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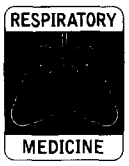

Original Articles

Plasma levels of von Willebrand factor antigen in acute bronchitis and in a normal population

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von Willebrand factor (vWF) is a large glycoprotein secreted predominantly by endothelial cells in both the systemic and pulmonary circulations and has a central role in the formation of the platelet plug. It has been put forward as a possible marker of endothelial cell injury, but is not ideal in that it is not specific for either the pulmonary or systemic circulation and may be released as part of the acute phase response from otherwise healthy endothelial cells.

We undertook two studies (i) to assess within-subject variation in plasma von Willebrand factor antigen (vWF:Ag) levels over time and to assess between-subject variation in a healthy patient population, and (ii) as part of a descriptive study of acute bronchitis, to assess whether plasma vWF:Ag levels altered in such a common and minor insult.

A random sample of patients aged 45–74 years were taken from a local general practice. vWF:Ag levels were measured on three occasions, and spirometry was performed. The descriptive study was undertaken on patients in the general practice diagnosed with acute bronchitis without pre-existing pulmonary disease. Plasma vWF:Ag was measured on presentation and 14 and 42 days later.

In 219 randomly selected patients the mean plasma vWF:Ag was similar at all three visits, the within-subject standard deviation being 0·09 U ml^{-1}. vWF:Ag levels rose significantly with age (r^2=0·29, P<0·01). In 39 patients with acute bronchitis, the plasma vWF:Ag level at presentation (1·51 U ml^{-1}) was significantly higher than at 2 and 6 weeks later (1·06 U ml^{-1} and 1·12 U ml^{-1} respectively). There was no correlation between plasma vWF:Ag and C-reactive protein on presentation.

We conclude that there is relatively little variation in an individual's plasma vWF:Ag level but that levels increase significantly with age. The observed elevation occurring with acute bronchitis is a true phenomenon; the absence of an associated acute phase response suggests that endothelial cell injury is the mechanism for the rise. These observations are important in the context of vWF as a marker of endothelial cell damage, as a common and supposedly minor insult such as acute bronchitis may markedly raise plasma levels.

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Introduction

von Willebrand factor (vWF) is a large glycoprotein which is produced predominantly by endothelial cells in both the pulmonary and systemic vasculature, and in platelets (1). It is made up of subunits, varying in molecular weight from 0·5 to 20 × 10^3 kDa. Loss of the larger subunits results in loss of biological activity (e.g. in type II von Willebrand's disease) (2,3). Quantitative levels of vWF may be assayed as vWF antigen (vWF:Ag), while biological activity may be assessed with ristocetin cofactor activity.

In endothelial cells, vWF is stored in Wiebel-Palade bodies and is released predominantly in situations where haemostasis is threatened, when it binds to the platelet membrane glycoprotein GP1b, thus activating the platelet and leading to irreversible platelet binding. The resulting platelet plug is the primary unit of haemostasis (3).

vWF may also be released by direct injury to endothelial cells (systemic or pulmonary) or when endothelial dysfunction is present. Thus, increased levels of vWF:Ag have been found in numerous clinical settings where endothelial dysfunction or mechanical damage are presumed, including hypertension (both pulmonary (4–6) and systemic (7,8)), abnormal cardiovascular haemodynamics associated with

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valvular disease (9), hypercholesterolaemia (10) and high altitude pulmonary oedema (11).

vWF is also released by the action of mediators such as interleukin-1 during the acute phase response (12–14). There are therefore a number of clinical situations where increased vWF:Ag levels could result from either cytokine-mediated release or from direct endothelial damage (15). In diseases such as sepsis-related ARDS (16,17), acute respiratory failure with other aetiologies (18), in the vasculitides (especially systemic sclerosis (19,20)) and following cardio-pulmonary bypass (21), vWF:Ag levels have been shown to be raised. The mechanism in these cases is likely to be endothelial damage, but release by acute-phase mediators is difficult to exclude.

It has been suggested that vWF is a useful marker of endothelial damage (13). However, the major problems with its use in this respect are its release during the acute phase and its release from *both* systemic and pulmonary endothelium (15). vWF:Ag levels have been used to predict the development of ARDS in non-pulmonary sepsis with varying success, and increased specificity for pulmonary endothelial damage rather than systemic damage has been suggested by these studies (16,17,22,23). They have also been used to follow the progress of disease in patients with systemic sclerosis, and have recently been correlated with risk of re-infarction and mortality in patients surviving acute myocardial infarction (24).

In platelets, vWF is stored in *α* granules. It has been postulated that release of vWF from platelets during aggregation and degranulation may contribute to raised levels of vWF:Ag in the above clinical conditions, but the contribution to plasma levels by this mechanism is likely to be small (13,25). Jones *et al.* found that intravenous prostacyclin (a potent inhibitor of platelet aggregation) failed to suppress the release of vWF following cardio-pulmonary bypass (21).

As part of a large community-based study (26) we sought to assess (i) within-subject and between subject variation in vWF:Ag levels in healthy subjects, and (ii) the effect of a common and relatively minor insult such as acute bronchitis on vWF:Ag levels.

Methods

PATIENTS

Study 1 – Sample Population

A respiratory symptom questionnaire was sent to all 2242 patients aged 45–74 years, identified by the age/sex register in an inner city general practice with a practice population of 7700. A question was included on whether the patient would be willing to attend the surgery for simple breathing and blood tests. From the positive responders, a 1-in-6 sample was selected, using random number tables, to attend for further investigation. No restrictions were placed on individuals with respect to smoking status or activity level.

Of the 251 patients selected to attend, 219 (87·1%) attended. The mean (SD) age was 60 years (± 8·8) and 117 (53%) were female.

Study 2 – Acute Bronchitis

A descriptive study of acute bronchitis was undertaken between September 1986 and March 1987 in the same general practice. A total of 39 patients were recruited, all of whom satisfied the American Thoracic Society's definition of acute bronchitis (27). Episodes of acute bronchitis in patients with previously diagnosed pulmonary disease, such as asthma or chronic obstructive airways disease, were excluded from the study. Details of this study have been published previously (26).

The mean age of the 39 patients was 38·4 years (range 5–73 years) and 27 (69%) of the episodes occurred in women.

Both studies were approved by the local Ethics Committee.

INVESTIGATIONS

Study 1 – Sample Population

Venous blood was taken at three morning visits to the clinic, each separated from the other by at least 7 days, for plasma vWF:Ag estimation. In addition, simple pulmonary function tests (FEV_1, FVC) were performed using a Vitalograph dry bellows spirometer, with at least two attempts reproducible to within 200 ml or 5% (whichever was smaller). Peak expiratory flow (PEF) was recorded on a Wright mini-peak flow meter, with at least two attempts reproducible to within 20 l min^{-1}. Exhaled breath carbon monoxide levels were measured (Morgan Ecocheck) to confirm the smoking history.

Study 2 – Acute Bronchitis

Written informed consent was obtained from all patients. Spirometry was performed at all three visits. Pathogens were sought using nasopharyngeal washings, sputum culture where possible, and viral serology. Venous blood was taken for plasma vWF:Ag and CRP on presentation and 6 weeks later, and for plasma vWF:Ag alone at an interim visit 2 weeks after presentation.

LABORATORY METHODS

Five millilitres of citrated blood (one part 3·9% sodium citrate to nine parts blood) was taken for vWF:Ag measurement. After centrifugation at 1500 ***g*** for 15 min, platelet-poor plasma was separated and stored at −20°C, being thawed at 37°C immediately prior to assay. Plasma vWF:Ag was measured using an ELISA method (28), which has a coefficient of variation between batches and within batches of less than 5%. A single batch of standard was used throughout, having been re-constituted to a vWF:Ag level of 1·00 U ml^{-1}, and a control preparation of plasma with a vWF:Ag level of 1·00 U ml^{-1} (Immuno-diagnostics) was assayed with each batch of test samples. vWF:Ag multimers were examined as described previously (29). CRP was estimated turbidimetrically using a

mono-specific antiserum to CRP raised in sheep (BDS, Birmingham).

ANALYSIS

Study 1 – Healthy Population

vWF:Ag results are expressed as the mean and the standard deviation. Ninety-five and 99% CI were calculated for the effect of age on plasma vWF:Ag. The Shapiro–Wilk test was performed on the vWF:Ag data for each visit, which demonstrated no significant differences, so the data could be assumed to follow a normal distribution. An analysis of variance (ANOVA) was carried out to analyse vWF:Ag variation over the three measurements, and the residual mean square from this procedure taken to calculate the within-subject standard deviation (measurement error) and the repeatability (the difference between paired observations is expected to be less than this figure 95% of the time) as suggested by Bland and Altman (30). Student's unpaired *t*-test was used to assess the effect of smoking, gender and the presence of respiratory symptoms on plasma vWF:Ag. Pulmonary function results were analysed as actual values, the percentage predicted, and standardized residuals (31), and linear regression analysis was undertaken to assess whether there was a relationship between any of these variables or age and plasma vWF:Ag levels.

Study 2 – Acute Bronchitis

Plasma vWF:Ag results are expressed as the mean and the standard error of the mean. For the differences between two means, the 95% confidence intervals (CI) have also been calculated. A split-plot analysis of variance (ANOVA) was used for repeated measure of vWF:Ag, and Tukey's procedure was performed to determine the significance of time and smoking status on the mean plasma vWF:Ag. McNemars test was used to determine whether there was a relationship between a raised plasma vWF:Ag level and a raised plasma CRP result.

Results

Study 1 – Sample Population

In the study population of 219, the mean percent predicted FEV_1 was 92·2% and FVC 100·2%.

The mean (SD) plasma vWF:Ag levels were 1·09 (0·22), 1·10 (0·20) and 1·10 (0·19) U ml^{-1} for the three visits (n=212, 203 and 195 respectively). The results were normally distributed (Shapiro–Wilk test), and ANOVA demonstrated no difference between the vWF:Ag blood levels at any of the three visits. Only 14/610 (0·7%) measurements of plasma vWF:Ag were outside the normal range for our laboratory (0·5–1·5 U ml^{-1}). The within-subject standard deviation (measurement error) was moderate at 0·09 U ml^{-1} and the repeatability was 0·25 U ml^{-1}.

Plasma vWF:Ag levels increased with age (r^2=0·29, P<0·01: Fig. 3), but did not relate to smoking status, exhaled carbon monoxide level, or any measure of pulmonary function. Patients who responded positively to the question 'Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill?' had significantly higher plasma vWF:Ag levels at each of three visits than did those patients with a negative reply (1·12 *vs.* 1·06, 1·13 *vs.* 1·07 and 1·14 *vs.* 1·07: P<0·02) for each visit, but regression analysis revealed that this association was due to age. There was no relationship between plasma vWF:Ag levels and any other questionnaire responses.

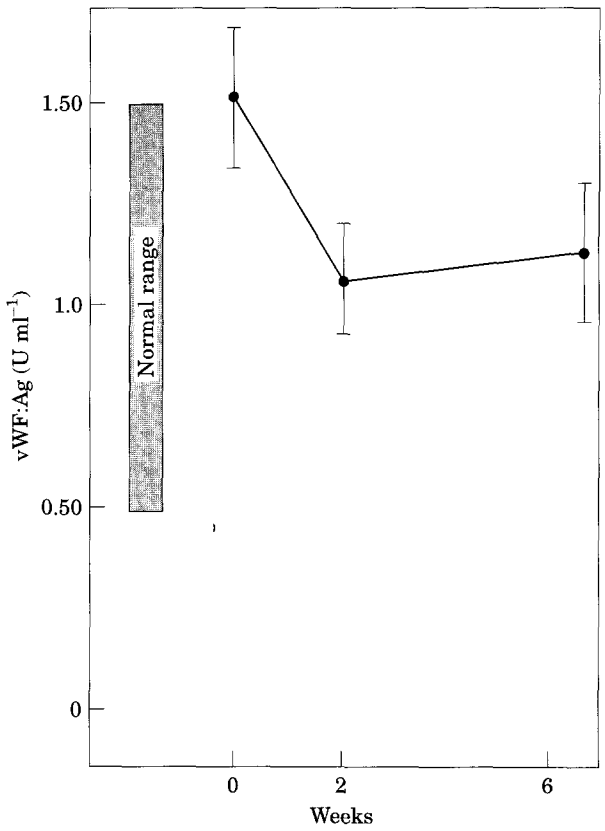

FIG. 1. vWF:Ag levels on presentation in patients with acute bronchitis and at follow-up 2 and 6 weeks later [means (SE)].

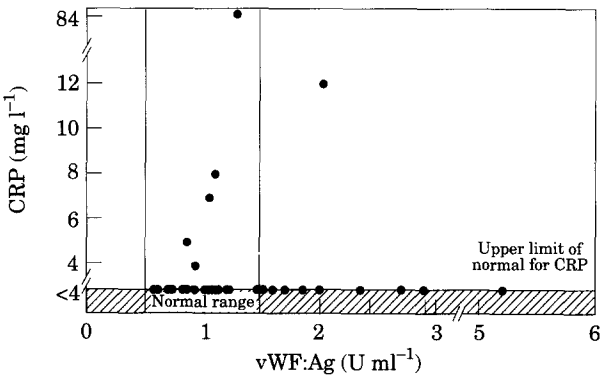

FIG. 2. CRP and plasma vWF:Ag levels on presentation in patients with acute bronchitis (data available on 32 of the 39 patients).

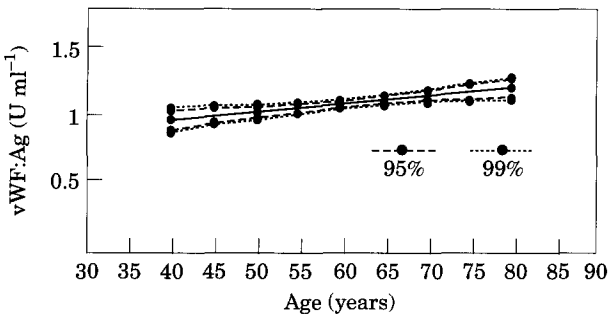

FIG. 3. Confidence intervals (CI) for the effect of age on plasma vWF:Ag level. $r^2=0{\cdot}29$, $P<0{\cdot}01$.

Study 2 – Acute Bronchitis

The mean percent predicted FEV_1 and FVC rose significantly between presentation and follow-up 2 weeks later (FEV_1 86·9% predicted to 91·6% predicted ($P<0{\cdot}01$) and FVC 90·8% predicted to 94·7% predicted ($P<0{\cdot}02$)).

Three of the 39 patients did not receive microbiological assessment; however, a potential pathogen was identified in 13 of the 36 (36%) fully investigated patients: eight viral infections (rhinovirus, influenza A and adenovirus), four *Mycoplasma pneumoniae* and three bacterial infections (*Haemophilus influenzae*, *Branhamella catarrhalis* and *Streptococcus pneumoniae*), two in association with a virus (26).

The mean (SEM) plasma vWF:Ag on presentation was 1·51 (0·15) U ml^{-1}, falling to 1·06 (0·11) U ml^{-1} at 2 weeks (mean difference 0·45 U ml^{-1}, 95% CI from 0·24 to 0·66). At 6 weeks, the value was 1·12 (0·11) U ml^{-1} (mean difference 0·36 U ml^{-1} from presentation, 95% CI 0·18 to 0·54). Split-plot ANOVA confirmed highly significant differences between the results at presentation, at 2 weeks and 6 weeks. Tukey's procedure demonstrated that the mean at presentation was significantly different from those at 2 weeks and at 6 weeks ($P<0{\cdot}05$), but there were no differences between the means at 2 and at 6 weeks. Plasma vWF:Ag levels exceeded the normal range in 11 (28%) at presentation. In contrast to the larger control population, split-plot ANOVA demonstrated a significant effect of smoking, with Tukey's procedure confirming that the means in ex-smokers were significantly greater than the means in current smokers and life-long non-smokers ($P<0{\cdot}05$). The plasma vWF:Ag levels at 6 weeks were higher in older patients regardless of their smoking status ($r=0{\cdot}543$; $P<0{\cdot}001$).

Multimeric analysis in three cases, two of whom had elevated levels on presentation, demonstrated a normal pattern of vWF:Ag multimers.

There was no relationship between a raised plasma vWF:Ag level and a raised serum CRP on presentation (Fig. 1). CRP was minimally elevated in five patients on presentation, and markedly raised in one case (84 mg l^{-1}); however, all of these patients had normal vWF:Ag levels. Equally, of the 11 patients who had elevated vWF:Ag levels, all had normal CRP.

Discussion

There was moderate within-subject variation in vWF:Ag levels (0·09 U ml^{-1}, repeatability 0·25 U ml^{-1} in healthy subjects, but less variation than has been observed previously with either exercise (32,33), or acute smoking (34). Work examining the effect of exercise has involved strenuous exercise, such as running up and down stairs for 3 min (33), or running half a mile as fast as possible (32) in young subjects. These studies have demonstrated quite considerable increases in plasma vWF:Ag levels ranging from 46 to 300%. It is believed that these changes are mediated via β_2-receptors (35) and that the rise is due to release from endothelial cells rather than activation in the plasma (36). In a previous study examining distributions of haemostatic variables in a population for the development of reference ranges (37), smokers were found to have lower plasma vWF:Ag levels than non-smokers. However, for regular smokers, smoking three cigarettes within 30 min produced a significant increase in plasma vWF:Ag (34). In our study, although ex-smokers with acute bronchitis had higher plasma vWF:Ag levels ($P<0{\cdot}05$), the much larger sample population of healthy individuals revealed plasma vWF:Ag levels which were similar in smokers, ex-smokers and life-long non-smokers. For current smokers, there was no correlation between plasma vWF:Ag and exhaled breath carbon monoxide, suggesting that, while cigarettes may have an effect in strictly controlled experimental situations, this may be of less relevance in a clinical setting.

Our study confirms work demonstrating that vWF:Ag levels rise with increasing age (37,38). As noted in these previous studies, this may be due to a cohort effect rather than chronological age, and longitudinal studies are required to examine this point. The 'normal' range, encompassing 95% of the values in our study is 0·64–1·54 U ml^{-1}, slightly higher than the reference range for our laboratory, and is probably due to the older age of our patients.

Patients with acute bronchitis showed a statistically significant 40% increase in vWF:Ag levels with their acute illness. The most obvious explanation is that this is simply part of the acute phase response: a non-specific cytokine-mediated response to microbial infection which includes an increase in the synthesis and secretion of a number of plasma proteins, predominantly by hepatocytes (39–41). CRP is the classic acute phase protein, secreted within 6 h of an acute insult and often in sufficient quantities to raise the plasma level over 100 fold (39,40). However, we found no correlation between CRP levels and vWF:Ag levels in patients with acute bronchitis, suggesting that vWF release was not caused by acute phase mediators.

Changes in CRP are useful in differentiating between bacterial and viral infections, particularly in children, since viral infections rarely produce a change in serum CRP levels (39,42–44). Since the majority of cases of acute bronchitis are presumed to be viral in origin, it is not surprising that CRP levels remained normal in most cases investigated in this study. A study involving inoculation of rhinovirus to assess the effect of interferon-γ in preventing the development of the common cold demonstrated

no correlation between peak CRP levels and vWF:Ag levels (12).

When vWF levels rise in the acute phase, the pattern of increase is slightly different from CRP during acute infection (12), is different from the acute phase protein α-1-antichymotrypsin following cardio-pulmonary bypass (21) and different from other coagulation proteins following acute tissue necrosis, such as acute myocardial infarction (45,46). It is possible, therefore, that given the different time course for secretion of vWF and CRP, a single measurement may miss either peak. More frequent measurements would be needed to discount this possibility.

The two other possible sources of vWF are platelets and damaged endothelium. As already described, platelets probably contribute only a very small amount of vWF to the plasma pool, especially in a minor insult such as acute bronchitis where widespread platelet degranulation is extremely unlikely. We therefore postulate that the infective agents in acute bronchitis have a direct effect on endothelial cells to cause release of vWF. The bronchial tree from bronchi down to respiratory bronchioles receives its arterial supply from the systemic circulation via the bronchial arteries. Endothelial cells damaged during acute bronchitis would therefore be of systemic origin.

In summary, plasma vWF:Ag shows relatively little within-subject variation in the general population, and, in particular, does not appear to be influenced by normal daily activity, or by smoking status. Levels increase with age, although longitudinal data are required to confirm this observation. A minor insult such as acute bronchitis raises plasma vWF:Ag levels by 40%. Our data suggest that this is not simply a part of the acute phase response, but a result of systemic endothelial damage in the bronchial circulation. These observations are important in the context of vWF as a putative marker of endothelial cell damage.

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