

## Preanalytical conditions that affect coagulation testing, including hormonal status and therapy

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**To cite this article:** Blombäck M, Konkle BA, Manco-Johnson MJ, Bremme K, Hellgren M, Kaaja R, on behalf of the ISTH SSC Subcommittee on Women's Health Issues. Preanalytical conditions that affect coagulation testing, including hormonal status and therapy. *J Thromb Haemost* 2007; 5: 855–8.

Preanalytical conditions, be they due to the individual's physiologic state or to exogenous factors, can affect coagulation factors, in either a transient or a persistent manner, and need to be considered in laboratory testing. These conditions include physical and mental stress, diurnal variation, hormone levels and posture at the time of blood drawing. While testing of these factors has not been exhaustive and some results are conflicting, guidelines for testing conditions can be given.

### Physical stress (exercise)

Exercise alters measures of coagulation and fibrinolysis to a variable extent depending on the intensity of the exercise. The effect depends in part on the age and physical condition of the subject [1,2]. Platelet aggregation in response to adenosine diphosphate and epinephrine is enhanced during, and within 1 h after, moderate exercise. Plasma levels of serotonin and  $\beta$ -thromboglobulin are increased after moderate exercise, suggesting platelet activation [3]. Concentrations of factor (F) VIII, von Willebrand factor antigen (VWF:Ag) and VWF ristocetin cofactor activity (VWF:RCo) are increased up to 2.5-fold, starting within 2–10 min and lasting for longer than 10 h after finishing exercise, with greater effects with more intense exercise [1,4]. No changes were observed in concentrations of FXII, FV, FVII, FII or fibrinogen after corrections for hemoconcentration [4].

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Activation of coagulation is reflected by an increase in markers such as thrombin–antithrombin complexes (TAT), prothrombin fragment 1 + 2 ( $F_1 + 2$ ) and fibrinopeptide A (FPA). Increased thrombin generation starts within 30 min of moderate exercise. However, these increases do not exceed reference intervals even during intense exercise [1,5].

An increase in global fibrinolysis is observed during exercise and immediately afterwards but soon returns to normal [4,6]. An increase in tissue plasminogen activator (t-PA) occurs early and disappears within 2 h, while plasmin- $\alpha$ 2-antiplasmin complexes (PAP) are formed within 30 min and last for more than 2 h. Concentrations of D-dimer rise quickly and the increase persists for more than 1 h [1,5].

### Mental stress

Mental stress can affect coagulation, although studies have found conflicting results. With acute mental stress, FVIII, VWF:Ag and VWF:RCo, fibrinogen, and t-PA increase [7,8,9]. Changes in menstruating women were greatest in the luteal phase [9]. FVII was found to increase in men, but not in women [8]. Another study found a decrease in VWF and fibrinogen, but this may have been due to a change in plasma volume [10]. Prolonged mental stress (continuously for 77 h) led to decreases in FV, FVIII and FIX. Only FIX had recovered 5 days after the end of the stress. No increase was observed in fibrinolysis [11].

### Hormonal influence

#### Pregnancy

Concentrations of most hemostatic proteins change to effect an overall prothrombotic state during pregnancy [12], presumably to protect women from bleeding at parturition. Modest increases are found for FVII and FX. Fibrinogen

and FVIII increase approximately 2-fold, and VWF 3-fold, and remain elevated for some period post partum; limited data suggest individual variability in the length of time before the levels return to baseline. The free protein S level decreases by about 30% and may remain decreased for at least up to 2 months post partum [13–15]. Protein C remains within the reference normal limits [12,13]. Classic and modified (using FV-deficient plasma) activated protein C (APC) ratios decrease; the most pronounced changes are found with the classic method [13,15]. The increased coagulation is reflected by increased F1+2, TAT and soluble fibrin [12,13]. With regard to the fibrinolytic system, both plasminogen activator inhibitor-1 (PAI-1) and the placenta-produced PAI-2, as well as D-dimer increase [12,13].

#### *Contraceptives, hormonal replacement therapy (HRT) and changes during menstrual cycle*

The need to consider the phase of the menstrual cycle depends upon what is being investigated. Estradiol concentrations are lowest on cycle days (cd) 1–3 and highest on cd 13–15, followed by a decrease. Progesterone concentrations are lowest on cd 1–8 and highest on cd 21–25.

Most hemostatic variables, such as FII, FX, FVII, anti-thrombin, APC resistance, F1+2, plasminogen,  $\alpha$ 2-antiplasmin, PAP, and D-dimer, show small or negligible changes with the menstrual cycle [16–18]. Fibrinogen concentrations are lowest during menstruation and highest during the luteal phase. The concentrations of FVII and FVIIa increase up to cd 14, while protein S decreases between cd 1 and 14 [19,20]. The bleeding time is longest during menstruation in most reports [21].

A number of reports on changes in FVIII, VWF:Ag and VWF:RCO [16,22–24] suggest that the concentrations are highest during the luteal phase and lowest during the follicular phase, while one study found no change [25]. However, preanalytical conditions and cycle days for sampling differed between these studies. VWF levels appear to be lowest on cd 1–4 [23].

In terms of hormonal therapy, influences on coagulation depend in particular on estrogen content [26]. The influence of progestins alone is not well-known. The degree of classic APC resistance and other hemostatic changes induced by combined hormonal contraceptives is modified by the progestin, with levonorgestrel being the most effective in countering the estrogen effect [27]. Currently prescribed hormonal contraceptives dampen the cyclic variation of VWF and fibrinogen seen in women not on hormonal contraceptives [22]. Regarding combined contraceptives, no data support differences in hemostasis by the mode of administration, be it oral, transdermal or the vaginal ring, although there may be variable absorption of hormones with a transdermal preparation.

Baseline epidemiological studies in healthy women undergoing menopause have demonstrated increased levels of several coagulation factors, including FVIII and fibrinogen. These changes are due to both estrogen status and aging [28]. Several

studies have shown that initiation of estrogen therapy activates the coagulation system in healthy postmenopausal women, with increases in F1+2, FPA, decreased APC resistance (classic method) and decreased free protein S noted [29–32]. The effect of transdermal HRT on coagulation is significantly less than that seen with oral preparations [31,32].

#### **Circadian variation**

Platelet aggregation is most pronounced and beta-thromboglobulin concentrations are highest in the morning [33–36]. FV activity, VWF:Ag and FVII antigen are unchanged, while FVIII activity, FVIIa and F1+2 are highest in the morning, as are protein C and S concentrations. Antithrombin is either unchanged or peaks in the afternoon [34–36].

Global fibrinolysis is lowest [6,37,38] in the early morning, as is t-PA activity, while PAI-1 activity and antigen are highest at that time. PAP is low in the morning and plasminogen is unchanged [35,38]. Circadian variations in fibrinolytic components are not affected by lifestyle, dietary habits or ethnic differences.

#### **Posture**

Posture during blood drawing is of importance, as the hematocrit increases by up to 15% between lying down and assuming a sitting position [39].

#### **Dietary influences and smoking**

The influences of fasting (totally overnight or only fat-fasting in the morning), caffeine-containing beverages and smoking have not been sufficiently investigated. However, lipemic plasma interferes with many assays. Abstaining from smoking for 2 h prior to the venipuncture is recommended, because of a potential effect on platelet aggregation.

#### **Recommendations to control preanalytical conditions in obtaining coagulation studies**

For venipuncture for coagulation studies, the patient should:

- 1 Abstain from intense physical exercise for at least 24 h and from physical activity (rushing) just prior to venipuncture.
- 2 Be provided with an environment where physical stress and mental stress are lessened.
- 3 Abstain from fatty foods and smoking on the morning of the venipuncture; the patient should be fasting for PAI-1 and global fibrinolysis assays.
- 4 Have samples obtained early in the morning (7.00–9.00 AM) after sitting in a relaxed position for 20–30 min.

Specifically for women:

- 1 For the diagnosis of von Willebrand disease (VWD) in fertile women, blood samples should be obtained on cd 1–4. This may aid in the diagnosis in women with borderline values obtained at other times.

- 2 Combined oral contraceptives and HRT should be withdrawn for at least 2 months before testing. This is especially true for testing of free protein S, protein S activity and APC resistance (classic method).
- 3 For the diagnosis of inherited disorders of hemostasis (particularly VWD, FVIII deficiency, and protein S deficiency) samples should be obtained when normal menstrual cycles have returned or at least 2 months post partum. All abnormal values obtained in connection with pregnancy, including testing for phospholipid antibodies, should be verified with repeat blood sampling.

### Areas of study needed

Standardized international studies of potential influences on coagulation, especially hormonal influences, should be performed using newer global hemostatic assays. Because of the significance of VWD and disorders of platelet function resulting in bleeding symptoms in women, improved understanding of hormonal effects on these factors, and improved laboratory testing for such, is needed.

### Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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