Background: Recently, a growing body of evidence on the possible role of residual platelet reactivity (RPR) in affecting clinical events has accumulated. The aim of this study was to systematically assess the relationship between RPR on acetylic salicylic acid (ASA) therapy and the occurrence of recurrent events in a meta-analysis of prospective studies.

Methods: A systematic literature search of MEDLINE, EMBASE, Science Citation Index, the Cochrane Systematic Review Database and bibliographies of retrieved articles through May 2007 was conducted. Studies were included if they analysed RPR in coronary heart disease patients in relation to the occurrence of adverse coronary events during follow-up.

Results: Eleven prospective studies, incorporating 1952 patients with coronary heart disease followed for a time ranging from 6 days to 4 years, met the inclusion criteria. The pooled analysis demonstrated a significantly increased relative risk of adverse clinical events during follow-up for patients with RPR on ASA therapy (RR: 3.11, 95%CI 1.88–5.15; p < 0.0001). Moreover, the association between RPR and cardiovascular recurrences remained to be statistically significant even when subgroup analyses performed according to the duration of follow-up, ASA dosage, characteristics of the study population, and laboratory method were conducted.

Conclusions: The present meta-analysis documents a significant association between RPR on ASA treatment and recurrent cardiovascular events. More prospective studies are needed to determine the independent prognostic importance of RPR during aspirin therapy and possible benefit of individually tailored anti-platelet treatment strategies in these patients.

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1. Introduction

Antiplatelet drugs have an established place in the secondary prevention of a variety of vascular events, such as fatal and non-fatal coronary heart disease (CHD), stroke and peripheral arterial disease [1]. Indeed, results from the meta-analysis of the “Antiplatelet Trialists’ Collaboration” demonstrated that acetyl salicylic acid (ASA) therapy is able to reduce the risk of non-fatal myocardial infarction by 35% and the risk of total vascular events by 18% in patients with manifest coronary heart and cerebrovascular disease [1].

However, in a consistent proportion of vascular patients on ASA therapy residual platelet reactivity (RPR), the so-called aspirin resistance, is detectable [2]. A systematic review that recently assessed the prevalence of RPR in patients with CHD on ASA therapy reported an overall prevalence of 25% among...
all the published studies [3]. The question whether this phenomenon has a significant clinical implication on the occurrence of atherothrombotic events has not been, to date, definitely answered. Some studies evaluating the occurrence of clinical events during follow-up in patients with RPR have been conducted [4–6], and a recent meta-analysis by Snoep et al., evidenced an association between a laboratory-defined aspirin resistance and a higher risk of recurrent cardiovascular events [7]. The aim of this study is to systematically review all the available studies that prospectively investigated the possible association between RPR on ASA therapy and the occurrence of major adverse cardiovascular events in patients with CHD.

2. Methods

2.1. Search strategy

An extensive systematic literature search in order to identify studies evaluating the association between RPR and the occurrence of secondary cardiovascular events was conducted. The search was performed by using a combined text word and MeSH search strategy of the terms “acetyl salicylic acid”, “aspirin”, “antiplatelet”, “residual platelet reactivity”, and “persistent platelet reactivity” in combination with “resistance”, “failure”, “recurrence” and “major adverse cardiovascular events”, “cardiovascular disease”, “coronary artery disease”, “coronary heart disease”, “ischemic heart disease”, “myocardial infarction”, and “acute coronary syndromes” through the electronic databases MEDLINE (from 1966 to May 2007), EMBASE (from 1974 to May 2007), Science Citation Index (from 1994 to May 2007), and the Cochrane Systematic Review databases. Furthermore, a manual search of citations from relevant original studies and review articles was performed.

2.2. Study eligibility

To be included in the analysis, studies had to fulfil the following criteria:

- Prospective study design
- CHD patients
- Patients on ASA therapy for secondary prevention of cardiovascular events
- Clear definition of RPR
- Clear definition of laboratory methods used to identify RPR
- Relative risk, hazard ratio, or odds ratio and their corresponding 95% confidence intervals (or data to calculate them)

2.3. Study selection and data collection

All data were independently extracted by 2 investigators (F.S., and R.M.) through the use of a standardized data extraction tool and entered into separate databases. Results were compared, and disagreements were resolved by discussion with a third investigator (A.M.G.). The interobserver agreement for the study selection was 0.90. Relevant data included the first author’s name, year of publication, country of origin of the centres, number of participants and number of patients with RPR, ASA dosage, duration of follow-up, participants’ age, gender of participants, number of patients with clinical recurrences at follow-up, laboratory method used to measure RPR, definition of RPR, relative risks or hazard ratios of CHD and corresponding 95% CI, and covariates adjusted in the statistical analysis.

2.4. Statistical analyses

Data were analyzed by using Review Manager (RevMan) software for Windows (version 4.2) by the Cochrane Collaboration, 2003 and Statistical Package for Social Sciences (SPSS) software for Windows (version 13.0). The \( \kappa \) statistic was used to assess agreement between reviewers for study selection. The results of each study were reported as relative risk (RR), hazard ratio, or dichotomous frequency data. Hazard ratios were treated as RR, whereas frequency data were algebraically converted into RR. When available, we used the results of the original studies from multivariable models with the most complete adjustment for potential confounders; the confounding variables included in this analysis are shown in Table 1. We used a random-effects model which accounts for interstudy variation and provides a more conservative effect than the fixed model. Thus, we calculated random-summary RR with 95% confidence intervals (CI), by using inverse-variance method. The potential sources of heterogeneity were assessed by using the Cochran’s \( Q \) test to assess between-study differences and the \( I^2 \) statistic to quantify the proportion of inconsistency across the study results. Publication bias was assessed using a funnel plot of effect size against standard error.

3. Results

3.1. Search results

The search strategy generated 26 potentially relevant studies. Eight studies were eliminated because they were not prospective studies, 4 because they reported only clopidogrel resistance, 2 because they did not evaluate clinical cardiovascular recurrences during the follow-up but only surrogate markers, and 1 because only a meeting abstract was available. As a result, a total of 11 prospective studies were included in the meta-analysis [8–18].

3.2. Studies’ characteristics

Characteristics of the included studies are presented in Table 1. The included studies comprehended a total of 1952 patients with CHD followed for a time ranging from 6 days to 4 years, with study sample sizes ranging from 71 to 326
patients. There were 3 studies involving patients with stable cardiovascular disease whereas the remaining studies included patients with acute coronary syndromes who underwent primary percutaneous intervention. With regard to the laboratory method used for identifying patients with RPR, most of the included studies (n=8) used the PFA-100 system. As regarding statistical analysis, in addition, 6 out of 11 studies reported statistical data not adjusted for potential confounders.

### 3.3. Meta-analyses

The cumulative analysis for patients with RPR on ASA therapy showed a significant increased risk of cardiovascular
recurrences during follow-up (RR: 3.11, 95%CI 1.88–5.15; \(p = 0.00001\)) (Fig. 1). However, a significant heterogeneity across studies was reported (\(I^2: 62.7\%; p = 0.003\)). The study by Poulsen et al. [18] resulted to significantly influence the heterogeneity of the analysis; after exclusion of this study the association was confirmed (RR: 3.56, 95%CI 2.36–5.36; \(p = 0.0001\)) and no significant heterogeneity was reported (\(I^2: 30.3\%; p = 0.17\)). The funnel plot of effect size against standard error was broadly symmetric, so consistent with the conclusion that there was no publication bias. In addition, the association remained statistically significant even after exclusion of studies that reported only crude unadjusted data (\(n = 5\)) (RR: 3.19, 95%CI 1.97–5.19, \(p < 0.00001\)) (\(I^2: 0\%\); test of homogeneity \(p = 0.59\)).

3.4. Sensitivity analyses

To further examine the association between RPR and occurrence of cardiovascular recurrences we performed subgroup analyses, by selecting studies according to some specific variables. Accordingly, an increased risk of clinical recurrences was observed when studies were subgrouped according to the duration of follow-up \(\geq 1\) year, studies \(n = 8\); RR: 2.51 (95%CI 1.46–4.32), \(p = 0.0009\); < 1 year, studies \(n = 3\); RR: 7.25 (95%CI 3.12–16.81), \(p < 0.00001\), ASA dosage \(\leq 100\) mg, studies \(n = 3\); RR: 5.69 (95%CI 2.81–11.54), \(p < 0.00001\); > 100 mg, studies \(n = 6\); RR: 2.45 (95%CI 1.45–4.19), \(p = 0.002\), patients’ characteristics [acute coronary syndromes, studies \(n = 8\); RR: 3.17 (95%CI 1.68–6.00), \(p = 0.0001\); stable CHD, studies \(n = 3\); RR: 3.02 (95%CI 1.26–7.26), \(p < 0.0001\), and method used for determining RPR [PFA-100, studies \(n = 8\); RR: 2.52 (95%CI 1.44–4.41), \(p = 0.001\); platelet aggregation, studies \(n = 3\); RR: 5.82 (95%CI 2.99–11.34), \(p < 0.0001\)].

4. Discussion

The present meta-analysis conducted in 11 prospective studies, with an overall population of 1952 CHD patients followed for a time ranging from 6 days to 4 years, showed a significant increased risk of clinical recurrences for patients who manifested RPR on ASA treatment. This association remained statistically significant even when subgroup analyses according to the duration of follow-up, ASA dosage, patients’ characteristics, and laboratory method were performed.

ASA is currently the most effective therapeutic option for secondary prevention of fatal and non fatal cardiovascular events in patients with cardiovascular disease. Its clinical effectiveness for patients suffering from various vascular diseases has been well established in many clinical trials and in meta-analyses [1]. However, in a proportion of patients with cardiovascular disease under ASA treatment, RPR, the so-called aspirin resistance, is detectable [2]. Estimates of the prevalence of RPR in clinical trials vary enormously, ranging from 0% to over than 50% [4–6]. In a recent systematic review of all the clinical trials that evaluated persistent platelet reactivity in patients under ASA therapy, the mean prevalence of RPR was nearly 25% [3]. Some clinical prospective studies...
reported a significant association between RPR and the occurrence of secondary cardiovascular events [4–6], and a recent meta-analysis by Snoep et al. [7] reported a significant role for the laboratory-defined aspirin resistance on the risk of clinical cardiovascular recurrences.

In the present meta-analysis we confirm the data recently published by Snoep et al. [7], by systematically analysing all the published clinical studies that evaluated platelet function among patients with CHD on ASA therapy. This in order to give further insights on this matter that is arousing increasing clinical interest among physicians and researchers. The main result of our study is that CHD patients with a laboratory evidence of RPR on ASA therapy were at an increased risk of clinical recurrences. This datum, though not conclusive, gives strong stimulus to researchers to continue working in this field.

To date, the mechanism of RPR on ASA therapy is still uncertain. Some investigators reported a nonadherence to therapy as the main cause of persistent platelet activation, by hypothesising this phenomenon only as “non-compliance” [19]. Others, instead, hypothesised that the interaction of platelets with other cells, as well as the presence of polymorphisms in genes encoding for proteins involved in the platelet function may help to explain the pathophysiological basis for RPR in these patients [20–24]. Additionally, diabetes, duration of ASA therapy and dosage have been also demonstrated to likely contribute in determining RPR [25].

Our meta-analysis suffers from some limitations that strictly derive from the studies included in the final analysis. Actually, a number of laboratory methods for the measurement of RPR have been used, and there is not an accepted uniform measure for screening platelet function in the clinical setting. Furthermore, the studies differ substantially for several features such as clinical setting of patients enrolled, ASA dosage, duration of treatment, laboratory method, and definition of RPR, so determining a statistically significance for the heterogeneity test of the cumulative analysis. However, after exclusion of the study by Poulsen et al. [18], which was not designed to investigate the prognostic role of residual platelet reactivity on the occurrence of atherothrombotic events, the heterogeneity test lost its significance. Finally, adherence to ASA therapy has not been systematically assessed, and most of the included studies lack of adjustment for confounding factors in the multivariable statistical models. However, subgroup analyses conducted according to some specific variables, i.e. laboratory method, duration of ASA treatment and ASA dosage confirmed the significant association observed between such phenomenon and the occurrence of secondary clinical events, so being in line with the results of our previous study documenting a concordance between different methods used for the measurement of RPR on ASA [26].

In conclusion, the present meta-analysis conducted in 11 prospective studies and including over than 1900 patients with CHD demonstrated that RPR is significantly associated with an increased risk of recurrent cardiovascular events, by possibly suggesting a clinical implication of RPR, though in apparent contrast with the indications of the scientific associations [27]. However, more prospective studies are needed to determine the independent prognostic importance of RPR during aspirin therapy and the possible benefit of individually tailored anti-platelet treatment strategies in these patients.

References