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ADAMTS13 and the risk of myocardial infarction: an individual patient data meta-analysis

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Keywords: myocardial infarction, ADAMTS13, risk factors, blood coagulation, meta-analysis, von Willebrand Factor

Abstract

Background: Low levels of ADAMTS13 have been repeatedly associated with an increased risk of ischemic stroke but results for risk of myocardial infarction (MI) are inconclusive.

Objective: To perform an individual patient data meta-analysis from observational studies investigating the association between ADAMTS13 levels and MI.

Methods: A one step meta-analytic approach with random treatment effects was used to estimate pooled odds ratios (OR) and corresponding 95% confidence intervals (CI) adjusted for confounding. Analyses were based on dichotomous exposures, with the 5th and 1st percentile of ADAMTS13 antigen levels as cut off values. Quartile analyses, with the highest quartile as a reference category, were used to assess a graded association between levels and risk ('dose' relationship). Additionally, we assessed the risk of the combined presence of low ADAMTS13 and high von Willebrand Factor (VWF) levels.

Results: Five studies were included, yielding individual data of 1501 cases and 2258 controls. Low levels of ADAMTS13 were associated with myocardial infarction risk, with an OR of 1.89 (95% CI 1.15–3.12) for values below the 5th percentile versus above, and an OR of 4.21 (95% CI 1.73–10.21) for values below the 1st percentile versus above. Risk appeared restricted to these extreme levels, as there was no graded association between levels of ADAMTS13 and MI risk over quartiles. Finally, there was only a minor synergistic effect for the combination of low ADAMTS13 and high VWF levels.

Conclusion: Low levels of ADAMTS13 are associated with an increased risk of MI.

Introduction

The thirteenth member of a disintegrin-like and metalloprotease with thrombospondin type 1 motif family (ADAMTS13) is a circulating plasma enzyme responsible for cleavage of the platelet-adhesive ultra-large forms of von Willebrand factor (VWF) [1]. The cleavage of ultra-large VWF into smaller molecules by ADAMTS13 is an important regulatory mechanism in haemostasis since these smaller VWF molecules have a reduced platelet tethering capacity [1, 2]. Severe deficiency of ADAMTS13 promotes VWF- induced platelet aggregation, and can result in thrombotic thrombocytopenic purpura (TTP) [2]. Historically, after ADAMTS13 has been identified as the VWF-cleaving enzyme in 2001, research on this metalloprotease first focussed on the pathophysiology of TTP and the interactions of the enzyme with VWF [3]. In recent years, the focus shifted to the role of ADAMTS13 in the more common forms of thrombotic disease. This started with studies of VWF, the cleavage substrate of ADAMTS13, that have indicated an increase in risk of cardiovascular disease for high levels of VWF [4]. A similar association has been established for genetic factors influencing circulating VWF levels (most notably, ABO blood group) [5-8]. These data suggested that low levels of ADAMTS13, which diminishes the VWF cleavage capacity and therefore increases the VWF activity, might also increase the risk of arterial thrombosis. Moreover, murine studies have shown that deletion of the murine ortholog of ADAMTS13 results in an increased predisposition to atherosclerosis and arterial thrombosis [9-11]. With this background, a number of studies investigated the role of ADAMTS13 plasma levels in relation to the risk of arterial thrombosis, but with conflicting results for the two main forms of this disease [12, 13]. Several studies reported that low levels of ADAMTS13 increased the risk of ischemic stroke, but one study on myocardial infarction was negative [14]. A recent meta-analysis based on published results confirmed this association for ischemic stroke (relative risk of 2.72, 95% confidence intervals 1.52 – 4.85, for low vs high levels of ADAMTS13), but failed to give a definitive answer for myocardial infarction. The pooled estimate for myocardial infarction was

accompanied by wide confidence interval due to a lack of power (relative risk of 1.45, 95% CI 0.71-2.98) [15]. The lack of precision of this meta-analytic approach, as well as the inability to discriminate specific patients subgroups, and uniform confounder adjustment, hampers the ability to determine the relevance of low ADAMTS13 levels in myocardial infarction.

A more powerful and less biased approach collects individual patient data (IPD) directly from the researchers responsible for each study. Use of IPD has several advantages over the aggregate data approach, including standardization of statistical analyses, assessment of potential causes of heterogeneity, adjustment for confounding on individual information and the investigation of interactions and non-linear effects.

Methods

Search strategy and selection of studies

This systematic review and IPD meta-analysis of studies investigating ADAMTS13 levels in myocardial infarction were conducted according to the principles of the PRISMA statement [16]. We searched for all publications reporting the association between ADAMTS13 and myocardial infarction up to February 2014. Publications were identified with a systematic search in PubMed (1950-2014). The search strategy was composed by the following Boolean combination of search terms: ADAMTS13 "AND" myocardial infarction "OR" heart disease "OR" coronary disease. Publications were selected independently by two authors (AM, LAL) from the resulting listing.

Publications were included when: (1) they reported original data about the association between ADAMTS13 levels in humans (i.e., plasma antigen or activity) and incident myocardial infarction; (2) the outcome was myocardial infarction as an acute vascular event rather than surrogate (e.g., studies reporting only coronary artery plaque were excluded). Studies that combined several forms of arterial thrombosis (e.g. myocardial infarction and transient ischemic attack, ischemic stroke, peripheral artery disease) were included as long as, at the patient level data, it was possible to select myocardial infarction cases from the combined endpoint.

The reference list of the included studies was checked for relevant publications that were not identified by the literature search. For practical reasons, and in order to minimize the impact of publication bias, studies were eligible only when the sample size was reasonably large (predefined cut off of more than 50 cases).

Study design

Corresponding authors of the selected publications were contacted and asked to provide information on an individual level regarding clinical and demographic characteristics of cases and controls. Requested variables were: age, sex, height, weight, and known clinical cardiovascular risk factors. These included history of hypertension, diabetes, hypercholesterolemia (defined as total cholesterol greater than 200 mg/dl) and smoking habits. Moreover, the authors were asked to provide the following laboratory variables: time from the event to the blood collection, ADAMTS13 level (plasma antigen levels) and VWF level (plasma antigen levels).

Statistical analysis

ADAMTS13 and VWF levels from each study were standardized by dividing each single value by the mean value of the corresponding control group and expressed as percentage. The overall association between ADAMTS13 and myocardial infarction was determined using a one-step meta-analytic approach on individual patient data. This was applied by mixed logistic regression model with random treatment effects to obtain odds ratios (OR) and corresponding 95% confidence intervals (95% CI) as measures of relative risk [17]. All models included the variables age and sex. Additional adjustment for potential confounders (e.g., hypercholesterolemia, hypertension, diabetes mellitus, body mass index and smoking) was done in separate models. The main analyses were based on a dichotomous exposures, with cut-off values set at the 5^{th} and 1^{st} percentile of the ADAMTS13 distribution of the pooled control group. Dummy variables with predefined cut off points were created to assess the combined effect of high levels of VWF (above the 90^{th} percentile, already showed to be associated with myocardial infarction [12]) and low levels of ADAMTS13 (below the 5^{th} percentile). Quartile analyses, with the highest quartile as reference category, were used to determine the association over the full range of ADAMTS13 values. The presence of a non-linear effect of the ADAMTS13 levels distribution on the risk of myocardial infarction (expressed

as log odds) was also evaluate using a restricted cubic spline function with 3 knots, which was the one that maximize the Akaike's information criterion [(model likelihood ratio $\chi 2 - 2p$), with p equal to the number of parameters in the model aside from the intercept (i.e., the number of knots – 1)] [18]. Predefined subgroups analyses were based on sex (male vs female) and age (below vs above 45 years old at the time of the event). Because of the reduction of the number of studies available for each subgroup, a fixed effect model was used, which included the variables age, sex, hypercholesterolemia, hypertension, diabetes mellitus, body mass index, smoking, study indicator and interaction terms between exposure and study indicator. Statistical analyses were performed using STATA version 13.0, SPSS version 20.0 and software R version 3.0.2.

Results

A total of 69 publications were identified by the search strategy. Of these, six studies fulfilled the inclusion criteria and one additional study was included after checking the reference lists of the included publications (Fig. 1).

Studies had various conclusions regarding the association of low levels of ADAMTS13 with myocardial infarction, encompassing a protective effect (Chion et al. [14]), no effect (Horii et al. [19] and Peyvandi et al. [13]) and a risk-increasing effect (Kaikita et al.[20], Matsukawa et al. [21], Crawley et al. [22], Bongers et al. [23] and Andersson et al. [12]). Ultimately, five studies fulfilled the criterion of an adequate sample size, and therefore were included in the analysis. The main characteristics of all study populations, such as study design and moment of blood draw, are summarised in Table 1.

All corresponding authors from the selected studies proved willing to provide the requested information, yielding a total of 1501 myocardial infarction cases and of 2258 controls. Demographic and clinical characteristics of cases and controls are shown in Table 2. Mean age is similar for cases and controls (51 years vs 47 years), whereas there was a preponderance of men within cases (66% vs 49%). As expected, cardiovascular risk factors were more prevalent in cases than in controls.

Fig. 2 shows the ORs for the association between ADAMTS13 levels and the risk of myocardial infarction based on the 5th percentile comparison in each single study. When all the studies were pooled together, low levels of ADAMTS13 (i.e., below the 5th percentile vs above the 5th percentile) were associated with almost a twofold increase in risk of myocardial infarction (fully adjusted OR 1.89, 95% CI 1.15 – 3.12). This association became stronger with a more extreme cut-off (fully adjusted OR 4.09, 95% CI 1.73 – 10.21 for levels below vs above the 1st percentile), as shown in Table 3. Additional adjustment for VWF levels did not affect the estimates (OR 1.79, 95% CI 1.05

– 3.06 for the 5th percentile cut-off and OR 4.16, 95% CI 1.74 – 9.98 for the 1st percentile cut-off). When the comparison was made by quartiles of ADAMTS13 distribution, moderately low levels of ADAMTS13 conferred a small increase in risk of myocardial infarction (lowest quartile vs highest quartile, fully adjusted OR 1.28, 95% CI 0.68 – 2.45), and no trend was seen for intermediate quartiles, indicating a threshold rather than a 'dose' relationship (Table 3). The form of the relationship between levels of ADAMTS13 and the risk of myocardial infarction is depicted in Fig. 3. There is appreciably a non-linear component of this association (i.e., a slightly more rapid increase in the risk of myocardial infarction) for levels below 80%.

Table 4 shows the combined effect of ADAMTS13 and VWF levels on the risk of myocardial infarction. VWF levels above the 90th percentile compared with levels below the 90th percentile were associated with an increase in the risk of myocardial infarction (OR 1.72, 95% CI 1.22 – 2.42). When the analysis was restricted to levels of VWF below the 90th percentile, the risk associated with low ADAMTS13 levels was similar to that of the main analysis (OR 1.77, 95% CI 1.00 – 3.25 for ADAMTS13 levels below the 5th percentile vs above the 5th percentile). The risk of myocardial infarction conferred by a combination of low levels of ADAMTS13 and high levels of VWF was only slightly higher than could be expected by the separate effect, without showing evidence of a strong interaction (expected OR 1+0.77+0.72=2.49; calculated OR 3.17, 95% CI 1.18 – 8.63).

The results from subgroup analyses are shown in Table 5. There was a similar relative effect of ADAMTS13 on the risk of myocardial infarction for subject below and above 45 years old, whereas there was a considerable difference between women and men. Women with low levels of ADAMTS13 (i.e. levels below the 5th percentile compared with levels above the 5th percentile) had an almost 3 fold increased risk of myocardial infarction (OR 2.78, 95% CI 1.61 – 4.88), while in men a 1.7 fold increase was observed (OR 1.66, 95% CI 1.08 – 2.56). When, to remove centre effects in this comparison, the analysis was restricted to studies including both sexes (i.e. GLAMIS

and ATTAC), this difference in relative rates persisted: for ADAMTS13 levels below vs above the 5th percentile OR in women 4.00, 95% CI 1.63 – 9.72; OR in men 2.50, 95% CI 1.48 – 4.15.

Finally, when the analysis was restricted to the studies in which the blood samples were collected after the acute phase (i.e. >1 month from the event), the results did not differ much from the main analysis (ADAMTS13 levels below vs above the 5th percentile, fully adjusted OR 1.96, 95% CI 1.08 – 3.55).

Discussion

Our analysis of 1501 cases and 2258 controls from 5 case-control studies indicates that only low levels of ADAMTS13 (i.e., standardized antigen levels below 64%, a cut-off that corresponds to values varying from 61% [ATTAC] to 78% [Milan] antigen levels relative to normal pooled plasma among the original studies) are associated with a moderate increase in risk of myocardial infarction. This association is not mediated by levels of VWF antigen.

Subgroups analysis showed that the association between ADAMTS13 and the risk of myocardial infarction was similar between ages, but, while present in both sexes, seemed to be more pronounced in women. Partly, this difference in relative risk could belongs to the selection of the studies in the two subgroups (i.e. SMILE, GLAMIS and ATTAC for men's subgroup and GLAMIS, RATIO, Milan, ATTAC for women's subgroup) and, because the incidence of myocardial infarction is higher in men than in women, a similar risk difference will lead to a higher relative risk. However, our data do not allow to further investigate if it reflects a true sex-specific effect, chance, or a difference in the presence of unmeasured confounding between men and women (for example alcohol consumption or physical activity).

The relationship between ADAMTS13 and other types of arterial thrombosis such as ischemic stroke has been investigated in some studies [12, 23-25]. Their results suggest that even moderately reduced levels of ADAMTS13 increased the risk of ischemic stroke. A meta-analysis of aggregate data, pooling these publications, showed a strong association between ADAMTS13 and ischemic stroke for the lowest quartile of the ADAMTS13 distribution (pooled OR for low vs high ADAMTS13 levels, 2.72, 95% CI 1.52 – 4.86) [15]. For myocardial infarction our IPD meta-analysis showed an association for the lowest levels of ADAMTS13 (OR for the lowest quartile vs the highest quartile 1.28, 95% CI 0.68 – 2.45, whereas OR for levels below vs above the 1st percentile 4.21, 95% CI 1.73 – 10.21). This indicates that, although ADAMTS13 levels are

associated with both forms of arterial thrombosis, the effects are different, which may reflect pathophysiological differences between the two disorders.

The pathophysiological mechanisms that underlie the association of low ADAMTS13 levels with myocardial infarction are not fully understood. We believe that this mechanism may be due to one, or a combination of, the following: 1) an effect of ADAMTS13 concentration upon the initiation and progression of the atherosclerotic plaque itself [9]; 2) the influence of ADAMTS13 concentration upon acute thrombus formation [10]; 3) the influence of ADAMTS13 upon amplification of the thrombus and deleterious post-thrombotic inflammation [11].

Murine models have revealed that complete ADAMTS13 deficiency augments the development of atherosclerotic lesions in a manner that is dependent on VWF [9, 26, 27]. Although not formally examined in these studies, it is likely that complete (rather than heterozygous) deficiency is necessary for this enhanced lesion development. This may argue against a role for more subtle variation in ADAMTS13 concentration upon plaque development. In humans, severe ADAMTS13 deficiency (<5% activity) is a cause for widespread microvascular thrombosis due to the lack of cleavage of the hyperactive ultra-large von Willebrand factor (ULVWF) multimers. This can also be associated with myocardial infarction in some patients with TTP [28]. However, it appears in both mice and humans that ADAMTS13 levels >50% are not associated with any detectable difference in plasma VWF multimer distribution [29].

In our analysis, we did not find that the association of ADAMTS13 with the risk of myocardial infarction was mediated by levels of VWF (i.e., adjustment for VWF levels did not materially affect the results). Moreover, we found only a minor synergistic effects between low levels of ADAMTS13 and high levels of VWF. We speculate that plasma levels of ADAMTS13 slightly below the lower bound of the normal range (i.e. ~60%) may more likely influence the kinetics of the thrombus growth, in a manner that is independent of circulating VWF levels. In physiologic conditions, the proteolysis of VWF is determined primarily by the unfolding of ULVWF multimers

rather than ADAMTS13 plasma levels. Conversely, at sites of injury or plaque rupture where the thrombus is consolidated by platelet binding through fibrinogen and fibrin, active VWF can become protected against the effects of ADAMTS13 cleavage [30]. Therefore, the plasma concentration of ADAMTS13 may be far more important during the acute phase control of thrombus formation. As such, low ADAMTS13 might impair this protective mechanism leading to the formation of a more extensive platelet plug that is further stabilized/consolidated by additional prothrombotic mechanisms. Such a mechanism may explain why there is a stronger association of low ADAMTS13 levels with ischaemic stroke than with myocardial infarction. Myocardial infarction could be more closely related to the extent of the underlying arterial disease, whereas ischemic stroke may in part be determined by thromboembolic events, the pathogenicity of which may be more directly influenced by ADAMTS13 plasma concentration.

Our study has some limitations. To increase the number of studies that could provide data and the homogeneity of the information, we intentionally limited the number of variables we used for our analyses. This, however, could have led to residual confounding and have limited the number of additional analysis (e.g. stratification for other blood parameters).

We also excluded studies with less than 50 cases. This might have led to a small reduction in power, but reduced the impact of publication bias, to which small studies are more prone than large studies. Given the considerable study sample size we have reached, it is unlikely that the inclusion of these studies would have altered our findings. Finally, because of the case-control design of the studies included, blood was collected after the event in the case groups. This might lead to reverse causation, which is when the consequence of an event is mistaken for the cause. We were not able to perform subgroups analysis based on time from the event to the blood sampling due to the little overlap of these time periods between the five studies. However, since only in one study blood was collected in the acute phase of myocardial infarction (i.e. the Milan study), and we did not find an

effect on timing of the blood draw in the individual patient data analysis, the results are unlikely to be explained by the transient effects of the acute phase.

In conclusion, with an IPD meta-analytic approach we demonstrated that low levels of ADAMTS13 increased the risk of myocardial infarction. This association is valid only for low levels of ADAMTS13, and therefore differs from its relation with ischemic stroke.

Addendum

A. Maino design the research, analysed the data, interpreted the results and drafted the manuscript; B. Siegerink design the research, provided individual patient data, analysed the data, interpreted the results and reviewed the manuscript; L. A. Lotta design the research, interpreted the results and reviewed the manuscript; J. T. B. Crawley provided individual patient data, interpreted the results and reviewed the manuscript; S. le Cessie provided statistical support, analysed the data, interpreted the results and reviewed the manuscript; F. W. G. Leebeek provided individual patient data, interpreted the results and reviewed the manuscript; D. A. Lane provided individual patient data, interpreted the results and reviewed the manuscript; G. D. O. Lowe provided individual patient data, interpreted the results and reviewed the manuscript; F. Peyvandi provided individual patient data, interpreted the results and reviewed the manuscript; F. R. Rosendaal design the research, analysed the data, interpreted the results and reviewed the manuscript. All authors read and approved the final version of the manuscript.

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Disclosure of Conflict of Interests

None to declare.

Figures and Tables

Table 1. Main characteristics of the studies included in the IPD meta-analysis

Study	Reference	Original study size (cases / controls)	Age	Sex	Recruitment period	Time of blood draw from the event (months)	Nation	Case-control matching variables
SMILE	Chion et al. 2007 [14]	560 / 646	>18	male	1994-1997	>3	The Netherlands	age
GLAMIS	Crawley et al. 2008 [22]	466 / 484	>18	both	1994-1995	>3	UK	sex and age
ATTAC	Bongers et al. 2009 [23]	169 / 332	<55 for men <40 for women	both	before 2005	>1	The Netherlands	age
Milan	Peyvandi et al. 2010 [13]	138 / 199	18-45	female	1998-2001	<1	Italy	age and geographic origin
RATIO	Andersson et al. 2012 [12]	202 / 626	<50	female	1990-1995	>38	The Netherlands	age, area of residence and index year

Table 2. Demographic and clinical characteristics for cases and controls with available ADAMTS13 levels, included in the IPD meta-analysis.

	SMILE		GLAMIS		ATTAC		Milan		RATIO		Overall	
	Cases (551)	Controls (635)	Cases (447)	Controls (472)	Cases (165)	Controls (329)	Cases (136)	Controls (196)	Cases (202)	Controls (628)	Cases (1501)	Controls (2258)
Age, mean (SD)	56.2 (9.1)	57.4 (10.8)	54.8 (7.5)	55.1 (7.5)	42.8 (5.6)	38.4 (7.9)	39.3 (5.6)	39.4 (5.2)	42.2 (6.1)	38.4 (7.9)	51.0 (10.1)	47.4 (12.3)
Sex, n (%)												
female	-	-	116 (26)	126 (27)	63 (38)	207 (63)	136 (100)	196 (100)	202 (100)	628 (100)	517 (34)	1155 (51)
male	551 (100)	635 (100)	331 (74)	346 (73)	102 (62)	122 (37)	-	-	-	-	984 (66)	1103 (49)
BMI (SD)	27.1 (3.4)	26.9 (3.5)	28.0 (4.8)	26.9 (4.6)	26.9 (4.8)	25.1 (4.3)	24.1 (4.3)	23.1 (4.7)	27 (5.2)	24.4 (4.1)	27.1 (4.4)	25.6 (4.3)
Months from MI, mean (min-max)	38 (3-72)	-	6 (3-9)*	-	2 (1-7)	-	<1 (0-1)	-	70 (38-112)	-	24 (0-112)	-
History of:												
Hypertension (%)	154 (28)	118 (18)	176 (39)	83 (18)	36 (24)	22 (8)	34 (26)	18 (9)	74 (37)	39 (6)	474 (32)	274 (12)
Diabetes (%)	26 (5)	21 (3)	45 (10)	10 (2)	15 (9)	5 (2)	7 (5)	2 (1)	10 (5)	9 (1)	103 (7)	47 (2)
Smoking (%)	345 (63)	208 (33)	197 (44)	131 (28)	145 (88)	166 (51)	95 (70)	92 (47)	167 (83)	264 (43)	949 (63)	864 (38)
Hypercholesterolemia (%)	164 (30)	10 (2)	142 (32)	153 (33)	81 (60)	133 (48)	52 (39)	73 (40)	20 (10)	19 (3)	458 (31)	388 (18)

MI, myocardial infarction; BMI, body mass index; SD, standard deviation;
*Details for single participants are not available, samples were drown between 3 and 9 months from the event.

Table 3. Risk of myocardial infarction in relation on various plasma levels of ADAMTS13.

Standardized levels ADAMTS13	Cases 1501	Controls 2258	OR (95% CI)	OR_1 (95% CI)
<= 5th percentile (<= 64%)	130	112	1.75 (0.98 – 3.12)	1.89 (1.15 – 3.12)
> 5th percentile (> 64%)	1371	2146	ref	ref
<= 1st percentile (<= 52%)	67	22	4.09 (1.41 – 11.83)	4.21 (1.73 – 10.21)
> 1st percentile (>52%)	1437	2236	ref	ref
Q1 (< 83%)	420	564	1.38 (0.69 – 2.78)	1.28 (0.68 – 2.45)
Q2 (from 83% to 97%)	379	565	1.23 (0.76 – 2.01)	1.25 (0.78 – 1.97)
Q3 (from 97% to 112%)	361	565	1.12 (0.83 – 1.52)	1.08 (0.81 – 1.46)
Q4 (> 112%)	341	564	ref	ref

ORs, as measure of relative risk, are calculated by mixed logistic regression model with random treatment effects and are all adjusted for age and sex. OR_1 values are also adjusted for body mass index and history of smoking, hypercholesterolemia, hypertension, diabetes. Q indicates quartile; and ref, reference.

Table 4. Risk of myocardial infarction in relation to the combination of low ADAMTS13 and high VWF plasma levels.

High VWF (> 90th percentile)	Low ADAMTS13 (< 5th percentile)	Cases, n (%)	Controls, n (%)	OR (95 % CI)
-	-	1137 (76)	1872 (86)	ref
+	-	225 (15)	201 (9)	1.72 (1.22 – 2.42)
-	+	94 (6)	92 (4)	1.77 (1.00 – 3.25)
+	+	35 (2)	17 (1)	3.17 (1.18 – 8.63)

ORs, as measure of relative risk, are calculated by mixed logistic regression model with random treatment effects and are adjusted for age, sex, body mass index and history of smoking, hypercholesterolemia, hypertension, diabetes. Relative risks are calculated for four strata, high plasma levels of VWF, i.e. above the 90th percentile, (+/-), low plasma levels of ADAMTS13, i.e. below the 5th percentile, (-/+), or both (+/+) with the –/– category as reference.
Data on VWF was available for 1491 cases (99% of total) and 2182 controls (97% of total). Ref indicates reference.

Table 5. Risk of myocardial infarction in relation on levels of ADAMTS13 for age and sex subgroups.

	ADAMTS13 <5th percentile		ADAMTS13 >5th percentile		
	Cases	Controls	Cases	Controls	OR (95% CI)
Age at the event					
below 45 years	57 (12%)	59 (6)	428 (88%)	966 (94%)	1.99 (0.93 – 4.26)
above 45 years	73 (7%)	53 (4)	943 (93%)	1180 (96%)	2.41 (0.74 – 7.86)
Sex					
female	59 (11%)	62 (5%)	458 (89%)	1093 (95%)	2.78 (1.61 – 4.88)
male	71 (7%)	50 (6%)	913 (93%)	1053 (94%)	1.66 (1.08 – 2.56)

ORs are calculated by multivariable logistic regression model with fixed effect and adjusted for age, sex, body mass index and history of smoking, hypercholesterolemia, hypertension, diabetes.

Fig. 1. Flow chart of the step of studies selection.

The figure shows the three steps in studies selection: identification of studies which reported the association between ADAMTS13 and incident myocardial infarction as acute vascular event and with an adequate sample size of more than 50 myocardial infarction cases.

Fig. 2. Forest plot for the association between low levels of ADAMTS13, which is below the 5th percentile, and the risk of myocardial infarction by studies.

Dots indicate ORs and solid bars indicate 95% confidence intervals. The scale is logarithmic. ORs were calculated for each study population by multivariable logistic regression model and were adjusted for age, sex, body mass index and history of smoking, hypercholesterolemia, hypertension, diabetes. The overall OR was calculated by one step individual patient data meta-analytic approach by mixed logistic regression with random treatment effects. NL, The Netherlands; UK, United Kingdom; IT, Italy.

Fig. 3. Restricted cubic spline curve showing the model-predicted probability of myocardial infarction against plasma levels of ADAMTS13.

The solid line represents the probability of myocardial infarction for levels of ADAMTS13 adjusted for age, sex, body mass index, study indicator and history of smoking, hypercholesterolemia, hypertension, diabetes. Dashed lines represent 95% confidence intervals**.**

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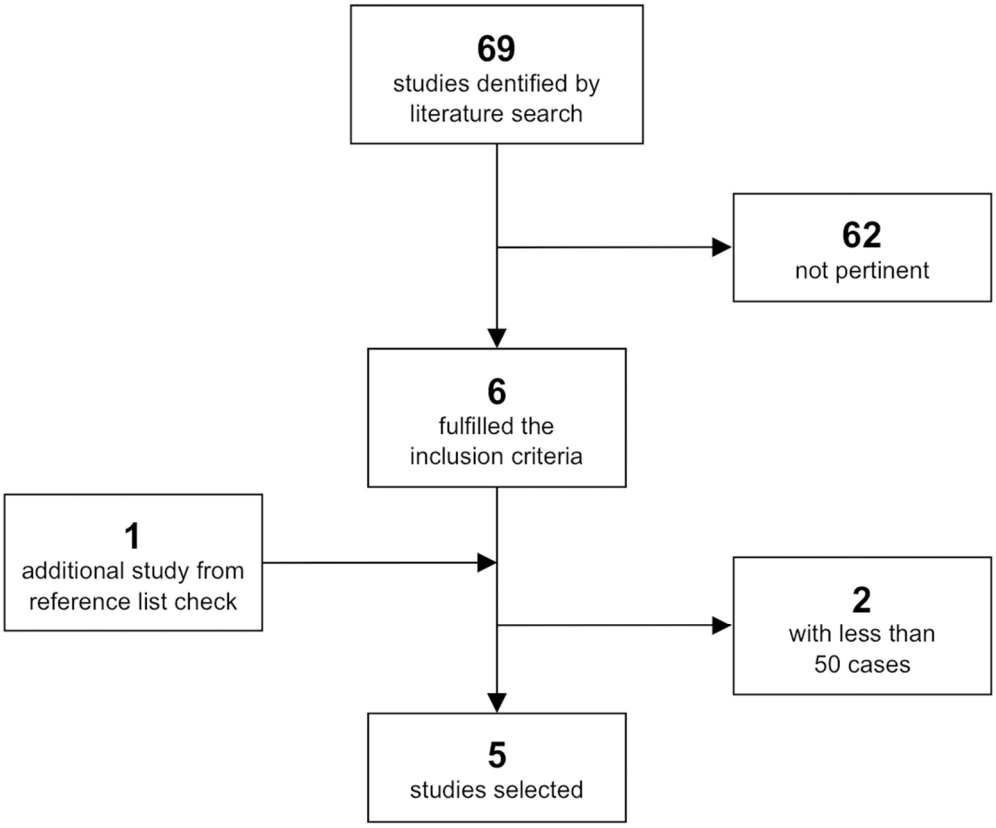

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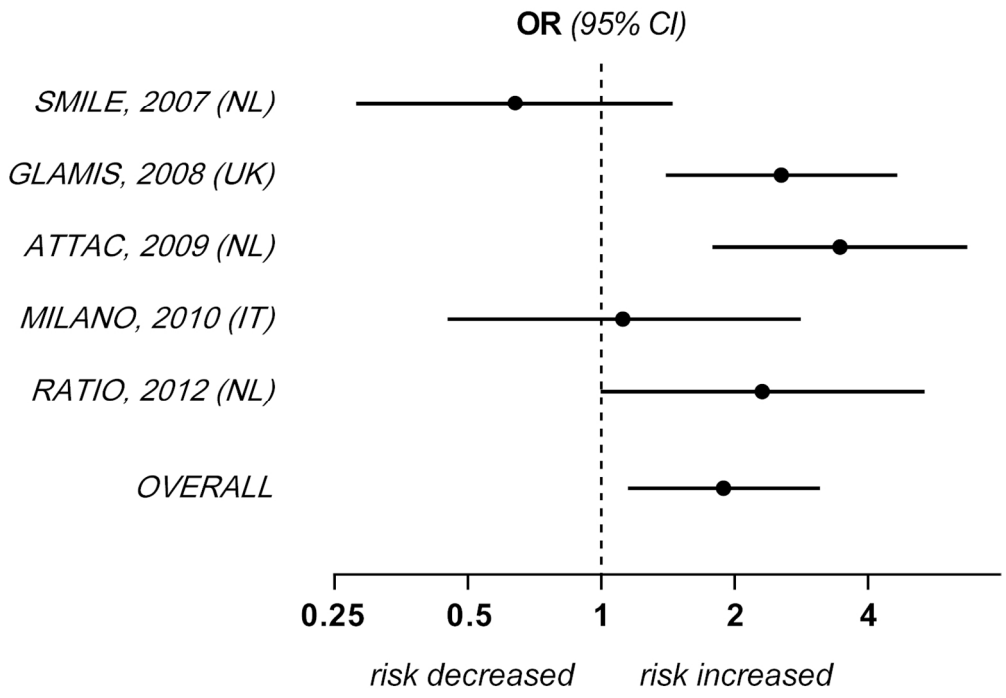

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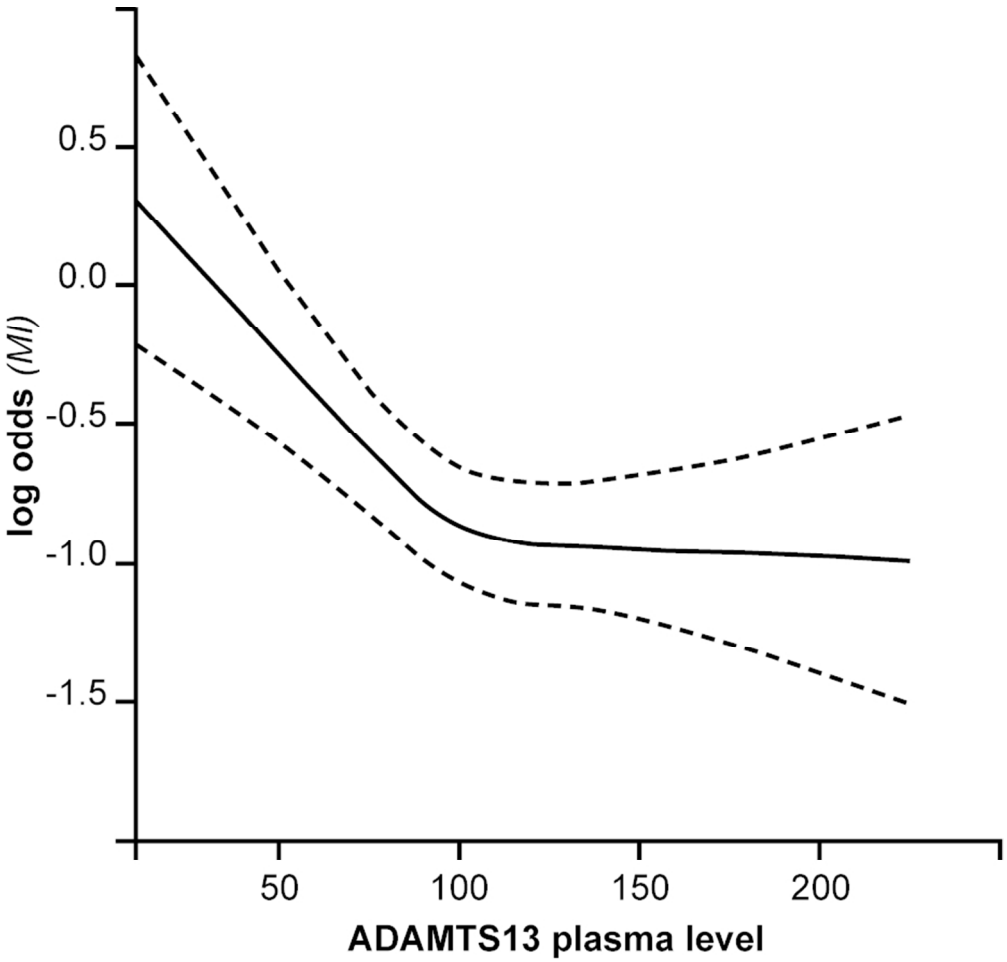

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8 and 17
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8 and 18
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8 and 18
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9 and 19
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9-10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15-16

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