MATERIALS AND METHODS

The main aims of our study were to provide an accurate estimate of the association between VTE and metabolic syndrome in different subgroups of patients and an accurate estimate of the association between VTE and the components of the metabolic syndrome, taken individually and then progressively clustered. Because a meta-analysis of aggregate data does not allow study of questions pertinent to patient subgroups and adjustment for potential confounders (1), we performed an individual patient data meta-analysis. Since we considered the role of the metabolic syndrome and of its components of greatest interest for the understanding of the pathogenesis of unprovoked VTE, we restricted our study to consideration of unprovoked VTE.

A protocol was prospectively developed. Specific objectives, criteria for study selection, approach to assess study quality, outcomes, and statistical methods were defined a priori (2).

Study Identification

We identified all published studies that evaluated the role of the metabolic syndrome as a risk factor for VTE using Medline (1946 to February week 1 2014) and EMBASE (1980 to February week 1 2014) databases. The search strategy used the keywords and medical subject headings presented in Appendix 1. We supplemented our search by reviewing the International Society of Thrombosis and Haemostasis and American Society of Hematology scientific meeting abstracts from 2003 to 2013 using metabolic syndrome, and venous thrombosis or pulmonary embolism as search terms and by manually reviewing the reference list of all articles retrieved for additional published or unpublished trials.

Study Selection

Study selection was performed independently by 2 reviewers (FD, WA), with disagreements resolved through discussion and by the opinion of a third reviewer (AS), if necessary.
Studies were included if they met the following criteria: 1) diagnosis of VTE was objectively confirmed according to established criteria (i.e. ultrasound of the lower limbs or CT scan for deep vein thrombosis and CT scan or high probability V/Q scan for pulmonary embolism); 2) Patients with unprovoked VTE were included, 3) Availability of quantitative data to adjudicate all individual components of the metabolic syndrome according to international definitions (see below).

To assess the agreement between reviewers for study selection, we used the \( \kappa \) statistic, which measures agreement beyond chance (3). According to Maclure and Willett, \( K \) values higher than 0.6 are considered to represent a substantial agreement and values higher than 0.8 an almost perfect agreement (4).

**Study Validity Assessment**

The same two investigators independently completed the assessment of study validity. Disagreement was resolved by consensus and by the opinion of a third reviewer, if necessary.

Although the use of quality scoring systems or quality scales in observational studies is controversial (2), we adapted the Newcastle-Ottawa Scale for assessing quality of non-randomized studies (25). This assesses the three broad areas of selection, comparability, and outcome or exposure, for case-control or cohort studies. The main items for case-control studies were the following: 1) definition of VTE (objective diagnosis); 2) consecutive selection of patients; 3) definition of control group (representativeness of the general population); 4) selection of control group (no previous history of VTE); 5) comparability on the basis of the design or analysis (age and sex matched or an adequate adjustment for age and sex in the statistical analysis); and 6) objective ascertainment of exposure (i.e. metabolic syndrome). The main items for the cohort studies were as follows: 1) representativeness of the exposed cohort; 2) objective ascertainment of exposure (i.e. metabolic syndrome); 3) selection of the non-exposed subjects; 4) comparability on the basis of the design or analysis (either exposed and non-exposed individuals must be matched in the design
and/or confounders must be adjusted for in the analysis); 5) objective definition of VTE as an outcome; 6) adequacy of follow-up of cohorts. The scoring system defined three quality categories as follows: a total of 6 points defined high-quality study; 4 and 5 points defined medium-quality studies; and 3 or less points defined low-quality studies. No attempts to mask for authorship, journal name, or institution were made.

**Development of Individual Patient Database**

We contacted the principal investigator of each eligible study to explain our meta-analysis objectives and analysis plan. After all investigators agreed to share their databases, the databases were transferred to a central location under the auspices of 2 reviewers (FD, WA). Data were checked, explanations for coding and uncertain or missing data were clarified, and a single pooled database was developed.

**Data Extraction**

Two reviewers (FD, WA) independently completed data extraction. Disagreement was resolved by consensus or by the opinion of a third reviewer (AS), if necessary. The following data were collected from each database: age, sex, body weight, height, body mass index (BMI), waist circumference, presence of hypertension, presence of diabetes mellitus or impaired glucose tolerance, hyperlipidemia, and concomitant drugs. In addition, total cholesterol, HDL cholesterol and triglycerides values were obtained, and LDL values were calculated using the Friedewald formula with the exception of subjects with plasma triglycerides above 400 mg/dL. The metabolic syndrome was defined by the presence of three or more of the following risk factors according to the revised National Cholesterol Education Program (NCEP) guidelines (6): abdominal obesity (i.e. waist circumference of greater than 102 cm for men and of greater than 88 cm for women); triglycerides levels equal to or greater than 150 mg dL⁻¹; HDL cholesterol of lower than 40 mg dL⁻¹ for men and of lower than 50 mg dL⁻¹ for women, blood pressure of equal to or greater than 130
mmHg for systolic and/or 85 mmHg for diastolic blood pressure, and fasting glucose levels equal to or greater than 100 mg dL\(^{-1}\). Patients currently receiving drug therapy for hypertension, diabetes, or dyslipidemia (only statins) were defined as having those disorders. Obesity was considered as a BMI greater than 30 kg/m\(^2\). According to the Asia–Pacific criteria (7), obesity in Asians was considered as a BMI greater than 25 kg/m\(^2\) and abdominal obesity as a waist circumference of greater than 90 cm for men and of greater than 80 cm for women.

**Statistical Analysis**

Separate analysis was carried out for case-control studies and for cohort studies. Descriptive statistics were used for variables in the pooled database. For continuous variables, we used mean and standard deviation (SD) for data with a normal distribution (Kolmogorov-Smirnov test) or median and inter-quartile range (IQR) for data with a non-normal distribution. For categorical data, we used frequencies and proportions.

We first calculated pooled odds ratios (OR) and 95% confidence intervals (CIs) of the aggregate data of the association between VTE and the metabolic syndrome for both case-control and cohort studies using a random-effects model (DerSimionan and Laird method) (8). The use of OR for cohort studies is due to the fact that in a study level meta-analysis differences in follow-up time cannot be considered. Statistical heterogeneity was evaluated using the \(I^2\) statistic, which assesses the appropriateness of pooling the individual study results (9).

The association between metabolic syndrome and VTE was subsequently calculated on an individual patient level using a study-stratified approach.

*Analysis of case-control studies*

Pooled results were reported as odds ratio (OR) with 95% confidence intervals (CI) and with 2-sided probability values. We analysed two pre-specified subgroups: male and female patients, and young and old patients using the median age of the entire group (i.e. 50 years) as the cut off. The presence of heterogeneity among the pre-specified subgroups was evaluated using the chi-square
for heterogeneity (Mantel–Haenszel method). Logistic regression analysis was used to evaluate the influence of individual components of the metabolic syndrome on the odds of VTE. We defined age, BMI, sex, and the metabolic syndrome as potential confounding variables a priori, and included them in our regression analysis. Obesity was categorized as a dichotomous variable. Age was expressed as a continuous variable. To further explore if the association between the metabolic syndrome and VTE depends on the presence of abdominal obesity, we repeated the multivariate analysis after the exclusion of all patients with an elevated waist circumference. Multivariate analysis was subsequently performed including all previous variables and the individual components of the metabolic syndrome in the place of the metabolic syndrome.

Analysis of cohort studies

Kaplan-Meier analysis was used to calculate the cumulative incidence of VTE, with associated 95% confidence intervals. Follow-up was calculated as time from baseline to time when one of the following events occurred: the subjects developed VTE, the subject died from another cause, the date an individual moved out of the VTE catchment area or the last follow-up occurred. The HR and 95% confidence intervals of VTE were calculated for the metabolic syndrome using multivariable Cox regression. We allowed for across study heterogeneity by initially running a Cox model with random effect (“shared frailty” $\gamma$ distributed) for the study variable. A study stratified Cox model under the fixed effect assumption was planned if no significant variance of $\gamma$ distribution was found. Other variables a priori defined in the regression model were age, BMI, and sex as potential confounding variables. Subjects age was handled as a continuous variable and as a dichotomous variable using the median age as the cut off and obesity was categorized as a dichotomous variable using the ethnic specific definitions previously reported. All variables were retained if the p value was less than 0.10 or if they significantly affected the regression coefficients of other variables. The proportional hazards assumption was assessed by analysis of Schoenfeld residuals and a sensitivity analysis around the primary diagnosis was performed. Subsequently, we carried out the analysis including all previous variables and the individual components of the
metabolic syndrome in the place of the metabolic syndrome. Furthermore, if an association between the metabolic syndrome and VTE was found, hazard regression models were to be used to investigate the impact of increasing number of individual components of the metabolic syndrome on the risk of VTE, and to explore the influence of abdominal obesity on this relationship using subjects with no components of the metabolic syndrome were as a reference population.

All the analyses were performed using Minitab and SPSS 18 (SPSS Inc., Chicago, IL, USA).

REFERENCES


