DB_random;
SET DB_random end=eof;
output;
if eof then do;
studyname="Overall DL random";
groupID=2;
      if "&type_measure"="OR" or "&type_measure"="HR" or
"&type_measure"="RR" then do;
      estimate=exp(&est_DL_random);
      lcl=exp(&lcl_DL_random);
      ucl=exp(&ucl_DL_random);
      end;
      else if "&type_measure"="MD" or "&type_measure"="SMD"  then
do;
      estimate=&est_DL_random;
      lcl=&lcl_DL_random;
      ucl=&ucl_DL_random;
      end;
output;
end;
run;

DATA DB_random;
SET DB_random;
format weight percent6.1;
weight=new_wi/&new_var_tot_2;
run;
```

```
%end;

/*****************************************************************/
/* End Summary estimate for random effects (Der Simonian and Laird)  */
/*****************************************************************/



/*****************************************************/
/* Matrix Variance-Covariance for indipendent measure */
/*****************************************************/


%if "&model"="YES" %then %do;

      proc sort data=db;by
      %if "&measure_correlated" = "" %then %do;
      &study
      %end;
      %else %if "&measure_correlated" ne "" %then %do;
      &study &block_measure &measure_correlated
      %end;
      ;
      run;

      proc sql;
      select count(*) into:nobs
      from db
      ;
      quit;

      %let nobs_new=%eval(&nobs+&random);

      %if "&measure_correlated" = "" %then %do;

            proc sort data=db;by &year &study;
            run;

            data aaa;
            set db;
            num+1;
            run;


            proc transpose data=aaa out=aaa2 prefix=covp;id num;var variance;
run;

            data fixed_&output;
            set aaa2;
            drop _name_;
            run;

            %if &random>0 %then %do;

                  data aaa3;
                  set aaa;
                  num+&random;
                  run;

                  proc sql;
                  select count(*) into:nobs
                  from aaa3
```

```
                    ;
                    quit;

                    %let nobs_new=%eval(&nobs+&random);

                    proc transpose data=aaa3 out=aaa4 prefix=covp;id num;var
variance; run;

                    data random_&output;
                    set aaa4;
                    array covp(*) covp1-covp&nobs_new;
                    drop _name_;
                    %do i=1 %to &random;
                          covp(&i)=&&random_value_&i;
                    %end;
                    run;
              %end;

      %end;

/*********************************************************/
/* End Matrix Variance-Covariance for indipendent measure */
/*********************************************************/

/*************************************************/
/* Matrix Variance-Covariance for dipendent measure */
/*************************************************/

      %if "&measure_correlated" ne "" %then %do;

            data db2;
            set db;
            format conc $500.;
            %if "&block_measure" = "&study" %then %do;
            conc=trim(left(&block_measure));
            %end;
            %else %if "&block_measure" ne "&study" %then %do;
            conc=trim(left(&study))||"_"||trim(left(&block_measure));
            %end;

            run;

            proc sort data=db2; by conc;run;

            proc transpose data=db2 out=transp_var(drop=_NAME_) prefix=var_;
            by conc;var variance;
            run;

            proc sql;
            create table aaa as
            select db2.*, transp_var.*
            from db2 left join transp_var
            on
            db2.conc = transp_var.conc
            ;
            run;

            data aaa_2;
            set aaa;
            do j=1 to &num_measure;
                              do i=1 to &num_measure;
                              output;
                        end;
```

```
                end;
          run;

          proc sort data=aaa_2 nodupkey out=aaa_3;by
          &block_measure &measure_correlated j i;
          run;

          data aaa_4;
          set aaa_3;
          if j=i or j>i;
          run;


          data aaa_4;
        set aaa_4;
          %do i=1 %to &num_measure;
            if j=i then cov = var_&i;
          %end;
            %do z=1 %to &num_measure;
                %do t=1 %to &num_measure;
                      %if &z=&t %then %do;
                      if (j=&Z and i=&T)  then cov=var_&z;
                      %end;
                      %else %if &t<&Z %then %do;
                    if (j=&Z and i=&T)  then
cov=&&corr&z&t*sqrt(var_&z)*sqrt(var_&t);
                      %end;
                %end;
            %end;
          if cov=. then cov=0.0001;
        run;

          proc sort data=aaa_4;by &year
          %if "&study"="&block_measure" %then %do;
          &study &measure_correlated
          %end;
          %else %if "&study" ne "&block_measure" %then %do;
          &study &block_measure &measure_correlated
          %end;
          ;
          run;


          %if "&block_measure" = "" %then %do;
          proc sort data=aaa_4 nodupkey out=aaa_5; by &study i j;run;
          %end;
          %else %if "&block_measure" ne "" %then %do;
          proc sort data=aaa_4 nodupkey out=aaa_5; by &block_measure i j;run;
          %end;

          data aaa_5;
          set aaa_5;
          num+1;
          run;

          /****************************/
          /* Matrix for fixed effects */
          /****************************/

          proc transpose data=aaa_5 out=fixed_&output prefix=covp;id num;var
cov; run;

          data fixed_&output;
```

```
            set fixed_&output;
            drop _name_;
            run;

            /*******************************/
            /* End Matrix for fixed effects */
            /*******************************/

            /*****************************/
            /*  Matrix for random effects */
            /*****************************/

            data aaa_5;
            set aaa_5;
            num+&random;
            run;

            proc sql;
            select count(*) into:nobs
            from aaa_5
            ;
            quit;

            %let nobs_new=%eval(&nobs+&random);

            proc transpose data=aaa_5 out=aaa_6 prefix=covp;id num;var cov; run;

            data random_&output;
            set aaa_6;
            array covp(*) covp1-covp&nobs_new;
            drop _name_;
            %do i=1 %to &random;
                  covp(&i)=&&random_value_&i;
            %end;
            run;

            /*********************************/
            /* End Matrix for random effects */
            /*********************************/
      %end;
%end;


/*********************************************/
/* FINE CREAZIONE MATRICE VARIANZE-COVARIANZE */
/*********************************************/


/***********************/
/* Linear Mixed Model  */
/***********************/


      proc sort data=db;
      by
      &year
      %if "&study"="&block_measure" %then %do;
      &study &measure_correlated
      %end;
      %else %if "&study" ne "&block_measure" %then %do;
      &study &block_measure &measure_correlated
      %end;
      ;
```

```
      run;

      %let n1=%length(&covariate);

      %if &n1 ne 0 %then %do;
                  %let n_covariate=%sysfunc(countw(&&covariate));
                        %do i = 1 %to &n_covariate;
                            %let covariate&i=%qscan(%superq(covariate),&i,%str(
));
                        %end;
      %end;
      %else %let n_covariate=0;

      %let n2=%length(&covcat);

      %if &n2 ne 0 %then %do;
                  %let n_covcat=%sysfunc(countw(&&covcat));
                        %do j = 1 %to &n_covcat;
                            %let covcat&j=%qscan(%superq(covcat),&j,%str( ));
                        %end;
      %end;

      %else %let n_covcat=0;

      /*%if &random=0 %then %do;*/


            Proc mixed  method=ml data=db order=data noinfo ;
            %if &n_covcat>0 %then %do;
                  class
                  %do i=1 %to &n_covcat;
                  &&covcat&i
                  %end;
                  ;
            %end;
            %if &n_covariate=0 %then %do;
                  model measure=  / s cl covb /*noint*/;
            %end;
            %else %if &n_covariate>0 %then %do;
                  model measure=
                  %do i=1 %to &n_covariate;
                   &&covariate&i
                  %end;
                  / s cl covb
                  %if "&intercept"="NO" %then %do;
                  noint
                  %end;
                  ;
            %end;
            repeated /subject=&subject_repeat group=&group_repeat
type=&type_matrix_R;
            parms / parmsdata=fixed_&output eqcons=1 to &nobs;
            ods output  SolutionF=result_model_fixed;
            ods output  ConvergenceStatus=convergence_fixed;
            %if &random>0  and "&intercept"="NO" %then %do;
            ods output  CovB=cov_matrix_f;
            %end;
            ;
            run;

      /*%end;*/
```

```
%if &random>0 %then %do;


      Proc mixed  method=ml data=db order=data noinfo;
      %if &n_covcat>0 %then %do;
            class
            %do i=1 %to &n_covcat;
            &&covcat&i
            %end;
            ;
      %end;
      %if &n_covariate=0 %then %do;
            model measure=  / s cl covb;
      %end;
      %else %if &n_covariate>0 %then %do;
            model measure=
            %do i=1 %to &n_covariate;
             &&covariate&i
            %end;
            / s cl covb
            %if "&intercept"="NO" %then %do;
            noint
            %end;
            ;
      %end;
      %do i=1 %to &random;
            random &&eff_random_&i/ subject=&&subject_random_&i
type=&type_matrix_G;
      %end;
      repeated /subject=&subject_repeat group=&group_repeat
type=&type_matrix_R;
      parms / parmsdata=random_&output eqcons=%eval(&random+1) to
&nobs_new;
      ods output  SolutionF=result_model_random;
      ods output  ConvergenceStatus=convergence_random;
      %if &random>0  and "&intercept"="NO" %then %do;
      ods output  CovB=cov_matrix_r;
      %end;
      ;
      run;


%end;

/*************************************************************/
/*
Macrovariable STATUS, pdG,  pdH.
If status=0 then "The model converge".
If pdG=1 then "Estimated G matrix is positive definite".
If pdH=1 then "Hessian matrix is positive definite".
*/
/*************************************************************/

data convergence_fixed;
set convergence_fixed;
call symputx('status', status);
call symputx('pdG', pdG);
call symputx('pdH', pdH);
run;

data result_model;
set result_model_fixed;
N+1;
```

```
      run;

      %if "&model"="YES" and &status=0 and &pdG=1 and &pdH=1 %then %do;
            %if &num_measure<=2 and "&intercept"="YES" %then %do;
                  proc sql noprint;
                  select estimate into:est_model_fixed trimmed
                  from result_model
                  %if "&measure_correlated" ne "" and "&covariate" ne "" %then
%do;
                  where effect="&measure_correlated" and stderr ne .
                  %end;
                  %else %if "&measure_correlated" ne "" and "&covariate" = ""
%then %do;
                  where effect="Intercept"
                  %end;
                  ;
                  quit;

                  proc sql noprint;
                  select lower into:lcl_model_fixed trimmed
                  from result_model
                  %if "&measure_correlated" ne "" and "&covariate" ne "" %then
%do;
                  where effect="&measure_correlated" and stderr ne .
                  %end;
                  %else %if "&measure_correlated" ne "" and "&covariate" = ""
%then %do;
                  where effect="Intercept"
                  %end;
                  ;
                  quit;

                  proc sql noprint;
                  select upper into:ucl_model_fixed trimmed
                  from result_model
                  %if "&measure_correlated" ne "" and "&covariate" ne "" %then
%do;
                  where effect="&measure_correlated" and stderr ne .
                  %end;
                  %else %if "&measure_correlated" ne "" and "&covariate" = ""
%then %do;
                  where effect="Intercept"
                  %end;
                  ;
                  quit;
            %end;
            %else %if &num_measure>2 and "&intercept"="YES" %then %do;
                  %do i=2 %to &num_measure;
                        proc sql noprint;
                        select estimate into:est_model_fixed_&i trimmed
                        from result_model
                        %if "&measure_correlated" ne "" %then %do;
                        where effect="&measure_correlated" and stderr ne . and
N=&i
                        %end;
                        ;
                        quit;

                        proc sql noprint;
                        select lower into:lcl_model_fixed_&i trimmed
                        from result_model
                        %if "&measure_correlated" ne "" %then %do;
```

```
                        where effect="&measure_correlated" and stderr ne . and
N=&i
                        %end;
                        ;
                        quit;

                        proc sql noprint;
                        select upper into:ucl_model_fixed_&i trimmed
                        from result_model
                        %if "&measure_correlated" ne "" %then %do;
                        where effect="&measure_correlated" and stderr ne . and
N=&i
                        %end;
                        ;
                        quit;
                  %end;
            %end;
            %else %if &num_measure>=2 and "&intercept"="NO" %then %do;
                  %do i=1 %to &num_measure;
                        proc sql noprint;
                        select estimate into:est_model_fixed_&i trimmed
                        from result_model
                        %if "&measure_correlated" ne "" %then %do;
                        where effect="&measure_correlated" and stderr ne . and
N=&i
                        %end;
                        ;
                        quit;

                        proc sql noprint;
                        select lower into:lcl_model_fixed_&i trimmed
                        from result_model
                        %if "&measure_correlated" ne "" %then %do;
                        where effect="&measure_correlated" and stderr ne . and
N=&i
                        %end;
                        ;
                        quit;

                        proc sql noprint;
                        select upper into:ucl_model_fixed_&i trimmed
                        from result_model
                        %if "&measure_correlated" ne "" %then %do;
                        where effect="&measure_correlated" and stderr ne . and
N=&i
                        %end;
                        ;
                        quit;
                  %end;
            %end;
      %end;

      %if &random>0 %then %do;
            data result_model;
            set result_model_random;
            N+1;
            run;

            data convergence_random;
            set convergence_random;
            call symputx('status', status);
            call symputx('pdG', pdG);
            call symputx('pdH', pdH);
```

```
            run;
      %end;


            %if &random>0 and  "&model"="YES" and &status=0 and &pdG=1 and
&pdH=1 %then %do;
                  %if &num_measure<=2 and "&intercept"="YES" %then %do;
                        proc sql noprint;
                        select estimate into:est_model trimmed
                        from result_model
                        %if "&measure_correlated" ne "" and "&covariate" ne ""
%then %do;
                        where effect="&measure_correlated" and stderr ne .
                        %end;
                        %else %if "&measure_correlated" ne "" and "&covariate" =
"" %then %do;
                        where effect="Intercept"
                        %end;
                        ;
                        quit;

                        proc sql noprint;
                        select lower into:lcl_model trimmed
                        from result_model
                        %if "&measure_correlated" ne "" and "&covariate" ne ""
%then %do;
                        where effect="&measure_correlated" and stderr ne .
                        %end;
                        %else %if "&measure_correlated" ne "" and "&covariate" =
"" %then %do;
                        where effect="Intercept"
                        %end;
                        ;
                        quit;

                        proc sql noprint;
                        select upper into:ucl_model trimmed
                        from result_model
                        %if "&measure_correlated" ne "" and "&covariate" ne ""
%then %do;
                        where effect="&measure_correlated" and stderr ne .
                        %end;
                        %else %if "&measure_correlated" ne "" and "&covariate" =
"" %then %do;
                        where effect="Intercept"
                        %end;
                        ;
                        quit;
                  %end;
            %else %if &num_measure>2 and "&intercept"="YES" %then %do;
                        %do i=2 %to &num_measure;
                        proc sql noprint;
                        select estimate into:est_model_&i trimmed
                        from result_model
                        %if "&measure_correlated" ne "" %then %do;
                        where effect="&measure_correlated" and stderr ne . and
N=&i
                        %end;
                        ;
                        quit;

                        proc sql noprint;
                        select lower into:lcl_model_&i trimmed
```

```
                        from result_model
                        %if "&measure_correlated" ne "" %then %do;
                        where effect="&measure_correlated" and stderr ne . and
N=&i
                        %end;
                        ;
                        quit;

                        proc sql noprint;
                        select upper into:ucl_model_&i trimmed
                        from result_model
                        %if "&measure_correlated" ne "" %then %do;
                        where effect="&measure_correlated" and stderr ne . and
N=&i
                        %end;
                        ;
                        quit;
                  %end;
            %end;
            %else %if &num_measure>=2 and "&intercept"="NO" %then %do;
                  %do i=1 %to &num_measure;
                        proc sql noprint;
                        select estimate into:est_model_&i trimmed
                        from result_model
                        %if "&measure_correlated" ne "" %then %do;
                        where effect="&measure_correlated" and stderr ne . and
N=&i
                        %end;
                        ;
                        quit;

                        proc sql noprint;
                        select lower into:lcl_model_&i trimmed
                        from result_model
                        %if "&measure_correlated" ne "" %then %do;
                        where effect="&measure_correlated" and stderr ne . and
N=&i
                        %end;
                        ;
                        quit;

                        proc sql noprint;
                        select upper into:ucl_model_&i trimmed
                        from result_model
                        %if "&measure_correlated" ne "" %then %do;
                        where effect="&measure_correlated" and stderr ne . and
N=&i
                        %end;
                        ;
                        quit;
                  %end;
            %end;
      %end;



/*************************/
/* End Linear Mixed Model  */
/*************************/

/*************************/
/* Database for FOREST PLOT */
/*************************/
```

```
/*****************************/
/* Database for fixed effects */
/*****************************/

/*%if &random=0 %then %do;*/
      %if "&model"="YES" %then %do;
            DATA DB_fixed;
            SET DB_fixed end=eof;
            output;
                  %if &status=0 and &pdG=1 and &pdH=1%then %do;
                        %if &num_measure<=2 and "&intercept"="YES" %then %do;
                              if eof then do;
                                    studyname='Overall model fixed';
                                    groupID=3;
                                          if "&type_measure"="OR" or
"&type_measure"="HR" or "&type_measure"="RR" then do;
                                          estimate=exp(&est_model_fixed);
                                          lcl=exp(&lcl_model_fixed);
                                          ucl=exp(&ucl_model_fixed);
                                          end;
                                          else if "&type_measure"="MD" or
"&type_measure"="SMD"  then do;
                                          estimate=&est_model_fixed;
                                          lcl=&lcl_model_fixed;
                                          ucl=&ucl_model_fixed;
                                          end;
                                          output;
                              end;
                        %end;
                        %else %if &num_measure>2 and "&intercept"="YES" %then
%do;
                              %do i=2 %to &num_measure;
                                    if eof then do;
                                          studyname='Overall model fixed';
                                          groupID=3;
                                                if "&type_measure"="OR" or
"&type_measure"="HR" or "&type_measure"="RR" then do;

      estimate=exp(&&est_model_fixed_&i);
                                                lcl=exp(&&lcl_model_fixed_&i);
                                                ucl=exp(&&ucl_model_fixed_&i);
                                                end;
                                                else if "&type_measure"="MD" or
"&type_measure"="SMD"  then do;
                                                estimate=&&est_model_fixed_&i;
                                                lcl=&&lcl_model_fixed_&i;
                                                ucl=&&ucl_model_fixed_&i;
                                                end;
                                                output;
                                    end;
                              %end;
                        %end;
                        %else %if &num_measure>=2 and "&intercept"="NO" %then
%do;
                              %do i=1 %to &num_measure;
                                    if eof then do;
                                          studyname='Overall model fixed';
                                          groupID=3;
                                                if "&type_measure"="OR" or
"&type_measure"="HR" or "&type_measure"="RR" then do;

      estimate=exp(&&est_model_fixed_&i);
```

```
                                                    lcl=exp(&&lcl_model_fixed_&i);
                                                    ucl=exp(&&ucl_model_fixed_&i);
                                                    end;
                                                    else if "&type_measure"="MD" or
"&type_measure"="SMD"  then do;
                                                    estimate=&&est_model_fixed_&i;
                                                    lcl=&&lcl_model_fixed_&i;
                                                    ucl=&&ucl_model_fixed_&i;
                                                    end;
                                                    output;
                                          end;
                                    %end;
                              %end;
                        %end;
                  run;
            %end;

                  %if "&estimate_model"="YES" and "&model" ne "YES" %then %do;
                  proc print data=db_fixed;var studyname estimate lcl ucl;where
groupid=2;run;
                  %end;
                  %if "&estimate_model"="YES" and "&model"="YES" and &num_measure=1
%then %do;
                  proc print data=db_fixed;var studyname estimate lcl ucl;where
groupid>=2;run;
                  %end;
                  %if "&estimate_model"="YES" and "&model"="YES" and &num_measure> 1
%then %do;
                  proc print data=db_fixed;var studyname estimate lcl ucl;where
groupid=3;run;
                  %end;

                  data db_forest;
                  set db_fixed;
                  run;

                  proc sort data=db_forest;by groupid &year &study;run;

                  %if &num_measure>1 %then %do;
                  data db_forest;
                  set db_forest;
                  if    studyname="Overall IV fixed" then delete;
                  if    studyname="Overall model fixed" then groupID=2;
                  run;
                  %end;
            %end;



/*%end;*/

/*********************************/
/* End Database for fixed effects */
/*********************************/

/*****************************/
/* Database for random effects */
/*****************************/


%if &random>0 %then %do;
      %if "&model"="YES" %then %do;
            DATA DB_random;
```

```
            SET DB_random end=eof;
            output;
                  %if &status=0 and &pdG=1 and &pdH=1%then %do;
                        %if &num_measure<=2 and "&intercept"="YES" %then %do;
                              if eof then do;
                                    studyname='Overall model random';
                                    groupID=3;
                                          if "&type_measure"="OR" or
"&type_measure"="HR" or "&type_measure"="RR" then do;
                                          estimate=exp(&est_model);
                                          lcl=exp(&lcl_model);
                                          ucl=exp(&ucl_model);
                                          end;
                                          else if "&type_measure"="MD" or
"&type_measure"="SMD"  then do;
                                          estimate=&est_model;
                                          lcl=&lcl_model;
                                          ucl=&ucl_model;
                                          end;
                                          output;
                              end;
                        %end;
                        %else %if &num_measure>2 and "&intercept"="YES" %then
%do;
                              %do i=2 %to &num_measure;
                              if eof then do;
                                    studyname='Overall model random';
                                    groupID=3;
                                          if "&type_measure"="OR" or
"&type_measure"="HR" or "&type_measure"="RR" then do;
                                          estimate=exp(&&est_model_&i);
                                          lcl=exp(&&lcl_model_&i);
                                          ucl=exp(&&ucl_model_&i);
                                          end;
                                          else if "&type_measure"="MD" or
"&type_measure"="SMD"  then do;
                                          estimate=&&est_model_&i;
                                          lcl=&&lcl_model_&i;
                                          ucl=&&ucl_model_&i;
                                          end;
                                          output;
                              end;
                              %end;
                        %end;
                        %else %if &num_measure>=2 and "&intercept"="NO" %then
%do;
                              %do i=1 %to &num_measure;
                                    if eof then do;
                                          studyname='Overall model random';
                                          groupID=3;
                                                if "&type_measure"="OR" or
"&type_measure"="HR" or "&type_measure"="RR" then do;
                                                estimate=exp(&&est_model_&i);
                                                lcl=exp(&&lcl_model_&i);
                                                ucl=exp(&&ucl_model_&i);
                                                end;
                                                else if "&type_measure"="MD" or
"&type_measure"="SMD"  then do;
                                                estimate=&&est_model_&i;
                                                lcl=&&lcl_model_&i;
                                                ucl=&&ucl_model_&i;
                                                end;
                                                output;
```

```
                                    end;
                                %end;
                            %end;
                        %end;
                run;
        %end;

                %if "&estimate_model"="YES" and "&model" ne "YES" %then %do;
                proc print data=db_random;var studyname estimate lcl ucl;where
groupid=2;run;
                %end;
                %if "&estimate_model"="YES" and "&model"="YES" and &num_measure=1
%then %do;
                proc print data=db_random;var studyname estimate lcl ucl;where
groupid>=2;run;
                %end;
                %if "&estimate_model"="YES" and "&model"="YES" and &num_measure > 1
%then %do;
                proc print data=db_random;var studyname estimate lcl ucl;where
groupid=3;run;
                %end;
                data db_forest;
                set db_random;
                run;

                proc sort data=db_forest;by groupid &year &study;run;

                %if &num_measure>1 %then %do;
                data db_forest;
                set db_forest;
                if    studyname="Overall DL random" then delete;
                if    studyname="Overall model random" then groupID=2;
                run;
                %end;
%end;

/***********************************/
/* End Database for random effects */
/***********************************/

%if "&model"="YES" %then %do;
                data warning;
                do i=1 to 3;
                count+1;
                output;
                end;
                drop i;
                run;
                data warning;
                length variable $ 200;
                set warning;
                format variable $200.;
                if count=1 then variable="Convergence status";
                if count=2 then variable="matrix G";
                if count=3 then variable="Hessian matrix";
                drop count;
                run;

                data warning;
                set warning;
                format warning $400.;
                %if &random=0 %then %do;
                      %if &status=0 and &pdG=1 and &pdH=1 %then %do;
```

```
                    if variable="Convergence status" then warning="The model
converge";
                    if variable="matrix G" then warning="Estimated G matrix is
positive definite";
                    if variable="Hessian matrix" then warning="The Hessian matrix
is positive definite";
                    %end;
                    %else %if &status=0 and &pdG=0 and &pdH=1 %then %do;
                    if variable="Convergence status" then warning="The model
converge";
                    if variable="matrix G" then warning="ATTENTION: Estimated G
matrix is not positive definite";
                    if variable="Hessian matrix" then warning="The Hessian matrix
is positive definite";
                    %end;
                    %else %if &status=0 and &pdG=1 and &pdH=0 %then %do;
                    if variable="Convergence status" then warning="The model
converge";
                    if variable="matrix G" then warning="Estimated G matrix is
positive definite";
                    if variable="Hessian matrix" then warning="ATTENTION: The
Hessian matrix is not positive definite";
                    %end;
                    %else %if &status=0 and &pdG=0 and &pdH=0 %then %do;
                    if variable="Convergence status" then warning="The model
converge";
                    if variable="matrix G" then warning="ATTENTION: Estimated G
matrix is not positive definite";
                    if variable="Hessian matrix" then warning="ATTENTION: The
Hessian matrix is not positive definite";
                    %end;
                    %else %if &status=1 and &pdG=0 and &pdH=0 %then %do;
                    if variable="Convergence status" then warning="ATTENTION: The
model did not converge";
                    if variable="matrix G" then warning="ATTENTION: Estimated G
matrix is not positive definite";
                    if variable="Hessian matrix" then warning="ATTENTION: The
Hessian matrix is not positive definite";
                    %end;
                    %else %if &status=1 and &pdG=1 and &pdH=0 %then %do;
                    if variable="Convergence status" then warning="ATTENTION: The
model did not converge";
                    if variable="matrix G" then warning="Estimated G matrix is
positive definite";
                    if variable="Hessian matrix" then warning="ATTENTION: The
Hessian matrix is not positive definite";
                    %end;
                    %else %if &status=1 and &pdG=0 and &pdH=1 %then %do;
                    if variable="Convergence status" then warning="ATTENTION: The
model did not converge";
                    if variable="matrix G" then warning="ATTENTION: Estimated G
matrix is not positive definite";
                    if variable="Hessian matrix" then warning="The Hessian matrix
is positive definite";
                    %end;
                    %else %if &status=1 and &pdG=1 and &pdH=1 %then %do;
                    if variable="Convergence status" then warning="ATTENTION: The
model did not converge";
                    if variable="matrix G" then warning="Estimated G matrix is
positive definite";
                    if variable="Hessian matrix" then warning="The Hessian matrix
is positive definite";
                    %end;
```

```
            %end;
            %if &random>0 %then %do;
                  %if &status=0 and &pdG=1 and &pdH=1 %then %do;
                  if variable="Convergence status" then warning="The model
converge";
                  if variable="matrix G" then warning="Estimated G matrix is
positive definite";
                  if variable="Hessian matrix" then warning="The Hessian matrix
is positive definite";
                  %end;
                  %else %if &status=0 and &pdG=0 and &pdH=1 %then %do;
                  if variable="Convergence status" then warning="The model
converge";
                  if variable="matrix G" then warning="ATTENTION: Estimated G
matrix is not positive definite";
                  if variable="Hessian matrix" then warning="The Hessian matrix
is positive definite";
                  %end;
                  %else %if &status=0 and &pdG=1 and &pdH=0 %then %do;
                  if variable="Convergence status" then warning="The model
converge";
                  if variable="matrix G" then warning="Estimated G matrix is
positive definite";
                  if variable="Hessian matrix" then warning="ATTENTION: The
Hessian matrix is not positive definite";
                  %end;
                  %else %if &status=0 and &pdG=0 and &pdH=0 %then %do;
                  if variable="Convergence status" then warning="The model
converge";
                  if variable="matrix G" then warning="ATTENTION: Estimated G
matrix is not positive definite";
                  if variable="Hessian matrix" then warning="ATTENTION: The
Hessian matrix is not positive definite";
                  %end;
                  %else %if &status=1 and &pdG=0 and &pdH=0 %then %do;
                  if variable="Convergence status" then warning="ATTENTION: The
model did not converge";
                  if variable="matrix G" then warning="ATTENTION: Estimated G
matrix is not positive definite";
                  if variable="Hessian matrix" then warning="ATTENTION: The
Hessian matrix is not positive definite";
                  %end;
                  %else %if &status=1 and &pdG=1 and &pdH=0 %then %do;
                  if variable="Convergence status" then warning="ATTENTION: The
model did not converge";
                  if variable="matrix G" then warning="Estimated G matrix is
positive definite";
                  if variable="Hessian matrix" then warning="ATTENTION: The
Hessian matrix is not positive definite";
                  %end;
                  %else %if &status=1 and &pdG=0 and &pdH=1 %then %do;
                  if variable="Convergence status" then warning="ATTENTION: The
model did not converge";
                  if variable="matrix G" then warning="ATTENTION: Estimated G
matrix is not positive definite";
                  if variable="Hessian matrix" then warning="The Hessian matrix
is positive definite";
                  %end;
                  %else %if &status=1 and &pdG=1 and &pdH=1 %then %do;
                  if variable="Convergence status" then warning="ATTENTION: The
model did not converge";
                  if variable="matrix G" then warning="Estimated G matrix is
positive definite";
```

```
                    if variable="Hessian matrix" then warning="The Hessian matrix
is positive definite";
                    %end;
             %end;
             run;

             proc print data=warning;var warning;run;
%end;

/*******************************/
/* End database for FOrest Plot */
/*******************************/

/*****************************/
/* Macro to build Forest Plot */
/*****************************/

%if "&forest"="YES" %then %do;
%macro ForestMacro (
       Data=,          /*--Data Set Name (Required)--*/
       Study=,           /*--Variable name for Study (Required)--*/
       OddsRatio=,       /*--Variable name for Odds Ratio (Required)--*/
       LCL=,             /*--Variable name for Lower Confidence Limit (Required)-
-*/
       UCL=,             /*--Variable name for Upper Confidence Limit (Required)-
-*/
       Group=,           /*--Variable name for Study Type--*/
       Weight=,          /*--Variable name for Study Weight in %--*/
         StatCol1=,        /*--Variable name for Stat Column 1--*/
         StatCol2=,        /*--Variable name for Stat Column 2--*/
         StatCol3=,        /*--Variable name for Stat Column 3--*/
         StatCol4=,        /*--Variable name for Stat Column 4--*/
         DisplayCols=YES, /*--Display the columns for OR, LCL, UCL & Weight--*/
         WtFactor=,        /*--Multiplier factor for Study Weights--*/
                          /*--If not provided WtFactor is computed internally--*/
         Bands=YES,        /*--Draw Horizontal Alternating Bands--*/
         Borders=NO,       /*--Draw Borders--*/
         GraphWalls=NO,    /*--Draw Filled Walls behind the Graph--*/
         StatWalls=NO,     /*--Draw Filled Walls behind the Statistics Tables--*/
         Width=6.4in,      /*--Default width of the graph in pixels--*/
         Height=,          /*--Default height of the graph is computed based on
number of observations--*/
         LabelColWidth=0.2,               /*--Fractional width for Label
Column--*/
         Label1=,           /*--Favorable Label--*/
         Label2=,             /*--Unfavorable Label--*/
         PlotTitle=&subtitle_forest,           /*--Plot Title--*/
         FootNote=,                        /*--Graph Footnote--*/
         Title2=,                          /*--Graph title2--*/
         Title=&Title_forest /*--Graph Title--*/
);

%local  WeightVar MarkerSize GraphColWidth StatColWidth Border DisplaySecondary
GraphWallDisplay StatWallDisplay;
%local  OddsLabel LowerLabel UpperLabel WeightLabel SLabel1 SLabel2 SLabel3
SLabel4;
%local  GraphHeight Ratio RowHeight HeaderHeight Nobs;

/*--Data, Study, OddsRatio, LCL and UCL are required   --*/
/*--Group is optional                                  --*/
/*--Terminatethese required parameters are not supplied--*/
%if %length(&Data) eq 0 %then %do;
%put The parameter 'Data' is required - Forest Macro Terminated.;
```

```
%goto finished;
%end;
%else %if %length(&Study) eq 0 %then %do;
%put The parameter 'Study' is required - Forest Macro Terminated.;
%goto finished;
%end;
%else %if %length(&LCL) eq 0 %then %do;
%put The parameter 'LCL' is required - Forest Macro Terminated.;
%goto finished;
%end;
%else %if %length(&UCL) eq 0 %then %do;
%put The parameter 'UCL' is required - Forest Macro Terminated.;
%goto finished;
%end;
%else %if %length(&oddsratio) eq 0 %then %do;
%put The parameter 'Outcome' is required - Forest Macro Terminated.;
%goto finished;
%end;

/*--Initialize GraphHeight, Height per row and Height for other graph items--*/
%let GraphHeight=&Height;
%let RowHeight=22;
%let HeaderHeight=100;
%if %length(&Footnote) ne 0 %then %do;
  %let HeaderHeight=115;
%end;

/*--If the Weight column is not provided, use equal weights, and suppress
display of Weight stat--*/
%if &Weight eq %then %do;
  %let WeightVar = _Weight;
  %let MarkerSize = 7;
%end;
%else %do;
  %let WeightVar=&Weight;
  %let MarkerSize = 0;
%end;

/*--Set up GTL options for borders--*/
%let DisplaySecondary = displaysecondary=none;
%let Borders=%upcase(&Borders);
%if &Borders eq YES or &Borders eq Y %then %do;
  %let Border = line;
  %let DisplaySecondary = displaysecondary=(line);
%end;

/*--Set up GTL options for GraphWall Display--*/
%let GraphWallDisplay = walldisplay=none;
%let GraphWalls=%upcase(&GraphWalls);
%if &GraphWalls eq YES or &GraphWalls eq Y %then %do;
  %let GraphWallDisplay = walldisplay=(fill);
%end;

/*--Set up GTL options for StatWall Display--*/
%let StatWallDisplay = walldisplay=none;
%let StatWalls=%upcase(&StatWalls);
%if &StatWalls eq YES or &StatWalls eq Y %then %do;
  %let StatWallDisplay = walldisplay=(fill);
%end;

/*--Create Label Columns for standard and additional columns--*/

/*--Load Stat Column Label or name into macro for label column value--*/
```

```
%let dsid=%sysfunc(open(&Data));
%if &dsid %then %do;

    %let Nobs=%sysfunc(attrn(&dsid, nlobs));
      %if &Nobs eq 0 %then %do;
      %put The Data Set &Data has no observations - Forest Macro Terminated.;
      %let rc=%sysfunc(close(&dsid));
      %goto finished;
    %end;

    %if &Nobs gt 100 %then %do;
      %put The Data Set &Data has over 100 observations - Forest Macro
Terminated.;
      %let rc=%sysfunc(close(&dsid));
      %goto finished;
    %end;

    /*--Count the number of stat columns--*/
    %let idx=0;

      /*--Column display information for the OddsRatio column--*/
      %let DisplayCols=%upcase(&DisplayCols);

    %if &DisplayCols eq YES or &DisplayCols eq Y %then %do;
      %let OddsLabel=%sysfunc(varlabel(&dsid,
%sysfunc(varnum(&dsid,&oddsratio))));
        %if %length(&OddsLabel) eq 0 %then %let OddsLabel=&oddsratio;
        %let idx= %eval(&idx+1);

      %let LowerLabel=%sysfunc(varlabel(&dsid, %sysfunc(varnum(&dsid,&LCL))));
        %if %length(&LowerLabel) eq 0 %then %let LowerLabel=&LCL;
        %let idx= %eval(&idx+1);

      %let UpperLabel=%sysfunc(varlabel(&dsid, %sysfunc(varnum(&dsid,&UCL))));
        %if %length(&UpperLabel) eq 0 %then %let UpperLabel=&UCL;
        %let idx= %eval(&idx+1);

      %if &Weight ne %then %do;
        %let WeightLabel=%sysfunc(varlabel(&dsid,
%sysfunc(varnum(&dsid,&Weight))));
          %if %length(&WeightLabel) eq 0 %then %let WeightLabel=&Weight;
          %let idx= %eval(&idx+1);
        %end;
    %end;

      /*--Additional columns to be displayed--*/
    %if %length(&StatCol1) ne 0 %then %do;
      %let SLabel1=%sysfunc(varlabel(&dsid, %sysfunc(varnum(&dsid,&StatCol1))));
        %if %length(&SLabel1) eq 0 %then %let SLabel1=&StatCol1;
        %let idx= %eval(&idx+1);
    %end;

    %if %length(&StatCol2) ne 0 %then %do;
      %let SLabel2=%sysfunc(varlabel(&dsid, %sysfunc(varnum(&dsid,&StatCol2))));
        %if %length(&SLabel2) eq 0 %then %let SLabel2=&StatCol2;
        %let idx= %eval(&idx+1);
    %end;

    %if %length(&StatCol3) ne 0 %then %do;
      %let SLabel3=%sysfunc(varlabel(&dsid, %sysfunc(varnum(&dsid,&StatCol3))));
        %if %length(&SLabel3) eq 0 %then %let SLabel3=&StatCol3;
        %let idx= %eval(&idx+1);
    %end;
```

```
    %if %length(&StatCol4) ne 0 %then %do;
      %let SLabel4=%sysfunc(varlabel(&dsid, %sysfunc(varnum(&dsid,&StatCol4))));
        %if %length(&SLabel4) eq 0 %then %let SLabel4=&StatCol4;
        %let idx= %eval(&idx+1);
    %end;

    %let rc=%sysfunc(close(&dsid));

      /*--Set column weights based on number of stat columns--*/
    %let StatColWidth=%sysevalf(&idx * 0.075);
    %let GraphColWidth= %sysevalf(1.0 - &LabelColWidth - &StatColWidth);
%end;
%else %do;
    %put The data set &Data does not exist - Forest Macro Terminated.;
    %goto finished;
%end;

/*--Compute Weight Factor if not provided   --*/
/*--Estimate height of graph if not provided--*/
data _null_;
  set &Data end=last;
  retain totalweight 0;
  totalweight+&WeightVar;

  if last then do;
    %if &wtFactor eq %then %do;
      if totalweight <= 0 then totalweight=1;
      call symput ('wtFactor', 1 / totalweight);
    %end;
      /*--Estimate Ratio of Plot height by Graph Height--*/
      call symput ('Ratio', (_N_* &RowHeight)/(_N_* &RowHeight +
&HeaderHeight));

    /*--Estimate the optimal height of the graph based on obs count--*/
    %if &Height eq %then %do;
        call symput ('GraphHeight', _N_ * &RowHeight + &HeaderHeight);
      %end;
  end;
run;

/*--Append a PX only if this internally estimated--*/
%if &Height eq %then %do;
%let GraphHeight=&GraphHeight.px;
%end;

/*--Process Data--*/
data _forest;
  set &Data;
  format _wt PERCENT6.1;

  _ObsId=_N_;

  %if &Weight eq %then %do;
    &WeightVar=0;
  %end;

  label _wt=&WeightLabel;

  /*--If Group column is provided--*/
  %if %length(&Group) ne 0 %then %do;
    /*--Group=1 (Study) values will be drawn without a group role--*/
    if &group=1 then do;
```

```
        _wt=&WeightVar/* / 100*/;
        _grp=10;
      _or1 = &oddsratio;
      _lcl1=&LCL;
      _ucl1=&UCL;
      /*--Compute marker width--*/
      _x1=&oddsratio / (10 ** (&WeightVar*&WtFactor/2));
      _x2=&oddsratio * (10 ** (&WeightVar*&WtFactor/2));
    end;
  /*--Group=2 & 3 (SubGroup and Overall) values will be drawn with groupindex=2
& 3--*/
    else if &group > 1 then do;
      _grp=&group;
      _or2 = &oddsratio;
        _lcl1=&LCL;
      _ucl1=&UCL;
    end;
  %end;
 /* %else %do;
    _wt=&WeightVar / 100;
    _grp=10;
    _or1 = &oddsratio;
    _lcl1=&LCL;
    _ucl1=&UCL;
    /*--Compute marker width--*/
    /*_x1=&oddsratio / (10 ** (&WeightVar*&WtFactor/2));
    _x2=&oddsratio * (10 ** (&WeightVar*&WtFactor/2));
  %end;*/

  /*--Create label columns for standard and additional statistic--*/
  %if %length(&oddsratio) ne 0 %then %do;
    _OddsRatioLabel = symget('OddsLabel');
  %end;

  %if %length(&LCL) ne 0 %then %do;
    _LowerLabel = symget('LowerLabel');
  %end;

  %if %length(&UCL) ne 0 %then %do;
    _UpperLabel = symget('UpperLabel');
  %end;

  %if %length(&Weight) ne 0 %then %do;
    _WeightLabel = symget('WeightLabel');
  %end;

  %if %length(&StatCol1) ne 0 %then %do;
    _StatColLabel1 = symget('SLabel1');
      _StatCol1 = &StatCol1;
  %end;

  %if %length(&StatCol2) ne 0 %then %do;
    _StatColLabel2 = symget('SLabel2');
      _StatCol2 = &StatCol2;
  %end;

  %if %length(&StatCol3) ne 0 %then %do;
    _StatColLabel3 = symget('SLabel3');
      _StatCol3 = &StatCol3;
  %end;

  %if %length(&StatCol4) ne 0 %then %do;
    _StatColLabel4 = symget('SLabel4');
```

```
      _StatCol4 = &StatCol4;
  %end;

  run;

/*--Reverse the order to avoid putting axis reverse--*/
proc sort data=_forest out=_forest;
  by descending _ObsId;
  run;

/*--Add sequence numbers to each observation--*/
data _forest;
  set _forest;
  studyvalue=_n_;
run;

/*--Output values and formatted strings to data set--*/
data _forestFormat;
  set _forest end=last;
  keep label start end fmtname type hlo;
  retain fmtname '_Study' type 'n';
  label=&Study;
  start=studyvalue;
  end=studyvalue;
  output;
  if last then do;
    hlo='O';
      label='Other';
      output;
  end;
  run;

/*--Create Format from data set--*/
proc format library=work cntlin=_forestFormat;
  run;

/*--Apply format to study values--*/
/*--Compute width of box proportional to weight in log scale--*/
data _forest;
  format studyvalue _study.;
  set _forest;
  %let Bands=%upcase(&Bands);
  %if &Bands eq YES or &Bands eq Y %then %do;
    if mod(studyvalue, 2) = 0 then _StudyRef=StudyValue;
  %end;
  run;

/*--Compute top and bottom offsets--*/
data _null_;
  pct=&Ratio/nobs;
  thk=pct* 0.9 *100;
  call symputx("pct", pct);
  call symputx("pct2", 2*pct);
  call symputx("RefThickness", thk);
  call symputx("count", nobs);
  set _forest nobs=nobs;
run;

/*title;*/
/*options nodate nonumber;*/

/*--Define GTL template for graph--*/
proc template;
```

```
  define statgraph ForestMacro;
    begingraph / designwidth=&Width designheight=&GraphHeight;
        entrytitle "&Title";
        entryfootnote halign=left "&FootNote";
        %if %length(&title2) ne 0 %then %do;
          entrytitle "&title2" / textattrs=graphLabelText;
      %end;
        layout lattice / columns=3 columnweights=(&LabelColWidth &GraphColWidth
&StatColWidth) columngutter=0
                         rowdatarange=union;
        /*--Column # 1 contains the Study Labels using Secondary Y axis--*/
        layout overlay / walldisplay=none x2axisopts=(display=none)
                           yaxisopts=(linearopts=(tickvaluesequence=(start=1
end=&count increment=1))
                                      offsetmin=&pct2 offsetmax=&pct display=none
                                      displaysecondary=(tickvalues &border));
              scatterplot y=studyvalue x=_or1 / yaxis=Y xaxis=X2
markerattrs=(size=0) includemissinggroup=true;
              scatterplot y=studyvalue x=_or1 / yaxis=Y xaxis=X2
markerattrs=(size=0) includemissinggroup=true;
            endlayout;
          /*--Column # 2 contains the graph--*/
        layout overlay / &GraphWallDisplay border=false
                           xaxisopts=(offsetmin=0  type=log
logopts=(minorticks=true)
                                      label="&PlotTitle" display=(ticks tickvalues
line)
                                      displaysecondary=(label &border))
                           yaxisopts=(linearopts=(tickvaluesequence=(start=1
end=&count increment=1))
                                      offsetmin=&pct2 offsetmax=&pct
display=none);

              /*--Draw alternating bands using referenceline--*/
          %if &Bands eq YES or &Bands eq Y %then %do;
            referenceline y=_StudyRef / lineattrs=(thickness=&RefThickness.PCT)
datatransparency=0.9;
              %end;

          /*--Draw Markers for SubGroup and Overall values--*/
              %if %length(&Group) ne 0 %then %do;
          scatterplot y=studyvalue x=_or2 / xerrorupper=_ucl1 xerrorlower=_lcl1
markerattrs=(symbol=diamondfilled size=10) group=_grp
                       includemissinggroup=true index=_grp;
              %end;
          /*--Draw OddsRatio and Limits for Study Values--*/
            scatterplot y=studyvalue x=_or1 / xerrorupper=_ucl1
xerrorlower=_lcl1
                  markerattrs=graphdata1(symbol=squarefilled size=&MarkerSize);

             /*scatterplot y=studyvalue x=_or1 / xerrorupper=_ucl1
xerrorlower=_lcl1 sizeresponse=weight
                  markerattrs=graphdata1(symbol=squarefilled);*/

            /*--Draw box representing the weight of the study--*/
          vectorplot y=studyvalue x=_x2 xorigin=_x1 yorigin=studyvalue /
lineattrs=GraphData1(thickness=8)
                arrowheads=false;

          /*--Draw Reference lines and labels--*/
              %if "&type_measure"="OR" or "&type_measure"="HR" or
"&type_measure"="RR" %then %do;
          referenceline x=1;
```

```
          referenceline x=0 /  lineattrs=(pattern=shortdash)
datatransparency=0.5;
              referenceline x=3 /  lineattrs=(pattern=shortdash)
datatransparency=0.5;
              entry halign=left  "&Label1" / valign=bottom;
              entry halign=right "&Label2" / valign=bottom;
              %end;
              %else %if "&type_measure"="MD" or "&type_measure"="SMD"  %then
%do;
              referenceline x=0;
           referenceline x=-5 /  lineattrs=(pattern=shortdash)
datatransparency=0.5;
              referenceline x=5 /  lineattrs=(pattern=shortdash)
datatransparency=0.5;
              entry halign=left  "&Label1" / valign=bottom;
              entry halign=right "&Label2" / valign=bottom;
              %end;
            endlayout;

          /*--Column # 2 contains the statistics data--*/
        layout overlay / &StatWallDisplay border=false
                         x2axisopts=(display=(tickvalues &border)
displaysecondary=(line))
                         yaxisopts=(linearopts=(tickvaluesequence=(start=1
end=&count increment=1))
                                    offsetmin=&pct2 offsetmax=&pct
                                    display=none &DisplaySecondary.);
          /*--Draw alternating bands using referenceline--*/
          %if &Bands eq YES %then %do;
            referenceline y=_StudyRef / lineattrs=(thickness=&RefThickness.PCT)
datatransparency=0.9;
              %end;

          /*--Draw standard statistics columns--*/
          %if &DisplayCols eq YES or &DisplayCols eq Y %then %do;
            scatterplot y=studyvalue x=_OddsRatioLabel /
markercharacter=&oddsratio xaxis=x2;
                scatterplot y=studyvalue x=_LowerLabel / markercharacter=&LCL
xaxis=x2;
                scatterplot y=studyvalue x=_UpperLabel / markercharacter=&UCL
xaxis=x2;
                %if &Weight ne %then %do;
             scatterplot y=studyvalue x=_WeightLabel / markercharacter=_wt
xaxis=x2;
                %end;
              %end;

              /*--Draw additional statistics columns--*/
          %if %length(&StatCol1) ne 0 %then %do;
            scatterplot y=studyvalue x=_StatColLabel1 /
markercharacter=&StatCol1 xaxis=x2;
              %end;

              %if %length(&StatCol2) ne 0 %then %do;
            scatterplot y=studyvalue x=_StatColLabel2 /
markercharacter=&StatCol2 xaxis=x2;
              %end;

          %if %length(&StatCol3) ne 0 %then %do;
            scatterplot y=studyvalue x=_StatColLabel3 /
markercharacter=&StatCol3 xaxis=x2;
              %end;
```

```
          %if %length(&StatCol4) ne 0 %then %do;
            scatterplot y=studyvalue x=_StatColLabel4 /
markercharacter=&StatCol4 xaxis=x2;
             %end;

            endlayout;
        endlayout;
      endgraph;
  end;
run;

proc sgrender data=_forest template=ForestMacro description='Forest Plot';
  run;

%finished:

%mend ForestMacro;

%ForestMacro(data=db_forest, Study=StudyName, Group=GroupId, Oddsratio=estimate,
LCL=lcl, UCL=ucl,
         width=6.5in, Weight=Weight, Bands=YES, GraphWalls=YES,
DisplayCols=yes);

%end;

/*********************************/
/* End Macro to build FOREST PLOT */
/*********************************/

/*******************************************************/
/* Output heterogeinity only for indipendent estimates */
/*******************************************************/
%if &num_measure=1 %then %do;
      data stat_hetero;
      do i=1 to 4;
      count+1;
      output;
      end;
      drop i;
      run;

      data stat_hetero;
      length variable $ 20;
      set stat_hetero;
      format variable $20.;
      if count=1 then variable="N estimates = ";
      if count=2 then variable="Q = ";
      if count=3 then variable="P value = ";
      if count=4 then variable="I square = ";
      drop count;
      run;

      data stat_hetero;
      set stat_hetero;
      if variable="N estimates = " then value=&k;
      if variable="Q = " then value=&q;
      if variable="P value = " then value=&p_value;
      if variable="I square = " then value=&I_square;
      if value < 0 then value =0;
      run;

      proc print data=stat_hetero;run;
%end;
```

```
/*************************************************************************/
/* Output heterogeinity only for dipendent estimates and for random effects */
/*************************************************************************/

%if &random>0  and &num_measure>1 and ("&covariate"="" OR
("&covariate"="&measure_correlated" AND "&intercept"="YES")) %then %do;
      data temp1;
      set result_model_fixed;
      %if "&covariate" ne "" %then %do;
            if effect="&measure_correlated" and stderr ne . ;
            var_fixed=(stderr)**2;
            var_fixed2=(upper-lower);
            c=1;
      %end;
      %else %if "&covariate"="" %then %do;
            var_fixed=(stderr)**2;
            var_fixed2=(upper-lower);
            c=1;
      %end;
      keep c var_fixed var_fixed2 &measure_correlated;
      run;

      data temp2;
      set result_model_random;
      %if "&covariate" ne "" %then %do;
            if effect="&measure_correlated" and stderr ne . ;
            var_random=(stderr)**2;
            var_random2=(upper-lower);
            c=1;
      %end;
      %else %if "&covariate"="" %then %do;
            var_random=(stderr)**2;
            var_random2=(upper-lower);
            c=1;
      %end;
      keep c var_random var_random2;
      run;

      data hetero_dip_est;
      merge temp1 temp2;
      by c;
      R=var_random/var_fixed;
      Isquare_R=(((R**2)-1)/(R**2));
      if Isquare_R<0 then Isquare_R=0;
      R2=var_random2/var_fixed2;
      Isquare_R2=(((R2**2)-1)/(R2**2));
      if Isquare_R2<0 then Isquare_R2=0;
      keep &measure_correlated Isquare_R Isquare_R2;
      run;

      proc print data=hetero_dip_est;run;
%end;

%if &random>0 and &num_measure>1 and "&covariate"="&measure_correlated" AND
"&intercept"="NO" %then %do;

      data cov_matrix_r1;
      set cov_matrix_r;
      drop row effect &measure_correlated;
      run;

      data cov_matrix_f1;
```

```
      set cov_matrix_f;
      drop row effect &measure_correlated;
      run;

      proc iml;
      use cov_matrix_r1;
      read all var _NUM_ into Cr;
      use cov_matrix_f1;
      read all var _NUM_ into Cf;


      x=det(Cr);
      y=det(Cf);
      Isquare_R=(x**(1/&num_measure)-y**(1/&num_measure))/(x**(1/&num_measure));

      if Isquare_R<0 then Isquare_R=0;

      print Isquare_R;
      quit;
%end;

/*****************************/
/* End Output heterogeinity */
/*****************************/

%mend LMM_META;
```

Appendix C

("Angiotensin-converting Enzyme Inhibitors" [All Fields] OR "Angiotensin-converting Enzyme Inhibitors"[Mesh] OR "Angiotensin-Converting Enzyme Inhibitors" [Pharmacological Action] OR "Perindopril"[Mesh] OR "Ramipril"[Mesh] OR "Zofenopril"[Mesh] OR Quinapril"[Mesh] OR "Spirapril"[Mesh] OR "Trandolapril"[Mesh] OR "Benazepril"[Mesh] OR "Cilazapril"[Mesh] OR "Delapril"[Mesh] OR "Fosinorpil"[Mesh] OR "Lisinorpil"[Mesh] OR "Moexipril"[Mesh] OR "Captopril"[Mesh] OR "Enalapril"[Mesh] OR "Renin-Angiotensin System"[Mesh]) AND ("dementia"[Mesh Terms] OR "Alzheimer disease"[Mesh Terms] OR "dementia, vascular"[Mesh Terms] OR Alzheimer's disease [tiab] OR dementia [tiab] OR vascular dementia [tiab] OR severity of dementia [tiab]).

Appendix D

%MCSA

data_input=,
Input database. The database has to include the variable study(name or code for each estimate), estimate (measure of interest for example difference mean, Risk relative or Odds Ratio, in case of association measure the researcher has to insert the log of the association measure). The standard error of the measure of interest.

logRR=,
logarithm of risk relative or the association between confounder and outcome.

tauRR=,
standard deviation of the logarithm of risk relative or the association between confounder and outcome.

logitp1=,
logit of the prevalence of the unmeasured confounder in the exposure group.

taup1=,
standard deviation of the logit of the prevalence of the unmeasured confounder in the exposure group.

logitp0=,
logit of the prevalence of the unmeasured confounder in the no exposure group.

taup0=,
standard deviation of the logit of the prevalence of the unmeasured confounder in the no exposure group.

niter=,
number of simulations.

outcome=,
measure of interest (obviously bias estimate).

sd_outcome=,
standard deviation of measure of interest.

```
%macro MCSA (data_input=, logRR=, tauRR=, logitp1=, taup1=, logitp0=, taup0=,
niter=, outcome=, sd_outcome= );

data simulation;
set &data_input;
run;

%do i=1 %to &niter;

      data aaa_&i;
      set &data_input;
      log_RR=rand("Normal",&logRR,&tauRR);
      logit_p1=rand("Normal",&logitp1,&taup1);
      logit_p0=rand("Normal",&logitp0,&taup0);
      gamma_x=logit_p1-logit_p0;
      p1=exp(logit_p1)/(1+exp(logit_p1));
      p0=exp(logit_p0)/(1+exp(logit_p0));
      bias=log(((1+exp(log_RR+logit_p0+gamma_x))*(1+exp(logit_p0)))/
      ((1+exp(log_RR+logit_p0))*(1+exp(logit_p0+gamma_x))));
      bias2=log((exp(log_RR)*p1+(1-p1))/(exp(log_RR)*p0+(1-p0)));
      theta_adj=&outcome-bias;
      theta_adj1=rand("Normal",theta_adj,&sd_outcome);
      iter=&i;
```

```
	run;

	data simulation;
	set simulation aaa_&i;
	run;

	proc delete data=aaa_&i;run;

%end;

	data simulation;
	set simulation;
	if iter=. then delete;
	run;

	proc sort data=simulation;by studyid;run;
	ods results off;
	proc univariate data=simulation;var theta_adj1;
	output out=pctls pctlpts=2.5 50 97.5 pctlpre=P pctlname=_2_5 _50 _97_5;
	by studyid;
	run;
	ods results on;
	proc sql;
	create table output as
	select &data_input..*, pctls.P_50 as theta, pctls.P_2_5 as inf,
pctls.P_97_5 as sup
	from &data_input left join pctls
	on &data_input..studyid=pctls.studyid
	;
	quit;

%mend;
```

Appendix E

(“Left ventricular mass” OR “LVM” OR “Left ventricular hypertrophy” OR “LV hypertrophy” OR “LVH” OR “LV mass”) AND (“reserpine” OR “rescinnamine“ OR “combinations of rauwolfia alkaloids“ OR “rauwolfia alkaloids, whole root “ OR “deserpidine“ OR “methoserpidine“ OR “bietaserpine“ OR “reserpine, combinations“ OR “bietaserpine,combinations” OR “Methyldopa” OR “clonidine“ OR “guanfacine“ OR “tolonidine” OR “moxonidine“ OR “rilmenidine“ OR “trimetaphan“ OR “mecamylamine“ OR “Bisquaternary ammonium compounds” OR “prazosin“ OR “indoramin“ OR “trimazosin“ OR “doxazosin“ OR “urapidil“ OR ”betanidine“ OR “guanethidine“ OR “guanoxan“ OR “debrisoquine“ OR “guanoclor“ OR “guanazodine “ OR “guanoxabenz “ OR “diazoxide “ OR “dihydralazine “ OR “hydralazine“ OR “endralazine“ OR “cadralazine“ OR “minoxidil“ OR “nitroprusside“ OR “pinacidil“ OR “veratrum“ OR “metirosine“ OR “pargyline“ OR “ketanserin“ OR “bosentan“ OR “ambrisentan“ OR “sitaxentan“ OR “macitentan“ OR “riociguat“ OR “methyldopa (levorotatory)” OR “picodralazine” OR “Serotonin antagonists” OR “combinations of antihypertensives” or “low-ceiling diuretics, thiazides” OR “bendroflumethiazide” OR “hydroflumethiazide” OR “hydrochlorothiazide” OR “chlorothiazide” OR “polythiazide” OR “trichlormethiazide” OR “cyclopenthiazide” OR “methyclothiazide” OR “cyclothiazide” OR “mebutizide “ OR “potassium” OR “chlorothiazide, combinations” OR “hydroflumethiazide, combinations” OR “hydrochlorothiazide, combinations” OR “quinethazone” OR “clopamide” OR “chlortalidone” OR “mefruside” OR “clofenamide” OR “metolazone” OR “meticrane“ OR “xipamide “ OR “indapamide“ OR “clorexolone “ OR ”fenquizone ” OR “mersalyl” OR “theobromine” OR “Sulfonamides, combinations” OR “cicletanine” OR “furosemide” OR “bumetanide” OR “piretanide” OR “torasemide” OR “etacrynic acid” OR “tienilic acid” OR “muzolimine”

OR “etozolin” OR “spironolactone” OR “potassium canrenoate” OR “canrenone” OR “eplerenone” OR “amiloride” OR “triamterene” OR “epitizide ” OR “altizide” OR “butizide” OR “furosemide” OR “tolvaptan” OR “conivaptan” OR “alprenolol” OR “oxprenolol” OR “pindolol” OR “propranolol” OR “timolol” OR “sotalol” OR nadolol OR mepindolol OR carteolol OR tertatolol OR bopindolol OR bupranolol OR penbutolol OR cloranolol OR “sotalol, combinations “ OR practolol OR metoprolol OR atenolol OR acebutolol OR betaxolol OR bevantolol OR bisoprolol OR celiprolol OR esmolol OR epanolol OR “s-atenolol” OR nebivolol OR talinolol OR “metoprolol, combinations” OR “bisoprolol, combinations”

OR labetalol OR carvedilol OR “thiazides” OR “metipranolol, combinations” OR “bevantolol” OR “bisoprolol” OR “abetalol” OR “Beta blocking agents, non-selective, and vasodilators” OR “Beta blocking agents, selective, and vasodilators” OR amlodipine OR felodipine OR isradipine OR nicardipine OR nifedipine OR nimodipine OR nisoldipine OR nitrendipine OR lacidipine OR nilvadipine OR manidipine OR barnidipine OR lercanidipine OR cilnidipine OR benidipine OR clevidipine OR “nifedipine, combinations” OR mibefradil OR verapamil OR gallopamil OR “verapamil, combinations” OR “diltiazem” OR “fendiline” OR “bepridil” OR “lidoflazine” OR “perhexiline” OR captopril OR enalapril OR lisinopril OR perindopril OR ramipril OR quinapril OR benazepril OR cilazapril OR fosinopril OR trandolapril OR spirapril OR delapril OR moexipril OR temocapril OR zofenopril OR imidapril OR manidipine OR “indapamide “ OR “losartan” OR “eprosartan “ OR “valsartan” OR irbesartan OR tasosartan OR candesartan OR telmisartan OR “olmesartan medoxomil “ OR “azilsartan medoxomil “ OR fimasartan OR “irbesartan“ OR remikiren OR aliskiren OR “diuretics” OR “β-blockers” OR “β-blocker” OR “beta-blockers” OR “beta-blocker” OR “calcium channel blockers” OR “calcium channel blocker” OR “CCB” OR “ACE ihibitors” OR “angiotensin receptor blockers” OR “angiotensin receptor blocker”)