Interleukin-6 and Antiphospholipid Antibodies in Women With Contraceptive-Related Thromboembolic Disease

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OBJECTIVE: The aim of this study was to explore the possible (joint) contributing role of interleukin-6 (IL-6) and antiphospholipid antibodies to the occurrence of the venous thromboembolism in women using oral contraceptives.

METHODS: Interleukin-6 and antiphospholipid antibodies (anti-β2-glycoprotein I antibody–immunoglobulin M [IgM], G [IgG], and A [IgA]; anticardiolipin-IgM and IgG; antiphosphatidylserine-IgM and IgG) were measured in 30 women (median age 41, range 28–49 years) in the stable period (on average 3.5 years) after first venous thromboembolism. Sixteen patients used oral contraceptives during the episode of venous thromboembolism (oral contraceptives group), whereas 14 patients did not (non–oral contraceptives group). Thirty-seven age-matched, healthy women served as controls

RESULTS: Compared with controls, the oral contraceptives group had elevated IL-6 (median interquartile range 2.3 [1.1–4.3] versus 1.4 [0–2.0] pg/mL, $P < .05$). The oral contraceptives group had elevated anti-β2-glycoprotein I antibody–IgM in comparison with both the non–oral contraceptives group (median interquartile range 47.5 [2.0–77.0] versus 29.50 [11.00–45.50] OD_{450}, $P < .06$) and controls (47.5 [2.0–77.0] versus 17.5 [3.5–30.0] OD_{450}, $P < .001$). Interleukin-6 level in the non–oral contraceptives group was related to obesity, whereas such a relation was not found in the oral contraceptives group, suggesting the presence of another factor (oral contraceptive use), which stimulates IL-6 production. Of particular interest is our finding that elevated IL-6 levels correlated significantly positively with elevated anti-β2-glycoprotein I antibody–IgG in patients who were users of oral contraceptives (but not overweight, n = 10) ($r = 0.56$, $P < .05$)

CONCLUSION: The results suggest a new hypothesis that, in susceptible women, use of oral contraceptives induces production of IL-6, which stimulates production of anti-β2-glycoprotein I. Thus, the prothrombotic profile is aggravated and could facilitate occurrence of venous thromboembolism. This remains to be elucidated in further studies. (Obstet Gynecol 2004;104:564–70. © 2004 by The American College of Obstetricians and Gynecologists.)

LEVEL OF EVIDENCE: III

A number of epidemiological and clinical studies have consistently shown that oral contraceptives are associated with an increased incidence of venous thromboembolism in women of childbearing age.[1–3] However, it is becoming more and more obvious that women who develop venous thromboembolism must have some underlying predisposition that leads to hypercoagulability after the introduction of oral contraceptives.[4] It is already known that inherited thrombophilia, such as possession of factor V Leiden, G20210A prothrombin, or deficiency of antithrombin, protein C, or protein S, substantially increases the risk of venous thromboembolism in women using oral contraceptives.[5–7] Several other novel risk factors, such as inflammatory cytokines and antiphospholipid antibodies, which have already been shown to be associated with venous thromboembolism,[8,9] might also be involved in the development of hypercoagulability and venous thromboembolism in users of oral contraceptives. However, data on such associations that might be clinically relevant are lacking.

Thus, the aim of our study was to test the hypothesis that interleukin-6 (IL-6), a procoagulant cytokine,[10] and antiphospholipid antibodies, which also have prothrombotic capacity,[9] would be elevated and associated with each other in young women who had developed first venous thromboembolism during oral contraceptive use (in the absence of other risk factors of venous thromboembolism such as malignancy, trauma/operation/immobilization, or inherited thrombophilia) in comparison with young women, who had developed first venous

thromboembolism when not on oral contraceptives, and with a control group of healthy women.

PATIENTS AND METHODS

The patients and controls were selected from the World Health Organization Collaborative hospital-based, case-control Study of Cardiovascular Diseases and Steroid Hormone Contraception, in which Slovenia was one of the participating countries.[1,11] The study was undertaken in 21 centers in 17 countries. The study investigated the risk of venous thromboembolism, myocardial infarction, and stroke associated with combined oral contraceptives. The principal aim of the venous thromboembolism component was to evaluate whether current oral contraceptive use was associated with increased risk of a first deep vein thrombosis, pulmonary embolism, or both. Secondary aims were to evaluate the risk of venous thromboembolism according to type, duration, and past use of oral contraceptives and in different subgroups of women. In each center, a monitoring system was set up to identify all eligible cases. Women were eligible as cases if they were aged 15–49 years, had been admitted to a collaborating hospital between February 1, 1989, and January 31, 1995, and had a discharge diagnosis of deep venous thrombosis (DVT) and/or pulmonary embolism. Those who died within 24 hours of admission, who had a history of stroke, DVT, pulmonary embolism, or acute myocardial infarction, or natural or surgical menopause, or who had a recent history (within 6 weeks) of pregnancy, major illness causing prolonged bed rest, or surgery were excluded. Cases were categorized according to methods used to confirm the diagnosis of DVT or pulmonary embolism as definite (DVT confirmed by venography or duplex scanning or radioisotope studies, and pulmonary embolism confirmed by a ventilation/perfusion scan or angiography), probable, possible, or other. Approximately 50% of DVT and 25% of pulmonary embolism cases were categorized as definite in European centers. For each case, an attempt was made to recruit 3 female controls, matched within a 5-year age band. Controls with 27 predefined diagnoses not associated with oral contraceptives had to be admitted to the same hospital as the given case within the period of 2 weeks before and up to 4 months after the case's admission. They were recruited by the investigators surveying the wards that accepted women with any of the control diagnoses in a predefined random order. Controls were subject to the same exclusion criteria as the cases. The study confirmed an association between oral contraceptive use and venous thromboembolism in European and developing countries (in Africa, Asia, and Latin America): oral contraceptives users had a 3- to 4-fold increased risk of venous thromboembolism. Risk was higher with use of oral contraceptives containing third-generation progesterone, compared with the use of oral contraceptives containing first- or second-generation progesterone. Increased risk was apparent within 4 months of starting oral contraceptives, was unaffected by the duration of the current period of oral contraceptives use, and had disappeared within 3 months of stopping oral contraceptives. In addition, increased body mass index above 25 kg/m^2, but not age, smoking, or a history of hypertension (excluding hypertension in pregnancy), was an independent risk factor for venous thromboembolism.

In our Slovenian center, 120 eligible cases with venous thromboembolism were identified and included in the World Health Organization study, of which 48% were on oral contraceptives. We reviewed all of the 120 protocols and selected all those who had objectively confirmed venous thromboembolism (n = 54). We invited them and 108 controls for further analysis. Thirty patients (22 had DVT, 7 DVT and pulmonary embolism, and 1 only pulmonary embolism) and 52 controls responded to our invitation and were willing to participate. The study was approved by the State Ethical Committee on Human Research, and full informed consent was obtained from all patients and controls.

Women were recruited to the study in the chronic stable phase of disease 6 months to 6 years (mean 3.5 years) after the acute event. Clinical examination was performed on the same day as blood sampling in 1995. All patients and controls were examined within a period of 2 months.

Blood samples were drawn from the antecubital vein between 7 and 9 AM, with minimal venous stasis. Women were fasting overnight and rested before blood sampling for 20 minutes in a sedentary position. Plasma was prepared by centrifugation at 2,000g and 4°C for 30 minutes, snap frozen in liquid nitrogen, and stored at −70°C until analyzed. Routine laboratory measurements and measurements of fibrinolytic and coagulation tests were made on sera and/or plasma of all patients and controls (we performed the tests in 1995). However, measurements of IL-6 and antiphospholipid antibodies were made in 2001. Sera of all patients and of 37 controls were available for these measurements.

The metabolic variables (fasting glucose, triglycerides, total cholesterol, high-density lipoprotein [HDL] cholesterol, and low-density lipoprotein [LDL] cholesterol) were determined by routine biochemical methods. Patients and controls were screened for the presence of antinuclear antibodies (ANA), extractable nuclear antigens (ENA), and anti-DNA antibodies by routine screening tests from our Laboratory of Immunology. Tests for antithrom-

bin, protein C deficiency were done by a kinetic spectro-photometric assay (Berichrom-Protein C and Berichrom Antithrombin II, Behring, Marburg/Lahn, Germany), and the presence of resistance to activated protein C was determined according to Dahlback et al.[12] Plasminogen activator inhibitor-1 (PAI-1) antigen was determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (Imulyse PAI-1, Biopool, Sweden) according to the instruction manual. Interleukin-6 was measured with commercial ELISA kits (Endogen, Pierce, Rockford, IL) according to the instruction manual. The detection limit of the assay was 1 pg/mL. Anticardiolipin and antiphosphatidylserine antibodies of immunoglobulin G (IgG) and M (IgM) isotypes were measured by ELISA kits used for routine measurements in our immunological laboratory as described previously.[13] Anti-β2-glycoprotein I antibodies of IgG and IgM isotypes were measured by modified ELISA as described previously.[14] Results for all measurements of antiphospholipid antibodies were expressed in optical density units $OD_{405} \times 1,000$ (OD_{405}). The individuals doing these analyses were masked with respect to the patient's status.

Normally distributed continuous clinical, biochemical, and hemostatic variables of patients and controls were compared by the t test for independent samples. Normality was evaluated by visual inspection of histograms and by means of the Kolmogorov-Smirnov test. Continuous variables that were not distributed normally were expressed as medians with ranges between the first and third quartile, and differences between groups were assessed by the Mann Whitney U test. The χ^2 or Fisher exact test, depending on the number of cases included in the analysis, was used for comparison of differences between patients and controls for discrete variables. Depending on the distribution, Pearson or Spearman correlation coefficients were calculated to test associations between different variables. Women from the oral contraceptive group were younger than women from the non–oral contraceptive group at the time of blood sampling, and thus all comparisons were adjusted for age. Analysis of covariance was used to test the influence of age and other covariates on the differences in measured parameters between groups. Statistical analyses were performed by the Statistica for Windows computer program (StatSoft, Inc, Tulsa, OK). A P value $< .05$ was considered statistically significant.

RESULTS

In patients (n = 30), no significant differences were found compared with controls (n = 52) in the frequency of hypertension (7% versus 20%), hyperlipidemia (3% versus 0%), or smoking (33% versus 36%) at the time of the acute event. At the time of clinical examination none of the women was acutely ill or had clinical evidence of autoimmune disease. Neither patients nor controls were using aspirin or oral anticoagulants at least 1 month before blood sampling. Neither patients nor controls were taking oral contraceptives at the time of blood sampling, and there were no differences between patients and controls in either smoking or menstrual status at the time of blood sampling. However, before the acute event, significantly more patients had been taking oral contraceptives compared with controls (16/30, 56% versus 2/37, 5.4%, $P < .000$). All women had been taking second-generation oral contraceptives (containing low doses of ethynylestradiol and levonorgestrel); neither patients nor controls had used third-generation oral contraceptives containing desogestrel. No women had taken first-generation oral contraceptives in their lifetimes. For further analysis, patients were divided into 2 subgroups: the oral contraceptives group, women who were users of oral contraceptives before the acute event (n = 16), and non–oral contraceptives group, women with venous thromboembolism who had not used oral contraceptives before the acute event (n = 14).

Anthropometric and metabolic parameters of patients and controls at the time of clinical examination and blood sampling are presented in Table 1. Women from the oral contraceptives group were younger, leaner, and had lower levels of insulin compared with controls, whereas women from the non–oral contraceptives group were more obese and had higher insulin and PAI-1 concentrations compared with both controls and women from the oral contraceptives group (Table 1). Women from the oral contraceptives group did not have significantly different anthropometric and metabolic parameters compared with their age-matched controls (data not shown).

Autoimmune screening tests (ANA, ENA, and anti-DNA antibodies) were negative in all patients and controls. Tests for thrombophilia showed that neither patients nor controls had antithrombin or protein C deficiency; 3 patients and 1 control were resistant to activated protein C (difference not significant).

Concentrations of IL-6 are shown in Table 2. They were above the detection limit in 68% of controls and 75% of patients. In the oral contraceptives group, values of IL-6 were higher than in controls ($P = .05$), the difference remaining significant after adjustment for parameters measured at the time of blood sampling: body mass index (BMI), waist/hip ratio, age, smoking status, arterial blood pressure, cholesterol, HDL and LDL cholesterol, triglycerides, and blood glucose (Table 2). In the oral contraceptives group, IL-6 did not correlate with either metabolic parameters or PAI-1 antigen, whereas in

Table 1. Anthropometric and Metabolic Parameters and Plasminogen Activator Inhibitor-1 in Controls, OC Group, and Non-OC Group

	Controls (n = 37)	OC group (n = 16)	Non-OC group (n = 14)	P (OC vs non-OC)
Age (y)	41 (28–49)	33 (23–45)*	39 (31–49)	.046
Body mass index (kg/m^2)	25 ± 4	24 ± 4†	32 ± 8*	.000
Waist/hip ratio (rel)	0.80 ± 0.06	0.80 ± 0.10	0.79 ± 0.04	.72
Systolic blood pressure (mm Hg)	128 ± 3	123 ± 20	126 ± 22	.52
Diastolic blood pressure (mm Hg)	82 ± 11	81 ± 11	84 ± 13	.48
Total cholesterol (mmol/L)	5.8 ± 1.5	5.5 ± 1.3	5.5 ± 0.9	.90
LDL cholesterol (mmol/L)	3.6 ± 1.1	3.4 ± 1.2	3.4 ± 0.9	.96
HDL cholesterol (mmol/L)	1.6 ± 0.4	1.8 ± 0.5	1.6 ± 0.5	.32
Triglycerides (mmol/L)	1.0 (0.7–1.3)	0.8 (0.6–1.0)	1.0 (0.5–1.4)	.44
Blood glucose (mmol/L)	5.1 ± 0.6	4.8 ± 0.5	5.1 ± 0.8	.33
Insulin level (mIE/L)	11.4 ± 5.4	8.0 ± 2.3*	12.5 ± 3.8	.002
PAI-1 antigen (ng/mL)	9.0 (6.0–13.0)	6.0 (3.9–17.4)	17.6 (7.8–25.1)†	.012

OC group, users of oral contraceptives at the time of the acute venous thromboembolic event; non-OC group, women who had not used oral contraceptives at the time of acute venous thromboembolic event; rel, relative; LDL, low-density lipoprotein; HDL, high-density lipoprotein; PAI-1, plasminogen activator inhibitor-1.

Values are expressed as mean ± standard deviation or as median (with range between the first and third quartile), with the exception of age, which is shown as mean (range).

* $P < .01$ vs controls.

† $P < .05$ vs controls.

the non–oral contraceptives group, IL-6 correlated with BMI ($r = 0.53$, $P = .06$).

Median concentrations of antiphospholipid antibodies with ranges between the first and third quartiles are shown in Table 3. Immunoglobulin M anti-β2-glycoprotein I levels were significantly higher in the oral contraceptives group and anticardiolipin IgG in the non–oral contraceptives group compared with controls. Immunoglobulin M anti-β2-glycoprotein I correlated significantly negatively with BMI ($r = -0.59$, $P < .001$), waist/hip ratio ($r = -0.51$, $P < .005$), insulin ($r = -0.57$, $P < .001$), and PAI-1 antigen ($r = -0.62$, $P < .001$). Anticardiolipin IgG correlated significantly negatively with HDL cholesterol in the non–oral contraceptives group ($r = -0.68$, $P < .01$).

When we tested associations between antiphospholipid antibodies and IL-6, we found a positive correlation between IL-6 and IgG anti-β2-glycoprotein I in lean patients (BMI ≤ 25 kg/m^2) who used oral contraceptives at the time of the acute event (n = 10) ($r = 0.56$, $P < .05$). In the oral contraceptives group, lean women who had high levels of IL-6 (over the third quartile) (n = 5) also had

higher IgG anti-β2-glycoprotein I compared with women with low levels of IL-6 (n = 5) (median interquartile range 20 [0 –26] versus 6 [–13 to –6.5] OD$_{450}$, $P < .05$) and with lean controls (n = 18) (median interquartile range 20 [0– 26] versus 3 [–14 to –9] OD$_{450}$, $P < .05$).

DISCUSSION

In our study we explored the presence of increased levels of IL-6 and antiphospholipid antibodies, both of which have prothrombotic capacity,[9,10] in young women who had developed first venous thromboembolism during use or non-use of oral contraceptives. In addition, we explored whether any associations between IL-6 and antiphospholipid antibodies exist.

Our results lead us to construct a new hypothesis about thrombosis potential in young women with oral contraceptive–related venous thromboembolism. However, we are aware of the possible difficulties of interpreting these results because of 2 important points that can occur in epidemiological studies. The first is that significant associations might be found only by chance because

Table 2. Interleukin-6 Concentrations in Controls, OC Group, and Non-OC Group

	Controls (n = 37)	OC group (n = 15)	Non-OC group (n = 13)	P (OC vs non-OC)
IL-6 concentrations (pg/mL)	1.4 (0–2.0)	2.3 (1.1–4.3)*	1.6 (0–2.3)	.18

OC group, users of oral contraceptives at the time of the acute venous thromboembolic event; non-OC group, women who had not used oral contraceptives at the time of acute venous thromboembolic event; IL-6, interleukin-6.

Values are expressed as median (interquartile range).

* $P = .05$ vs controls.

Table 3. Differences in Optical Density Units (OD_{405}) of Antiphospholipid Antibodies in Controls, OC Group, and Non-OC Group

	Controls (n = 37)	OC group (n = 16)	Non-OC group (n = 14)	P (OC vs non-OC)
anti-β2GPI-IgG	3.50 (−4.0–12.0)	8.00 (−13.0–79.0)	9.00 (2.00–21.50)	.48
anti-β2GPI-IgM	17.50 (3.50–30.0)	47.50 (2.0–77.0)*	29.50 (11.0–45.50)†	.06
anti-β2GPI-IgA	22.50 (6.50–37.5)	20.50 (−10.0–97.0)	24.00 (11.0–43.0)	.39
aCL IgG	0.14 (0.09–0.17)	0.18 (0.10–0.31)†	0.17 (0.12–0.22)‡	.90
aPS IgG	0.15 (0.10–0.17)	0.12 (0.1–0.23)	0.14 (0.08–0.17)	.35
aCL IgM	0.09 (0.04–0.14)	0.10 (0.0–0.27)	0.09 (0.06–0.14)	.72
aPS IgM	0.10 (0.06–0.16)	0.12 (0.0–0.28)	0.12 (0.04–0.14)	.60

OD_{405}, $OD_{405nm} \times 1,000$; OC group, users of oral contraceptives at the time of the acute venous thromboembolic event; non-OC group, women who had not used oral contraceptives at the time of acute venous thromboembolic event; anti-β2GPI, anti-β2-glycoprotein I antibody; Ig, immunoglobulin; aCL, anticardiolipin; aPS, antiphosphatidylserine.

Values are expressed as median (interquartile range).

* $P < .001$ vs control.

† $P = .09$ vs control.

‡ $P < .05$ vs control.

of the measurement of a large number of variables in several groups with small numbers of patients. The second is that, in patients with a history of thrombosis, it is impossible to know whether differences between patients and controls are related to or the consequence of the clinical event.
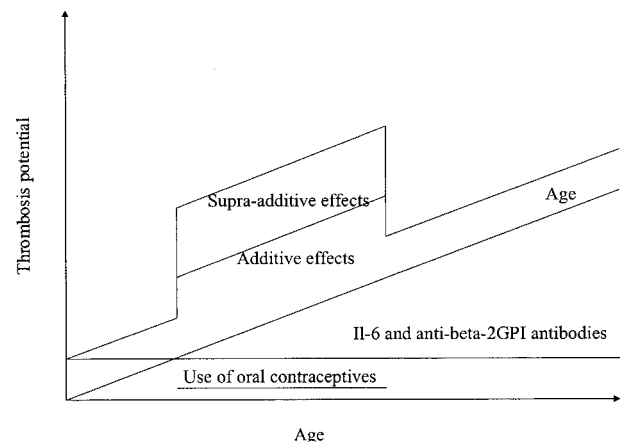
In young women who had suffered venous thromboembolism during the use of oral contraceptives, we found increased levels of IL-6 and anti-β2-glycoprotein I antibodies, whereas, in women who had suffered idiopathic venous thromboembolism (and did not use oral contraceptives) and in controls, IL-6 and anti-β2-glycoprotein I antibodies were not increased. Because venous thromboembolism is a multicausal disease as proposed by Rosendaal[15] in his dynamic, age-dependent model of thrombosis potential, we hypothesized that increased production of IL-6 and antiphospholipid antibodies in women with oral contraceptive–related venous thromboembolism could represent additional risk factors for development of venous thromboembolism (Fig. 1).

Besides its relation to bacterial infections, IL-6 was found to be related to age, parameters of obesity, smoking status, arterial blood pressure, cholesterol, HDL and LDL-cholesterol, triglycerides, and blood glucose.[16–23] Although, in our cohort of women with idiopathic venous thromboembolism, IL-6 was related to obesity, increased levels of IL-6 in women with oral contraceptive–related venous thromboembolism were not associated with obesity and other factors known to increase IL-6. Thus, it appears that other factors must be involved in high IL-6 production in these women.

Recently, it has been shown that increased IL-6 production could be induced by oral contraceptive use and activation of endothelial cells with anti-β2-glycoprotein I antibodies.[24–26] Furthermore, it has been shown that IL-6 induces production of antiphospholipid antibodies

by B lymphocytes.[27–28] Taking this into account, we hypothesized that in women with oral contraceptive–related venous thromboembolism who had increased IL-6 and anti-β2-glycoprotein I antibodies, production of IL-6 and anti-β2-glycoprotein I antibodies could be triggered by oral contraceptives (Fig. 2). This mechanism might lead to additive or supra-additive effects of IL-6 and anti-β2-glycoprotein I antibodies with oral contraceptive use (Fig. 1).

According to this hypothesis, it is of interest whether a correlation between IL-6 and anti-β2-glycoprotein I antibodies exists in our patients. To avoid the influence of increased BMI, which is associated with increased IL-6, we selected only "lean" patients and controls (with BMI

Fig. 1. Hypothetical model of thrombosis potential in young women who developed venous thromboembolic event during oral contraceptive use, according to the Rosendaal dynamic age-dependent model of thrombosis risk, with interaction between different risk factors.

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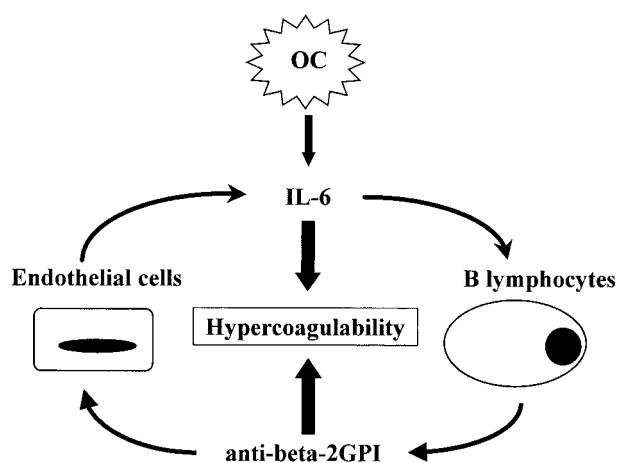
Fig. 2. Possible vicious circle of hypercoagulability during oral contraceptive (OC) use caused by increased interleukin-6 (IL-6), which promotes induction of anti-β2-glycoprotein I (anti-β2-GPI) antibodies from lymphocytes, which in turn stimulate endothelial cells to produce IL-6.

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≤ 25 kg/m^2) to test this association. We found a significant association between IL-6 and anti-β2-glycoprotein I antibodies in patients who used oral contraceptives at the time of the acute event, but not in non–oral contraceptive users and controls. Further significant differences in anti-β2-glycoprotein I antibodies were found between lean oral contraceptive patients who had IL-6 above and below the third quartile of the controls. These results are consistent with our hypothesis on the supra-additive thrombosis potential of a combination of increased IL-6 and anti-β2-glycoprotein I in women with oral contraceptive–related venous thromboembolism (Fig. 1), as well as with a possible explanation of increased IL-6 and anti-β2-glycoprotein I production during oral contraceptive use in high IL-6 producers (Fig. 2). However, we are aware that this hypothesis is not convincingly supported by our data because of the small sample size. New studies on a larger number of patients should be conducted in the future.

We found significantly increased IL-6 and anti-β2-glycoprotein I levels in young women with oral contraceptive–related venous thromboembolism. Further, in lean patients, increased levels of IL-6 correlated with anti-β2-glycoprotein I. These findings support the hypothesis that, in susceptible women, use of oral contraceptives induces production of IL-6, which stimulates production of anti-β2-glycoprotein I, which in turn further stimulates IL-6 production. Thus, the prothrombotic profile is aggravated and could facilitate the occurrence of venous thromboembolism. This new hypothesis should be further explored to try to define the group of young women susceptible to development of venous thromboembolism during the use of oral contraceptives. It might be that high levels of IL-6 before or after the introduction of oral contraceptives could identify these patients. This hypothesis remains to be elucidated in further prospective studies.

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