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Letter to the Editors-in-Chief

Combined oral contraceptives do not influence von Willebrand factor related biomarkers despite an induced acute phase response

Dear Editors,

Introduction

Von Willebrand's disease (VWD) is the most common inherited bleeding disorder, caused by defects in von Willebrand factor (VWF) [1]. VWF is released from activated platelets and damaged endothelial cells, and facilitates platelet adhesion to subendothelial collagens and binds circulating factor VIII (FVIII). Mutations in the *VWF* gene cause a deficiency of VWF (type 1 and 3 VWD) or a structural defect with impaired function but normal circulating amounts (type 2 VWD) [2]. This impairs platelet adhesion and primary haemostasis.

A frequent manifestation of VWD in fertile women is primary menorrhagia [3,4]. Menorrhagia is common in adolescents; nonetheless, it is estimated that 5-20% of women presenting with primary menorrhagia suffer from VWD not previously diagnosed [5]. Since combined oral contraceptives (COCs) are the first choice treatment for menorrhagia [6], a substantial proportion of women are receiving COC treatment when referred for VWD investigation.

Few previous studies have assessed changes in VWF after COC start, and results are conflicting. Some guidelines recommend withdrawal of COCs before VWD investigation [7] or performing multiple tests [8] because of the possible influence on VWF levels, while other guidelines do not contain such recommendations [9,10]. We specifically investigated the effect of COCs on VWF to improve the knowledge on this topic and help standardise VWD diagnosing in women taking COCs. Since FVIII and C-reactive protein (CRP) are part of routine VWD diagnosis in some laboratories [9], we also assessed these VWF related parameters.

Methods and Materials

We included a group of women wishing to use COCs (COC group, n = 16), recruited from Central Aarhus, Denmark through general practitioners, and a group of women not using COCs during the study (control group, n = 28) via notices at Aarhus University and Aarhus University Hospital, Denmark. Inclusion took place January-August 2013.

The inclusion criteria were healthy females aged 18-34, of European origin and able to give informed consent. Exclusion criteria were COC use, other systemic hormonal treatment or pregnancy less than three months before the first blood sample (baseline), known systemic infectious or inflammatory disease, known hepatic or renal disease, and known thrombophilia or bleeding disorders, including VWD. This was assessed by a questionnaire. Only women prescribed second generation COCs (150 mg levonorgestrel/250 mg norgestimate and 30-35 μg ethinyl estradiol), were included in the COC group.

Blood samples were obtained at the time of inclusion (baseline) and three and six months after baseline. The COC group started COCs after the first sampling. Thus, three full COC or menstrual cycles were completed between samplings. Samples were obtained in the late luteal phase (control group) and during the one-week COC-free interval (COC group). Blood was drawn from a 21G needle into 3.2% sodium citrate or 4% lithium heparin vacuum tubes (Terumo Europe, Leuwen, Belgium), centrifuged within one hour after sampling at 3300 g for 25 minutes, and plasma was stored at -80 °C until analysed. The participant was resting for at least five minutes before sampling. The laboratory analyses performed were VWF ristocetin cofactor (VWF:RCo), VWF antigen (VWF:Ag), VWF collagen binding (VWF:CB), FVIII clot (FVIII:C), CRP, human chorionic gonadotropin (hCG) and ABO blood typing.

VWF:RCo and VWF:Ag were analysed by latex immunoturbidimetry (HemosIL®) with coefficients of variation (CV) of 7% and 6%. FVIII:C was analysed by a one-stage clotting assay using Platelin LS reagent (BioMérieux, Herlev, Denmark) and FVIII-deficient plasma (Hart Biologicals, Hartlepool, UK), CV = 9%. For these three analyses, an ACL Top® (ILS Scandinavia, Allerød, Denmark) was employed. VWF:CB was analysed at Medilys Laborgesellschaft, Asklepios Klinik Altona, Hamburg, Germany by an in-house ELISA as described previously [11], CV = 12%, with type III collagen and peroxidase-labeled rabbit anti-human VWF antibody (Dako A228). CRP and hCG were analysed on a Cobas® 6000 (Roche, Mannheim, Germany). ABO determination was performed using an IH1000 (BioRad, Schwitzerland).

The primary outcome was change in VWF:RCo after COC start. With a minimum relevant difference of 15%, at least 11 individuals should be included in the COC group. To ensure a full data set, we aimed to include at least 15 women in the COC group and twice as many in the control group. Except for CRP, our data were normally distributed, or this could be achieved by logarithmic transformation. Since normal distribution was not achieved for all data, median with range was used for descriptive statistics. Unpaired t-test and one-way repeated-measures ANOVA with Geisser-Greenhouse correction were employed; for CRP, Mann-Whitney and Friedman's tests. Pearson's correlation coefficient was employed where both variables were normally distributed, otherwise, Spearman's was used. Data were analysed using GraphPad Prism® version 6.02 (GraphPad Software Inc., California, USA).

The study was approved by The Central Denmark Region Committees on Health Research Ethics (case no. 1-10-72-502-12) and The Danish Data Protection Agency (case no. 2007-58-0010).

Results

Nineteen women in the COC group and 35 in the control group were included (Fig. 1). Sixteen COC users and 28 controls completed all blood samplings. Demographic information and baseline values for VWF related biomarkers and CRP are presented in Table 1. The two groups did not differ significantly with regards to these. None of the women were pregnant at sampling time, evaluated by hCG. As

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expected, no VWF and FVIII values were below the reference interval at baseline, except one VWF:CB measurement of 59 U/dL in the control group.

We observed no significant difference in VWF:RCo, VWF:Ag or VWF:CB between the COC group and the control group during six months of COC use (Fig. 2.a-c). FVIII:C and CRP were significantly higher in the COC group compared to the control group at three and six months (Fig. 2.d and e.). Within the COC group, we observed a significant increase in CRP, but not in VWF:RCo, VWF:Ag, VWF:CB and FVIII:C when tested by ANOVA. The mean increase in VWF related parameters after COC start is shown in table 1.

VWF:RCo, VWF:Ag, VWF:CB and FVIII:C levels were all significantly correlated with each other in both groups (all $r > 0.72$, $p < 0.01$), but no significant correlation between CRP and VWF or FVIII:C levels was observed in either group (all $r < 0.60$, $p > 0.11$).

Discussion

We found that VWF levels did not change after three or six months' use of COCs, indicating that it is not necessary to withdraw COC treatment before investigation for VWD.

Few studies have investigated the influence of COCs on VWF plasma levels [12–18], focusing primarily on the pro-thrombotic and pro-inflammatory effects of COCs. The results of these are conflicting, as two previous studies observed an increase in VWF levels after COC start [16,17]. Dumont et al. recently reviewed the previous literature and concluded that VWD can probably be investigated during COC use [19]. This is in accordance with our findings. The discrepancies between the present study and others may be explained by differences in study designs. Inflammatory disease was not an exclusion criterion in the above-mentioned studies, and neither included a control group. The different COCs used may also play a part. In the present study, second generation COCs were used, as these are currently recommended as first choice in Denmark [20].

We found that CRP levels increased after COC start, suggesting that COCs induce an acute phase response. This may contribute to the increased thrombosis risk observed in COC users, as argued by others [16,23], through activation of the haemostatic system [21,22]. Our results suggest that the acute phase response persists during several COC cycles, though less pronounced with time. This may partly explain the observation reported previously that the prevalence of venous thrombosis is highest during the first months of COC use [24]. Interestingly, though VWF is known to be an acute phase reactant [25], we observed no correlation between VWF and CRP.

To the best of our knowledge, the present study is the first one to specifically investigate the effect of COCs on multiple VWF related biomarkers, including VWF:CB. A major strength was the longitudinal, two-group design. We also designed the study specifically with regards to VWF measurements, fitting in-/exclusion criteria to avoid influence on VWF from pregnancy and inflammation [25,26] and timing blood samplings with the menstrual cycle to reduce its influence [26].

However, some limitations must be considered. The participants were healthy women, and we cannot exclude that COCs may influence VWF differently in women with VWD. Furthermore, women with subnormal VWF levels pose a special problem, since small changes in VWF could shift these patients between subnormal and normal levels.

In conclusion, the present study showed that second generation COCs did not influence VWF plasma levels significantly, though inducing an acute phase response. This indicates that investigation of VWD in fertile women can be performed without withdrawing COCs.

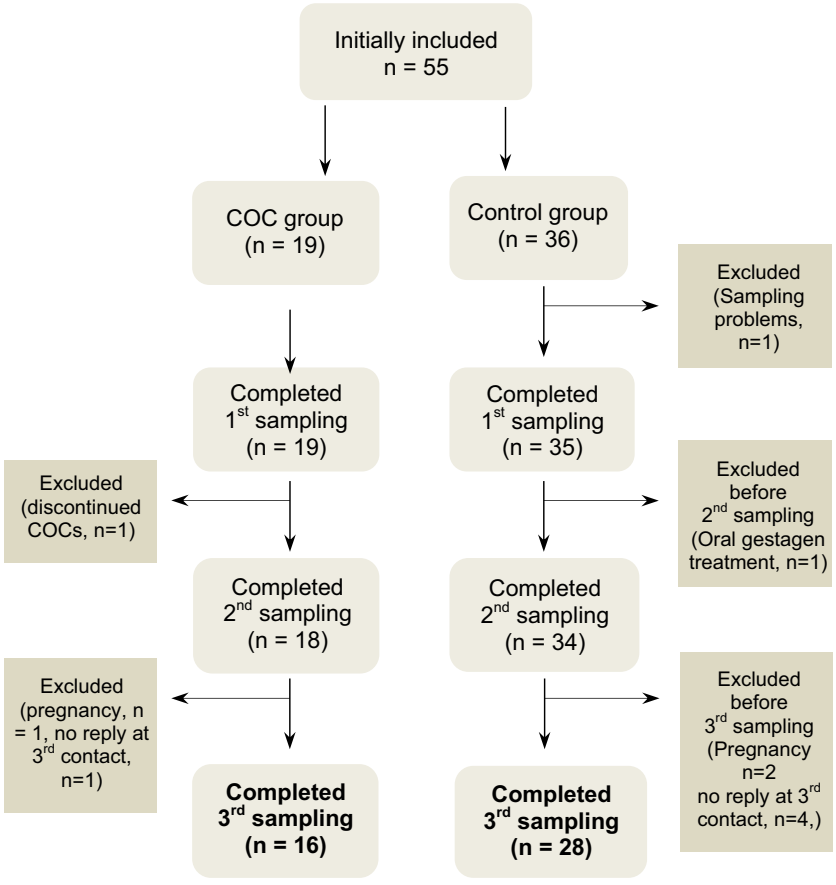

Fig. 1. Flow chart of in- and exclusion of study participants. COCs, combined oral contraceptives.