ADAMTS13, von Willebrand Factor and the Risk of Myocardial Infarction in Men

Chan K.N.K. Chion¹, Carine J.M. Doggen², James T.B. Crawley¹, David A. Lane¹ and Frits R Rosendaal²,³

¹) Department of Haematology, Imperial College London, Hammersmith Hospital Campus, London W12 ONN, UK
Department of ²) Clinical Epidemiology and Hemostasis and ³) Thrombosis Research Center, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands

Corresponding author:

J.T.B.C, Department of Haematology, Imperial College London, 5th Floor Commonwealth Building, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, UK
Tel ++442083832297; Fax ++442083832296;
e-mail: j.crawley@imperial.ac.uk

Short Title:

ADAMTS13, VWF and MI

C.K.N.K.C designed and performed research and analyzed data and wrote the paper,
C.J.M.D designed the original case-control study, analyzed data and wrote the paper,
J.T.B.C designed and performed research and analyzed data and wrote the paper,
D.A.L designed research, analyzed data and wrote the paper,
F.R.R designed research, analyzed data and wrote the paper

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Abstract
Von Willebrand factor (VWF) mediates the tethering/adhesion of platelets at sites of vascular injury. This function depends upon its multimeric size, which is controlled by ADAMTS13. We measured plasma ADAMTS13 and VWF antigen levels by ELISA in a large population-based case-control study (SMILE), consisting of 560 men with a first myocardial infarction (MI) and 646 control subjects. Although ABO blood groups influenced VWF levels, they had no influence, upon ADAMTS13. Furthermore, there was no relationship between plasma ADAMTS13 and VWF levels. Similar to VWF, the estimated risk of MI was increased for every quartile of ADAMTS13 when compared to the lowest quartile (odds ratio 1.5 to 1.6). If confirmed, the association of ADAMTS13 with MI may suggest an unexpected mechanistic action of ADAMTS13.

Introduction
Von Willebrand factor (VWF) binds exposed collagen to form a bridge between the site of vascular injury and platelets during the initiation of haemostasis. Due to its pivotal role in haemostatic plug formation, VWF function may directly influence the likelihood of a thrombotic event, as suggested by the association of VWF levels with an increased risk of ischaemic heart disease [odds ratio (OR) approximately 1.5].

Plasma VWF is comprised of multimers held together by intermolecular disulphide bonds. The molecular weight (MW)/multimeric composition of VWF is a key determinant of its platelet-tethering function. Larger multimers are the most reactive at the site of vessel injury. VWF multimeric size is modulated by ADAMTS13, which cleaves in the VWF A2 domain, thus reducing both its MW and platelet-tethering function. ADAMTS13-deficiency leads to VWF-induced platelet aggregation, resulting in thrombotic thrombocytopenia purpura (TTP). The level of ADAMTS13 in the blood may thus influence cardiovascular disease. Herein, we report on the relationships between ADAMTS13, VWF, and MI in a large population-based case-control study ‘Study of Myocardial Infarctions Leiden’ (SMILE).

Materials and Methods
Subjects. We employed the previously published population-based case-control, SMILE. We included 560 men (18 to 70 years), consecutively diagnosed with a first episode of MI, between 1994 and 1997, and 646 men without MI who had not received anticoagulants for more than 6 months. The control group was frequency matched to cases on 10-year age groups. Venous blood, anticoagulated using tri-sodium citrate (1:9 v/v), was collected at least 6 months post-MI (median 2.6 years). Plasma samples
were aliquotted and stored at -80°C. Approval for these studies was obtained from the institutional review boards of both the university and general hospitals in Leiden, the Netherlands. Informed consent was provided according to the Declaration of Helsinki.

**ELISAs for ADAMTS13, VWF and C-reactive protein (CRP).** The ADAMTS13 ELISA used polyclonal antibodies raised in rabbits immunised with recombinant (r)ADAMTS13\(^{17,18}\). Anti-ADAMTS13 thrombospondin type 1 repeat domains (2-4) [TSP1(2-4)] antibodies were affinity purified, and biotinylated for use as the detection antibody. Anti-ADAMTS13 antibodies that had been fully depleted of anti-TSP1(2-4) IgG (5\(\mu\)g/ml) were used as the capture antibody in 96-well plates. Wells were washed and blocked before the addition of 100\(\mu\)l/well of plasma samples (diluted 1:20 in PBS), or plasma standards in duplicate, and incubated overnight at 4°C. Wells were washed, and ADAMTS13 was detected using biotinylated anti-TSPR1(2-4) antibodies (0.1\(\mu\)g/ml), followed by a streptavidin-horseradish peroxidase conjugate (AmershamPharmacia). Highly purified rADAMTS13\(^{17,18}\) and pooled normal plasma (Technoclone) were used in standard curves. Normal pooled plasma was determined to contain 900ng/ml ADAMTS13, similar to previous estimates\(^{19-21}\). Specificity of our ELISA was confirmed by the lack of signal derived from plasma from a patient with severe congenital ADAMTS13-deficiency (provided by Dr R Leissner, Hospital for Sick Children, London, UK). Furthermore, this TTP plasma had no effect on standard curves when mixed with normal pooled plasma dilutions. Recovery of rADAMTS13 standards from TTP plasma was 94% ±9.1. The lower detection limit of the ELISA was 5% that of normal plasma. Intra- and inter-assay coefficients of variation were less than 5%. All outlying samples (less than 50% or greater than 200%) were confirmed by repetition. Multiple freeze-thawing of control plasmas was found not to influence the ADAMTS13 ELISA.

The VWF ELISA was performed according to manufacturer’s instructions (Diagnostica Stago, Asnieres, France). The CRP was measured as previously reported\(^{16}\). Blood group was determined by questionnaire. Due to sample depletion, both ADAMTS13 and VWF levels were both available for 534 out of 560 patients and 607 out of 646 control subjects.

**Statistical Analysis.** Quartiles of ADAMTS13 and VWF were defined by control subjects. The lowest quartile was used as a reference category for the OR. A 95% confidence interval (CI95) was calculated according to the method of Woolf\(^{22}\). Unconditional logistic regression was performed to adjust for age. Further adjustments for ADAMTS13 or VWF levels were performed by using these as continuous variables. All computations were carried out with the SPSS for Windows Version 12.0.1 (SPSS Inc, Chicago, Ill) statistical package.
Results and Discussion

The mean ADAMTS13 levels (standard deviation) in 551 male MI patient plasmas was 101 (22.9) % (range 4 to 350%, 5th to 95th percentile 70 to 136%), and was 100 (30.2) % (range 41 to 432%, 5th to 95th percentile 70 to 139%) in 635 male control subjects (mean difference 0.0 (CI95 -3.0 to 3.1) %). The frequency plots in cases and controls (Figure 1 A) showed normal distributions with tight groupings around the mean. Interestingly, one 45 year old MI patient exhibited less than 5% ADAMTS13 (confirmed by activity assay; not shown), with no signs of TTP.

Mean VWF levels in plasma were similar, 138%(51.4) (range 46 to 567%, 5th to 95th percentile 73 to 229%) in MI patients, and 135%(66.4) (range 43 to 813%, 5th to 95th percentile 69 to 199%) in control subjects (mean difference 3.50 (CI95 -3.4 to 10.4) %) with wide frequency distributions of results in both patients, and control subjects (Figure 1B).

There was no clear association between ADAMTS13 and VWF in either control subjects or MI patients (Figure 1C and 1D). An inverse relationship between these proteins was previously reported in a small number of heterogeneous patients. However, because of homogeneity and the size of our study these data strongly suggest that ADAMTS13 plasma levels are not associated with VWF plasma levels in normal men or male patients with MI. Blood group information was available for 387 control subjects. As previously reported, VWF levels were dependent upon blood group (Figure 1E). No relationship was evident between blood group and ADAMTS13 levels.

Using the lowest quartile as the reference group, we found an increase in risk for MI in men for both ADAMTS13 and VWF for each quartile, after adjusting for age (Table 1, i and ii, respectively), indicating a threshold rather than a dose relationship for ADAMTS13. Adjustment of ADAMTS13 results for VWF, and vice versa, did not materially change the risk estimate, suggesting that the proteins have separate effects on the risk of MI. As inflammation can modulate risk of MI, adjustment was also made for CRP levels, but this did not alter the risk estimate for either ADAMTS13 or VWF levels.

Although a recent study has reported reduced ADAMTS13 in a small number of acute MI patients, that study used data from samples taken upon hospital admission. Consequently, whether reduced ADAMTS13 was a cause or effect of MI in those individuals could not be determined. Our data, using plasma samples obtained more than 6 months after the MI, reveal a positive rather than negative association of ADAMTS13 levels with risk of MI in men, and renders a detrimental effect of low levels of ADAMTS13 unlikely. This result is unexpected, and consequently merits additional investigation to ascertain whether this is specific to our study, or a general finding. If the latter, a mechanistic basis for
the positive association must be identified, perhaps in the context of ADAMTS13 action in or around the atheromatous plaque.

Acknowledgments

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References


Legends

Figure 1: ADAMTS13 and VWF in male control and MI patients. Frequency distribution of ADAMTS13 (A) and VWF (B) levels in plasma of MI patients (black bars) and control subjects (grey bars). Data are presented as percentage of total plasma samples measured. Graphs do not show outlying samples greater than 200% for (A) or 300% for (B). Association between plasma levels of ADAMTS13 and VWF in (Ci) control and (Di) MI patients. The association between these proteins in the normal range (boxed areas) are enlarged (Cii and Dii). The regression line is shown. E) ADAMTS13 and VWF levels per blood group among control subjects. ADAMTS13 levels were not different between blood groups (ANOVA P equals 0.21). VWF levels in blood group A, B and AB were all significantly different from blood group O (ANOVA P less than 0.001).

Table 1. ADAMTS13 and VWF levels in relation to MI. Levels of ADAMTS13 (i) and VWF (ii) are depicted as quartiles and OR determined using the lowest quartiles as the reference groups. § reference group. * adjusted for age. † adjusted for age, VWF or ADAMTS13 as continuous variable. ‡ adjusted for age, log transformed CRP.
Figure 1
Table 1

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