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ORIGINAL ARTICLE

Plasma ADAMTS-13 levels and the risk of myocardial infarction: an individual patient data meta-analysis

A. MAINO,*† B. SIEGERINK,*‡§ L. A. LOTTA,† J. T. B. CRAWLEY,¶ S. LE CESSIE,**
F. W. G. LEEBEEK,‡‡ D. A. LANE,¶ G. D. O. LOWE,§§ F. PEYVANDI† and F. R. ROSENDAAL*‡¶¶
**Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands; †A. Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milano, Università degli Studi di Milano, Milan, Italy; ‡Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, the Netherlands; §Center for Stroke Research, Charité-Universitätsmedizin, Berlin, Germany; ¶Centre for Haematology, Faculty of Medicine, Imperial College London, London, UK; **Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden; ‡‡Department of Hematology, Erasmus University Medical Center, Rotterdam, the Netherlands; §§Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK; and ¶¶Department of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, the Netherlands*

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Summary. *Background:* Low ADAMTS-13 levels have been repeatedly associated with an increased risk of ischemic stroke, but results concerning the risk of myocardial infarction are inconclusive. *Objectives:* To perform an individual patient data meta-analysis from observational studies investigating the association between ADAMTS-13 levels and myocardial infarction. *Methods:* A one-step meta-analytic approach with random treatment effects was used to estimate pooled odds ratios (ORs) and corresponding 95% confidence intervals (CIs) adjusted for confounding. Analyses were based on dichotomous exposures, with the 5th and 1st percentiles of ADAMTS-13 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 ADAMTS-13 and high von Willebrand factor (VWF) levels. *Results:* Five studies were included, yielding individual data on 1501 cases and 2258 controls (mean age of 49 years). Low ADAMTS-13 levels 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 to be restricted to these extreme levels, as there was no graded association between ADAMTS-13 levels and myocardial infarction risk over quartiles. Finally, there was only a minor synergistic effect for the combination of low ADAMTS-13 and high VWF levels. *Conclusions:* Low ADAMTS-13 levels are associated with an increased risk of myocardial infarction.

Correspondence: Frits R. Rosendaal, Department of Clinical Epidemiology, C7-P, P.O. Box 9600, 2300 RC Leiden, the Netherlands.
Tel.: +31 71 5264037; fax: +31 71 5266994.
E-mail: f.r.rosendaal@lumc.nl

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Introduction

The 13th member of the ADAMTS family (ADAMTS-13) is a circulating plasma enzyme responsible for cleavage of the platelet-adhesive ultralarge forms of von Willebrand factor (VWF) [1]. The cleavage of ultralarge VWF (ULVWF) into smaller molecules by ADAMTS-13 is an important regulatory mechanism in hemostasis, as these smaller VWF molecules have reduced platelet-tethering capacity [1,2]. Severe deficiency of ADAMTS-13 promotes VWF-induced platelet aggregation, and can result in thrombotic thrombocytopenic purpura (TTP) [2]. Historically, after ADAMTS-13 had been identified as the VWF-cleaving enzyme in 2001, research on this metalloprotease first focused on the pathophysiology of TTP and the interactions of the enzyme with VWF [3]. In recent years, the focus has shifted to the role of ADAMTS-13 in the more common forms of thrombotic disease. This started with studies of VWF, the cleavage substrate of ADAMTS-13, that indicated an increase in the risk of cardiovascular disease with high VWF levels [4]. A similar association has been established for genetic factors influ-

encing circulating VWF levels (most notably, ABO blood group) [5–8]. These data suggested that low ADAMTS-13 levels, which diminish VWF cleavage capacity and therefore increase VWF activity, might also increase the risk of arterial thrombosis. Moreover, murine studies have shown that deletion of the murine ortholog of ADAMTS-13 results in an increased predisposition to atherosclerosis and arterial thrombosis [9–11].

With this background, a number of studies investigated the role of ADAMTS-13 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 ADAMTS-13 levels increased the risk of ischemic stroke, but one study on myocardial infarction gave a negative result [14]. A recent meta-analysis based on published results confirmed this association for ischemic stroke (relative risk of 2.72, 95% confidence interval [CI] 1.52–4.85, for low versus high ADAMTS-13 levels), but failed to give a definitive answer for myocardial infarction. The pooled estimate for myocardial infarction was accompanied by a wide CI, owing 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, the inability to discriminate specific patient subgroups and uniform confounder adjustment hamper the ability to determine the relevance of low ADAMTS-13 levels for myocardial infarction.

A more powerful and less biased approach involves the collection of individual patient data (IPD) directly from the researchers responsible for each study. The 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 [16].

Methods

Search strategy and selection of studies

This systematic review and IPD meta-analysis of studies investigating ADAMTS-13 levels in patients with myocardial infarction were conducted according to the principles of the PRISMA statement [17]. We searched for all publications reporting the association between ADAMTS-13 and myocardial infarction up to February 2014. Publications were identified with a systematic search in PubMed (1950–2014). The search strategy was composed of the following Boolean combination of search terms: ADAMTS-13 'AND' myocardial infarction 'OR' heart disease 'OR' coronary disease. Publications were selected independently by two authors (A.M. and L.A.L.) from the resulting listing.

Publications were included when: (i) they reported original data on the association between ADAMTS-13 levels in humans (i.e. plasma antigen or activity) and incident myocardial infarction; and (ii) the outcome was myocardial infarction as an acute vascular event rather than as a surrogate (for example, 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, and peripheral artery disease) were included as long as, at the level of patient data, it was possible to select myocardial infarction cases from the combined endpoint. No restriction was applied to study design, except that the studies had to be controlled.

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 > 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 a history of hypertension, diabetes, hypercholesterolemia (defined as total cholesterol of > 200 mg dL^{-1}), and smoking habits. Moreover, the authors were asked to provide the following laboratory variables: time from the event to the blood collection, ADAMTS-13 level (plasma antigen levels), and VWF level (plasma antigen levels).

Statistical analysis

ADAMTS-13 and VWF levels from each study were standardized by dividing each single value by the mean value of the corresponding control group and expressing the result as a percentage. The overall association between ADAMTS-13 and myocardial infarction was determined with a one-step meta-analytic approach on IPD. This was performed with a mixed logistic regression model with random treatment effects to obtain odds ratios (ORs) and corresponding 95% CIs as measures of relative risk [18]. 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 performed in separate models. The main analyses were based on a dichotomous exposure, with *a priori* cut-off values set at the 5th and 1st percentiles of the ADAMTS-13 distribution of the pooled control group. Dummy variables with predefined cut-off points were created to assess the combined effect of high VWF levels (above the 90th percentile, which have already been shown to be associated with myocardial infarction [12]) and low ADAMTS-13 levels (below the 5th percentile). Quartile analyses, with the highest quartile

as the reference category, were used to determine the association over the full range of ADAMTS-13 values. The presence of a non-linear effect of the ADAMTS-13 level distribution on the risk of myocardial infarction (expressed as log odds) was also evaluated by use of a restricted cubic spline function with three knots, which was the one that maximized Akaike's information criterion [(model likelihood ratio χ^2–2p), with p equal to the number of parameters in the model aside from the intercept (i.e. the number of knots – 1)] [19]. Predefined subgroup analyses were based on sex (male versus female) and age (below versus above 45 years at the time of the event). Because of the reduction in 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 (i.e. the original study population to which each patient belonged), and interaction terms between exposure and study indicator. Finally, in order to minimize the effect of an acute-phase reaction, we restricted the main analysis to those studies in which the blood draw was performed at least 1 month from the event. Statistical analyses were performed with STATA version 13.0, SPSS version 20.0 and R version 3.0.2.

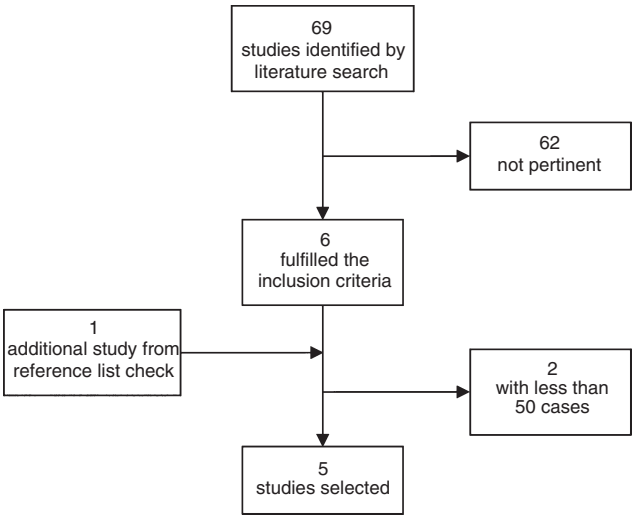

Fig. 1. Flow chart of the steps of study selection: identification of studies that reported the association between ADAMTS-13 levels and incident myocardial infarction as an acute vascular event, and with an adequate sample size of > 50 myocardial infarction cases.

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 of the reference lists of the included publications (Fig. 1). The studies that were identified were conducted with a case–control design and had various conclusions regarding the association of low ADAMTS-13 levels with myocardial infarction, encompassing a protective effect [14], no effect [13,20], and a risk-increasing effect [12,21–24]. Ultimately, five studies fulfilled the criterion of an adequate sample size, and were therefore included in the analysis. The main characteristics of all study populations, such as study design and moment of blood draw, are summarized in Table 1. In all of these studies, investigators measured ADAMTS-13 antigen levels with an ELISA, and, in only one study (ATTAC study), ADAMTS-13 activity was also measured.

All corresponding authors from the selected studies were willing to provide the requested information, yielding 1501 myocardial infarction cases and 2258 controls. All cases were recruited after their first event. Demographic and clinical characteristics of cases and controls are shown in Table 2. Overall, participants were young, with similar mean ages for cases and controls (51 years versus 47 years), whereas there was a preponderance of men among cases (66% versus 49%). As expected, cardiovascular risk factors were more prevalent in cases than in controls.

Table 1 Main characteristics of the studies included in the individual patient data meta-analysis

Study	Reference	Original study size (cases/controls)	Age (years)	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 [23]	466/484	> 18	Both	1994–1995	> 3	UK	Sex and age
ATTAC	Bongers *et al.* 2009 [24]	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 ADAMTS-13 levels, included in the individual patient data 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 (years), 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 (minimum–maximum)	38 (3–72)	–	6 (3–9)*	–	2 (1–7)	–	< 1 (0–1)	–	70 (38–112)	–	24 (0–112)	–
History of:												
Hypertension, *n* (%)	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, *n* (%)	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, *n* (%)	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, *n* (%)	164 (30)	10 (2)	142 (32)	153 (33)	81 (60)	133 (48)	52 (39)	73 (40)	20 (10)	19 (3)	458 (31)	388 (18)

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

Fig. 2. Forest plot for the association between low ADAMTS-13 levels, below the 5th percentile, and the risk of myocardial infarction by study. Dots indicate odds ratios (ORs), and solid bars indicate 95% confidence intervals (CIs). The scale is logarithmic. ORs were calculated for each study population with a multivariable logistic regression model, and were adjusted for age, sex, body mass index, and history of smoking, hypercholesterolemia, hypertension, and diabetes. The overall OR was calculated with a one-step individual patient data meta-analytic approach by mixed logistic regression with random treatment effects. IT, Italy; NL, the Netherlands.

Figure 2 shows the ORs for the association between ADAMTS-13 levels and the risk of myocardial infarction based on the 5th percentile comparison in each single study. When all of the studies were pooled together, low ADAMTS-13 levels (i.e. below the 5th percentile versus above the 5th percentile) were associated with an almost a two-fold increase in the 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 and 95% CI 1.73–10.21 for levels below versus above the 1st percentile), as shown in Table 3. Additional adjustment for VWF levels did not affect the estimates (OR 1.79 and 95% CI 1.05–3.06 for the 5th percentile cut-off, and OR 4.16 and 95% CI 1.74–9.98 for the 1st percentile cut-off). When the comparison was made by quartiles of ADAMTS-13 distribution, moderately low ADAMTS-13 levels were not associated with a substantial increase in the risk of myocardial infarction (lowest quartile versus 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 ADAMTS-13 levels and the risk of myocardial infarction is shown in Fig. 3. There is an appreciably 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 ADAMTS-13 and VWF levels on the risk of myocardial infarction. VWF levels above the 90th percentile as compared with levels below the 90th percentile were associated with an increase in the risk of myocardial infarction (OR 1.72,

Table 3 Risk of myocardial infarction in relation to various plasma levels of ADAMTS-13

Standardized ADAMTS-13 levels	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	Reference	Reference
≤ 1st percentile (≤ 52%)	67	22	4.09 (1.41–11.83)	4.21 (1.73–10.21)
> 1st percentile (> 52%)	1437	2236	Reference	Reference
Q1 (< 83%)	420	564	1.38 (0.69–2.78)	1.28 (0.68–2.45)
Q2 (83–97%)	379	565	1.23 (0.76–2.01)	1.25 (0.78–1.97)
Q3 (97–112%)	361	565	1.12 (0.83–1.52)	1.08 (0.81–1.46)
Q4 (> 112%)	341	564	Reference	Reference

CI, confidence interval; OR, odds ratio; Q, quartile. ORs, as measures of relative risk, were calculated with a mixed logistic regression model with random treatment effects, and were all adjusted for age and sex. OR_1 values were also adjusted for body mass index and history of smoking, hypercholesterolemia, hypertension, and diabetes.

95% CI 1.22–2.42). When the analysis was restricted to VWF levels below the 90th percentile, the risk associated with low ADAMTS-13 levels was similar to that of the main analysis (OR 1.77 and 95% CI 1.00–3.25 for ADAMTS-13 levels below the 5th percentile versus above the 5th percentile). The risk of myocardial infarction conferred by a combination of low ADAMTS-13 levels and high VWF levels was only slightly higher than could be expected by the separate effect, without evidence of a strong interaction (expected OR 1 + 0.77 + 0.72 = 2.49; calculated OR 3.17, 95% CI 1.18–8.63).

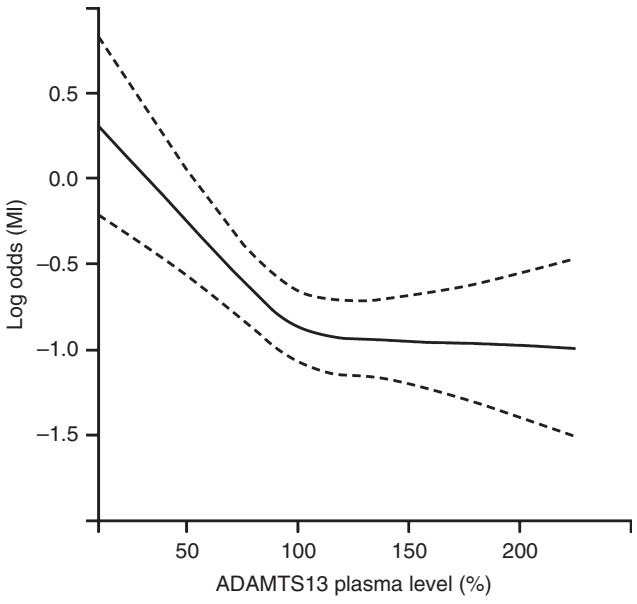

Fig. 3. Restricted cubic spline curve showing the model-predicted probability of myocardial infarction (MI) against ADAMTS-13 plasma levels. The solid line represents the model-predicted probability of MI (log odds [MI]) for each level of ADAMTS-13, adjusted for age, sex, body mass index, study indicator, and history of smoking, hypercholesterolemia, hypertension, and diabetes. Dashed lines represent 95% confidence intervals. Splines are the result of a curve-smoothing method based on a regression model that shapes a function of the log odds of MI associated with each value of ADAMTS-13. It does so by allowing different fitted curves for different ranges of the exposure of interest, with the constraint that the result can be plotted as a continuous curve.

The results from subgroup analyses are shown in Table 5. There was a similar relative effect of ADAMTS-13 levels on the risk of myocardial infarction for subjects below and above 45 years of age, whereas there was a

Table 4 Risk of myocardial infarction in relation to the combination of low ADAMTS-13 and high von Willebrand factor (VWF) plasma levels

High VWF (> 90th percentile)	Low ADAMTS-13 (< 5th percentile)	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)
−	−	1137 (76)	1872 (86)	Reference
+	−	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)

CI, confidence interval; OR, odds ratio. ORs, as measures of relative risk, were calculated with a mixed logistic regression model with random treatment effects, and were adjusted for age, sex, body mass index, and history of smoking, hypercholesterolemia, hypertension, and diabetes. Relative risks were calculated for four strata: high VWF plasma levels, i.e. above the 90th percentile (+/−); low ADAMTS-13 plasma levels, i.e. below the 5th percentile (−/+); or both (+/+) with the −/− category as reference.
Data on VWF were available for 1491 cases (99% of the total) and 2182 controls (97% of the total).

Table 5 Risk of myocardial infarction in relation to ADAMTS-13 levels for age and sex subgroups

	ADAMTS-13, < 5th percentile		ADAMTS-13, > 5th percentile		
	Cases	Controls	Cases	Controls	OR (95% CI)
Age at the event (years), *n* (%)					
< 45	57 (12)	59 (6)	428 (88)	966 (94)	1.99 (0.93–4.26)
> 45	73 (7)	53 (4)	943 (93)	1180 (96)	2.41 (0.74–7.86)
Sex, *n* (%)					
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)

CI, confidence interval; OR, odds ratio. ORs were calculated with a multivariable logistic regression model with fixed effect, and adjusted for age, sex, body mass index, and history of smoking, hypercholesterolemia, hypertension, and diabetes.

considerable difference between women and men. Women with low ADAMTS-13 levels (i.e. levels below the 5th percentile as compared with levels above the 5th percentile) had an almost three-fold increased risk of myocardial infarction (OR 2.78, 95% CI 1.61–4.88), whereas in men a 1.7-fold increase was observed (OR 1.66, 95% CI 1.08–2.56). When, to remove center 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 ADAMTS-13 levels below versus above the 5th percentile the OR in women was 4.00 (95% CI 1.63–9.72), and the OR in men was 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 those of the main analysis (ADAMTS-13 levels below versus 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 five case–control studies indicates that only low ADAMTS-13 levels (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 the risk of myocardial infarction. This association is not mediated by VWF antigen levels.

The relationship between ADAMTS-13 levels and myocardial infarction has been previously studied by Sonneveld *et al*. [15]. They summarized the same five studies in a meta-analysis of aggregate data. However, they pooled ORs measured with different cut-off levels of ADAMTS-13 (tertiles and quartiles) and with different adjustments, and were unable to give a definitive answer. By performing a meta-analysis based on IPD, we obtained a less biased estimate, we determined the dose–response relationship, and we investigated the association in specific subgroups of patients.

Subgroup analysis showed that the association between ADAMTS-13 levels and the risk of myocardial infarction was similar between ages, but, although present in both sexes, seemed to be more pronounced in women. Partly, this difference in relative risk could have resulted from the division of the studies into the two subgroups (i.e. SMILE, GLAMIS and ATTAC for men, and GLAMIS, RATIO, Milan and ATTAC for women), but could also have resulted from the incidence of myocardial infarction being higher in men than in women [25], meaning that a similar absolute risk difference between sexes for low versus high ADAMTS-13 levels will lead to a higher relative risk in women than in men. However, our data do not allow further investigation of whether this reflects a true sex-specific effect, chance, or a difference in the presence of unmeasured confounding between men and women (e.g. alcohol consumption or physical activity).

The relationship between ADAMTS-13 levels and other types of arterial thrombosis, such as ischemic stroke, has been investigated in some studies [12,24,26,27]. Their results suggest that even moderately reduced ADAMTS-13 levels increased the risk of ischemic stroke. A meta-analysis of aggregate data, pooling these publications, showed a strong association between ADAMTS-13 levels and ischemic stroke for the lowest quartile of the ADAMTS-13 distribution (pooled OR of 2.72 for low versus high ADAMTS-13 levels, 95% CI 1.52–4.86) [15]. For myocardial infarction, our IPD meta-analysis showed an association for the lowest ADAMTS-13 levels (OR of 1.28 for the lowest quartile versus the highest quartile, 95% CI 0.68–2.45; OR of 4.21 for levels below versus above the 1st percentile, 95% CI 1.73–10.21). This indicates that, although ADAMTS-13 levels are associated with both forms of arterial thrombosis, the effects are different, which may reflect pathophysiologic differences between the two disorders.

The pathophysiologic mechanisms that underlie the association of low ADAMTS-13 levels with myocardial infarction are not fully understood. We believe that this mechanism may be attributable to one, or a combination of, the following: (i) an effect of ADAMTS-13 level on the initiation and progression of the atherosclerotic plaque itself [9]; (ii) the influence of ADAMTS-13 level on acute thrombus formation [10]; and (iii) the influence of ADAMTS-13 level on amplification of the thrombus and deleterious post-thrombotic inflammation [11].

Murine models have revealed that complete ADAMTS-13 deficiency augments the development of atherosclerotic lesions in a manner that is dependent on VWF [9,28,29]. 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 variations in ADAMTS-13 levels in plaque development. In humans, severe ADAMTS-13 deficiency (< 5% activity) is a cause of widespread microvascular thrombosis, owing to the lack of cleavage of the hyperactive ULVWF multimers. This can also be associated with myocardial infarction in some patients with TTP [30]. However, it appears that, in both mice and humans, ADAMTS-13 levels of > 50% are not associated with any detectable difference in plasma VWF multimer distribution [31].

In our analysis, we did not find that the association of ADAMTS-13 levels with the risk of myocardial infarction was mediated by VWF levels (that is, adjustment for VWF levels did not materially affect the results). Moreover, we found only a minor synergistic effect between low ADAMTS-13 levels and high VWF levels. Therefore, it is possible that ADAMTS-13 plasma levels slightly below the lower bound of the normal range (i.e. ~ 60%) may influence the kinetics of thrombus growth, in a man-

ner that is independent of circulating VWF levels. In physiologic conditions, the proteolysis of circulating VWF is determined primarily by the unfolding of UL-VWF multimers rather than ADAMTS-13 plasma levels. Conversely, at sites of injury or plaque rupture, where the thrombus is consolidated by platelet binding through fibrinogen and fibrin, and active VWF can become protected against proteolysis [32], changes in ADAMTS-13 levels may be far more important in controlling thrombus formation. Therefore, low ADAMTS-13 levels might lead to the formation of a more extensive platelet plug that is further stabilized/consolidated by additional prothrombotic mechanisms.

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 that we used for our analyses. This, however, could have led to residual confounding and limited the number of additional analyses (e.g. stratification for other blood parameters). We also excluded studies with < 50 cases. This might have led to a small reduction in power, but it reduced the impact of publication bias, to which small studies are more prone than large studies. Given the considerable study sample size that we achieved, 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, i.e. the consequence of an event being mistaken for the cause. Several factors could have influenced ADAMTS-13 levels. The first is the acute-phase response to the arterial thrombotic event. We were not able to perform subgroup analysis based on time from the event to the blood sampling, owing to the small overlap of these time periods between the five studies. However, because in only one study was blood 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 IPD analysis, the results are unlikely to be explained by the transient effects of the acute phase. Second, it has to be considered whether ADAMTS-13 levels were influenced by the presence of chronic heart failure (CHF) in patients with myocardial infarction. There is no information in the literature on ADAMTS-13 levels in CHF patients as compared with healthy subjects, but one study found that low ADAMTS-13 activity levels were predictors of adverse functional outcomes in CHF patients [33]. Therefore, we cannot exclude the possibility that ADAMTS-13 levels are decreased in CHF patients. However, given that ADAMTS-13 is mainly produced in the liver, and is therefore not or hardly affected by endothelial dysfunction [1], it seems most likely that the relationship was ADAMTS-13 causing worsening of CHF rather than the converse. Moreover, the participants in our analysis were relatively young (mean age of 51 years for cases), in an age category in which CHF and other age-related risk factors such as endothelial lesions and dysfunction through atherosclerosis are uncommon. Third, some medications, such as the thienopyridines (e.g. ticlopidine, clopidogrel, and prasugrel), which are often prescribed after myocardial infarction, have been associated with acute TTP episodes [34]. This occurrence, however, is extremely rare, and the underlying mechanism seems to be an acute induced autoimmune reaction with dramatic clearance of ADAMTS-13, rather than a moderate decrease in its plasma concentration. It is therefore unlikely that *post hoc* use of thienopyridines offers an alternative explanation for our findings. Because only one of the five included studies investigated ADAMTS-13 and VWF activity (ATTAC study), we chose to focus on antigen levels, which not always are a good representation of the molecule's activity. However, plasma antigen levels of ADAMTS-13 correlate well with its activity [35]. Only in rare cases, when inhibitor antibodies against ADAMTS-13 are present, do antigen levels not reflect activity. Therefore, we do not believe that this could have influenced our main results.

In conclusion, with an IPD meta-analytic approach, we demonstrated that low ADAMTS-13 levels increased the risk of myocardial infarction. This association is valid only for low ADAMTS-13 levels, and therefore differs from the relationship of ADAMTS-13 with ischemic stroke.

Addendum

A. Maino designed the research, analyzed the data, interpreted the results, and drafted the manuscript. B. Siegerink designed the research, provided IPD, analyzed the data, interpreted the results, and reviewed the manuscript. L. A. Lotta designed the research, interpreted the results, and reviewed the manuscript. J. T. B. Crawley, F. W. Leebeek, D. A. Lane, G. D. O. Lowe and F. Peyvandi provided IPD, interpreted the results, and reviewed the manuscript. S. le Cessie provided statistical support, analyzed the data, interpreted the results, and reviewed the manuscript. F. R. Rosendaal designed the research, analyzed 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

The authors state that they have no conflict of interest.

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