OPEN

Comparative Study of the Effects of Combined Oral Contraceptives in Hemostatic Variables

An Observational Preliminary Study

Bianca Stocco, MSc, Helen F. Fumagalli, MSc, Silvio A. Franceschini, PhD, Edson Z. Martinez, PhD, Cleni M. Marzocchi-Machado, PhD, Marcos Felipe S. de Sá, PhD, and Maria Regina T. Toloi, PhD

Abstract: Thrombotic risk is associated with the estrogen dose and type of progestin in combined oral contraceptives. Studies published since 1990 showed that third-generation progestins have larger risk to contribute to thrombosis development than the second-generation. However, there are conflicts in the literature regarding the thrombotic risk associated to the drospirenone progestin. So, this study aimed to evaluate the effects of 3 formulations of contraceptives containing ethinylestradiol (EE) (20 and 30 μg) combined with drospirenone versus levonorgestrel combined with EE (30 μg) in hemostatic parameters.

This cross-sectional study included 70 healthy women between 18 and 30 years, BMI 19 to 30 kg/m^2, not pregnant, non-smokers, and users or non-users (control) of contraceptives for a minimum period of 6 months. The following parameters were assessed: prothrombin time (PT), Factor VII, activated partial thromboplastin time (aPTT), Factor XII, fibrinogen, Factor 1 + 2, Protein C, Protein S, antithrombin, D-dimers, and plasminogen activator inhibitor-1.

Significant alterations were found in PT, aPTT, fibrinogen, D-dimers, and protein S, all favoring a state of hypercoagulation for contraceptive containing DRSP/20EE. Both contraceptives containing DRSP/30EE and LNG/30EE promoted changes that favor the hypercoagulability in the coagulant variable PT and in the anticoagulant variables Protein S and Protein C, respectively.

We suggest that the progestin drospirenone can contribute to an inadequate balance among procoagulant, anticoagulant, and fibrinolytic factors, since that the contraceptive containing the lowest dose of estrogen and drospirenone (DRSP/20EE) caused a higher number of hemostatic changes.

(*Medicine* 94(4):e385)

Abbreviations: ANCOVA = analysis of covariance, ANOVA = analysis of variance, aPTT = activated partial thromboplastin time, BMI = body mass index, COCs = combined oral contraceptives, DRSP = drospirenone, EE = ethinylestradiol, FCFRP = Faculty of Pharmaceutical Sciences of Ribeirão Preto, FMRP = Faculty of Medicine of Ribeirão Preto, LNG = levonorgestrel, PAI-1 = plasminogen activator inhibitor-1, PT = prothrombin time.

INTRODUCTION

Over 100 million women around the world use hormonal contraceptive methods. Among them, 93 million use combined oral contraceptives (COCs).[1] COCs are well tolerated by most women and present high contraceptive efficacy when used properly.[2] Hormonal contraceptives cause several metabolic effects on lipids, carbohydrates, and hemostatic parameters.[3] These changes may lead to the formation of an obstructive clot in face of an inadequate balance among procoagulant, anticoagulant, and fibrinolytic factors.[4]

The association between the use of COCs and the incidence of venous thrombosis is well defined and has been confirmed in several observational studies.[5–10] A study that compared the incidence of venous thrombosis in non-users and users of oral contraceptives younger than 30 years found that the relative risk of thrombosis for women younger than 30 years who use COCs is 3.1.[10,11]

Initially, the estrogen contained in contraceptives was considered the only responsible for the prothrombotic effects of hormonal contraception. In fact, a progressive reduction in the dose of estrogen reduced these side effects.[12]

However, due to the increase in the incidence of thrombosis induced by contraceptives containing the same dose of estrogen and different progestagen, it was possible to observe that the hypercoagulant effect of the contraceptive is not strictly dependent on the dose of estrogen, but on the ''total estrogenicity'' of the estroprogestive formulation. The estrogenicity increases as the dose of estrogen increases, but decreases with the increase of the antiestrogenic activity of the progestagen.[13]

Studies published since 1990 showed that third-generation progestins have a lower antiestrogenic activity in relation to the second-generation ones (levonorgestrel), and therefore, they are less potent to counterbalance the prothrombotic effects caused by the estrogen Table 1.[7,10,14–16]

There are conflicts in the literature regarding the thrombotic risk associated to the drospirenone progestin. Some studies reported an increase in the risk of thrombosis in face of the use of drospirenone,[9,17,18] whereas others have not found significant results.[19,20]

Drospirenone is an analogue of the antagonist of aldosterone, the spirolactone, which has antimineralocorticoid and

Editor: Ganesh Cherala.
Received: September 8, 2014; revised and accepted: November 24, 2014.
From the Department of Clinical, Toxicological and Bromatological Analysis, Faculty of Pharmaceutical, Sciences of Ribeirão Preto, (BS, HFF, CMM-M, MRTT); Department of Gynecology and Obstetrics (SAF,MFSDS); and Department of Social Medicine, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil (EZM).
Correspondence: Bianca Stocco, Avenida do Café, S/N, Bloco M, Sala 52, Ribeirão Preto, São Paulo 14040–903, Brazil (e-mail: bianca.sto@hotmail.com).
This study was financially supported by FAPESP - São Paulo Research Foundation (grant 07/58578–7).
The authors declare that they have no conflicts of interests.
Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.
This is an open access article distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0, where it is permissible to download, share and reproduce the work in any medium, provided it is properly cited. The work cannot be changed in any way or used commercially.
ISSN: 0025-7974
DOI: 10.1097/MD.0000000000000385

TABLE 1. Published Studies that Showed the Risk of VTE in Users of Oral Contraceptives Containing Third-generation Progestogen Versus Second-generation (Levonorgestrel)

Third-generation Progestogen Type	Third-generation Progestogen vs Levonogestrel VTE Risk	References
Gestodene and desogestrel	1.9	Bloemenkamp et al, 1995[7]
Gestodene	0.95	Farmer et al, 1997[14]
Desogestrel 20 μg	2.93	
Desogestrel 30 μg	0.64	
Gestodene and desogestrel	1.7	Kemmeren et al, 2001[15]
Gestodene and desogestrel	1.5–1.8	Petitti, 2003[16]
Desogestrel	2.0	Van Hylckama et al, 2009[10]
Gestodene	1.6	
Cyproterone acetate	2.0	

VTE = venous thromboembolism.

antiandrogenic activity, besides being quite similar to the endogenous progesterone.[21]

In face of the facts presented, this study aimed at evaluating the incidence of hemostatic changes in the Brazilian female population using contraceptives containing different doses of estrogen and types of progestins (drospirenone and levonorgestrel).

MATERIALS AND METHODS

Subjects and Study Protocol

Cross-sectional study with 70 women was distributed into 4 groups: women who use oral contraceptive containing 20 or 30 μg EE combined with 3 mg DRSP 21/7 or 24/4 regimen and 30 μg EE combined with 0.15 mg of LNG 21/7. Control group consisted of non-users of hormonal contraceptive methods. (Figure 1). This study was developed from August 2009 to June 2010 in the Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, São Paulo, Brazil.

The present study is part of a research protocol designed to evaluate the effect of 3 formulations of Hormonal Contraceptives in the expression of soluble adhesion molecules.[22] Sample size calculations were performed by using Proc Power in SAS 9.1 (SAS Institute, Cary, NC, USA). Considering comparisons between population means of the Soluble Intercellular Adhesion Molecule 1 by a 1-way analysis of variance (ANOVA), it was found that a sample size of 20 individuals at each group is sufficient for detecting a minimum difference of 40 ng/mL in soluble adhesion molecules between 2 of 4 groups at a significance level of 0.05, power of 0.80, and a standard deviation of 40 ng/mL.

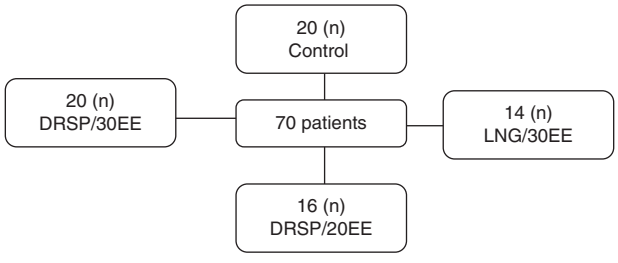

FIGURE 1. Study flow chart. DRSP = drospirenone, EE = ethinylestradiol, LNG = levonorgestrel, COC = combined oral contraceptive, n = number of patients.

Considering the limited number of participants (Figure 1), we assume that this is an underpowered study and all the results obtained in this study are considered as preliminary.

This study was approved by the Committee of Ethics in Research of the Faculty of Pharmaceutical Sciences of Ribeirão Preto (FCFRP). All women signed a Free and Clarified Consent Form agreeing to participate voluntarily.

This study had the participation of healthy Brazilian women, who were students and used the service of the department of Gynecology and Obstetrics offered by the Integrated Health System of the University of São Paulo campus Ribeirão Preto; and students who used the service of Gynecology and Obstetrics of the Basic Health Unit of Cuiabá-School Hospital of the Faculty of Medicine of Ribeirão Preto (FMRP).

The inclusion criteria adopted were: age between 18 and 30 years, body mass index (BMI) 19 to 30 kg/m^2, user or non-user (control group) of contraceptives for a minimum period of 6 months and a maximum period of 24 months. Smokers, under the prescription of anti-inflammatory drugs and with a personal history of cardiovascular disease, hypertension, thromboembolism, chronic or acute hepatopathy, diabetes, or autoimmune disease, were excluded from the study. To obtain information such as frequency in the practice of physical exercise and family history of thrombosis, the volunteers answered a questionnaire.

The blood sample was collected in standardized conditions, between 08:00 and 10:00 AM, after at least 12 h of fasting. Sodium citrate (0.11 mol/L) was used as anticoagulant and the samples were centrifuged for 20 minutes at 2500*g* and stored at −70°C, and after 6 months, the laboratorial parameters were analyzed in the plasma of the volunteers. The blood samples of all the groups were collected during the luteal phase of the menstrual cycle (21–28 days).

Measurements of Haemostatic Variables

The effect of the 3 formulations of contraceptives in the procoagulant parameters was analyzed through the determination of the activated partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen plasma concentration (Clauss method), and activity of the factors XII and VII in the plasma of the patients, with the use of an automatic coagulation analyzer (STart DIAGNOSTICA STAGO, Paris - France) according to the criteria of the manufacturer (Trinity Biotech-Ireland).

The determination of the variable marking coagulation F1 + 2 (prothrombin fragment 1 + 2) in the plasma was

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

established through immunoenzyme technique (Kit Enzygnost®, Siemens Germany).

The anticoagulant variables analyzed were the activity of the antithrombin and the plasmatic concentration of Protein C and S. In these analyses, a chromogenic method was used to determine the activity of the antithrombin (Kit Berichrom Antithrombin III) with the assistance of the equipment BCS®XP. The concentration Protein C and S was determined by ELISA (Helena Laboratories, Beaumont, Texas).

The quantification of the fibrinolytic variable D-dimer was performed through automatized immunoenzyme technique with the use of the equipment and reagent VIDAS®D-Dimer Exclusion (BioMériuex, France). The activity of the antifibrinolytic variable PAI-1 was determined through chromogenic method (Berichrom PAI-1, Siemens, Germany) in the equipment BCS®XP.

All samples were processed concomitantly in the end of the study to minimize the interference of inter-assay variations.

Statistical Analysis

The population means of the variables, age and BMI, in the 4 groups of volunteers were compared using an ANOVA. Previously, Levene test was used to compare equality of variances. When significant differences were detected between the variances, Welch ANOVA was used to compare the population means. Otherwise, the traditional ANOVA was used. Fisher exact test was used to compare the frequencies of the practice of physical exercise and family history of thrombosis between the 4 groups.

An analysis of covariance was used to compare population means of the variables associated to the procoagulants, anticoagulants, fibrinolytics, and antifibrinolytics variables between the 4 groups of volunteers, considering adjustments for possible confounding effects of the covariates age, BMI, practice of physical exercise, and family history of thrombosis.

RESULTS

The authors recruited 72 women for this study. Among these, 70 were distributed into 4 groups (Figure 1). During the period of study, 2 patients were excluded, as they presented incompatible values of BMI in relation to the values established as inclusion criteria.

The characteristics, age, BMI, frequency in the practice of physical exercise, and family history of thrombosis of the 4 groups, of women who participated in the study are presented in Table 1.

In Table 2, there is evidence that the age of the volunteers has approximate averages between populations of interest ($P = 0.07$), but different variances ($P < 0.01$). There is a greater dispersion of age in Group IV, which consisted of the youngest and oldest women of this study.

The results obtained in the analysis of the coagulant, anticoagulant, and fibrinolytic variables of the women in the 4 groups of this study are presented in Table 3.

The PT, in seconds, was significantly longer for patients in the control group in comparison with the patients who used the 3 formulations of contraceptives (DRSP/30EE, DRSP/20EE, and LNG/30EE). The contraceptive containing second-generation progestagen (LNG/30EE) presented a PT closer to that found in the control group. In the determination of the aPTT, there was a significant decrease in the value of this parameter for the group DRSP/20EE in comparison with the control group. In the quantification of the fibrinogen plasma concentration, the group DRSP/20EE presented a significantly increased value in relation to the control group. In the analysis of the activity percentage of the coagulation factors (FVII and FXII) and plasma concentration of F1 + 2, no significant differences were found.

Regarding the anticoagulant parameters, the level of Protein C found in the plasma of the users of LNG/30EE was lower than in the others 3 groups. A significant decrease in the level of Protein S for the users of DRSP/30EE and DRSP/20EE was observed. No significant differences were found for the quantification of the activity percentage of the antithrombin in the plasma of the patients in the 4 evaluated groups.

As for the fibrinolysis analysis, a significant increase was found in the fibrinolytic parameter D-dimer for the group DRSP/20EE in comparison with the control group. Still in the fibrinolysis evaluation, a significant decrease was found in the anti-fibrinolytic parameter PA1-1 for the group DRSP/30EE in comparison with the other 3 studied groups (Control, DRSP/20EE, LNG/30EE).

DISCUSSION

In this study, the changes caused in the hemostasis were more expressive for the users of DRSP/20EE, in which it is

TABLE 2. Baseline Characteristics of Subjects at Enrollment

Characteristics	Control n = 20	DRSP/30EE n = 20	DRSP/20EE n = 16	LNG/30EE n = 14	*P* value
Age, years	26.3 ± 2.5	24.5 ± 2.7	24.2 ± 3.4*	26.9 ± 5.1*	<0.01†
					0.07‡
BMI, kg/m^2	22.4 ± 2.9	22.7 ± 2.8	21.7 ± 2.3	23.7 ± 3.3	0.47†
					0.29§
Physical exercise‖, n (%)	6 (30)	10 (50)	4 (25)	1 (7)	0.06¶
Family history of thrombosis, n (%)	7 (35)	6 (30)	5 (31)	4 (29)	1.00¶

Values expressed as means ± standard deviation. BMI = body mass index, DRSP = drospirenone, EE = ethinylestradiol, LNG = levonorgestrel.
* Significant comparison between these values ($P < 0.01$).
† Comparison between population variances, Levene test.
‡ Comparison between population means, Welch ANOVA.
§ Comparison between population means, ANOVA.
‖ Practice of physical exercise ≥2 times per week.
¶ Comparison between proportions, Fisher exact test.

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

TABLE 3. Hemostatic Variables

Variable		Control	DRSP/30EE	DRSP/20EE	LNG/30EE	ANCOVA *P* value
PT (s)	Min–Max*	12–18				
	Mean (SD)	14.7 (1.0)	12.6 (1.1)	12.2 (0.6)	13.5 (0.9)	<0.01
APTT (s)	Min–Max*	25–34				
	Mean (SD)	31.5 (3.5)	29.6 (2.5)	29.2 (2.7)	29.3 (2)	0.02
Fibrinogen (mg/dL)	Min–Max*	175–400				
	Mean (SD)	254.6 (41.8)	294.4 (48.4)	302.6 (54)	290.6 (40.2)	0.02
Factor VII activity (%)	Min–Max*	50–50				
	Mean (SD)	120.1 (41.8)	150.3 (34.5)	150.2 (40.1)	133.4 (43.6)	0.12
Factor XII activity (%)	Min–Max*	50–150				
	Mean (SD)	88.7 (43.5)	106.8 (32.9)	107.9 (33.1)	102.8 (42.2)	0.37
Factor 1 + 2 (pmol/L)	Min–Max*	69–229				
	Mean (SD)	125 (88)	127.7 (54.9)	129.5 (47.4)	171.7 (49.8)	0.06
Protein C (%)	Min–Max*	72–160				
	Mean (SD)	111.3 (19.5)	118.1 (11.8)	106.4 (13.6)	93.6 (11.8)	<0.01
Protein S (%)	Min–Max*	60–150				
	Mean (SD)	145 (20.9)	114.7 (15.8)	103.2 (14.6)	140.3 (21.9)	<0.01
Antithrombin (%)	Min–Max*	75–125				
	Mean (SD)	94 (9.5)	89.8 (8.3)	91.4 (7)	95.5 (7.1)	0.25
D-dimer (μg/mL)	Min–Max*	0–0.5				
	Mean (SD)	0.22 (0.12)	0.27 (0.12)	0.38 (0.22)	0.27 (0.08)	<0.01
PAI-1 activity (%)	Min–Max*	0.3–3.5				
	Mean (SD)	2.61 (0.52)	1.77 (0.56)	2.38 (0.41)	2.75 (0.6)	<0.01

Values expressed as means (SD, standard deviation). aPTT = activated partial thromboplastin time, DRSP = drospirenone, EE = ethinylestradiol, LNG = levonorgestrel, PAI-1 = plasminogen activator inhibitor-1, PT = prothrombin time.
* Reference values for all groups.

possible to notice significant changes in 3 coagulant parameters (PT, aPTT, and fibrinogen), in the anticoagulant parameter Protein S, and in the fibrinolytic parameter (D-dimer), all favoring a state of hypercoagulation. The contraceptives containing DRSP/30EE and LNG/30EE promoted changes that favor hypercoagulability in the coagulant parameter PT and in the anticoagulant parameter Protein S and Protein C, respectively. These statistically significant findings were not followed by clinical significance.

The results found in this study agree with Kluft et al,[23] who reported a decrease in the values of PT and aPTT in the 3 groups evaluated in their study (DRSP/30EE, DRSP/20EE, DSG/30EE). Although this same author already reported in the year 2000 that global tests such as PT and aPTT present inconclusive results about the clinical relevance of a drug, since different factors such as differences of handling of samples, use of tetracycline and antihistamines drugs, and lipemic samples may influence these tests,[24] and the only way to obtain conclusive evidence would be to evaluate coagulation factors in isolation.[25] Klipping & Marr[26] demonstrated an increase in the fibrinogen plasma concentration in patients treated with DRSP/20EE in relation to nontreated patients. Previously published studies[5–10] have demonstrated that the use of oral contraceptives may favor hypercoagulation, increasing the chances of developing venous thrombosis by providing an increase in the coagulant factors and a decrease in anticoagulation and fibrinolysis.[27] Venous thrombosis is a multifactorial disease with an annual incidence around 50 per 100 000 in patients at 25 years of age and 120 per 100 000 in patients by age 50, and its major complication, the pulmonary embolism, cause death in 1% to 2% of patients.[28]

Numerous reports have indicated that the use of oral contraceptives increases the thrombotic risk 2- to 6-fold.[9,10] This increased risk is associated with the dose of estrogen and the type of progestogen. The second-generation progestins have been shown to be safer than the new progestins and this fact has generated extensive discussions. A large Danish national cohort study analyzed that among 3.3 million of women, 4213 venous thrombotic events were found, among which 2045 events were observed in current users of COCs.[9]

The Food and Drug Administration issued addition of information about the risk of thrombosis during the use of contraceptive containing DRSP on the labels, and very recently, a French medicines agency has suspended marketing authorization for pills containing cyproterone acetate combined with 35-μg ethinylestradiol (EE).[29]

In our study, users of DRSP/20EE had more hemostatic alterations than the users of DRSP/30EE. These results disagree with the studies that show that the estrogen is the responsible by cause alterations that favor the occurrence of thrombotic events,[5–10] since DRSP/20EE caused more hypercoagulant alterations. Studies[10,30] demonstrated that the decrease in the dose of EE (estrogen) from 50 μg to 30 to 20 μg has caused a decrease in the risk of developing thrombosis. Nevertheless, there is no evidence that the risk of thrombosis has decreased due to the changes done in the concentration of estrogen (30–20 μg). In this comparison, it was possible to observe that the hypercoagulant effect of the contraceptive is not strictly dependent on the dose of estrogen.[31] It is important to note that the women who used the contraceptive containing DRSP/20EE were the ones that showed an increase in the levels of D-dimers, which are fibrin degradation products, and therefore are dependent on fibrin generation and fibrinolysis, becoming

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

the best marker of fibrin turnover. Among all markers of thrombotic states, D-dimers are the ones who actually certify the presence of stabilized fibrin.[32]

When we evaluate the effects of different progestogens (DRSP/30EE vs LNG/30EE), both contraceptives caused the same number of hemostatic alterations. So, we can suggest that this comparison showed inconclusive results, since it was expected that the second-generation progestogen (levonorgestrel) had higher antiestrogenic activity,[14–16] causing less alterations in the hemostasis. Perhaps the generation of these inconclusive results can be related to the different characteristics observed in each group, for example, BMI, age, and practice of physical exercises.

In this study, we concluded that the contraceptive containing the lowest dose of estrogen DRSP/20EE caused changes in procoagulant, anticoagulant, and fibrinolytic factors, confirming the fact that the hemostatic changes caused by the contraceptives do not depend strictly on the dose of estrogen, being highly related to the anti-estrogenic activity of the progestin. In this case, the progestin drospirenone was found to have lower efficacy to counterbalance the prothrombotic effect provided by the estrogen present in the formulation. These findings can contribute to leave people aware of the risk related to the use of contraceptive containing DRSP.

Some limitations were found in this study, for example, it was difficult to find participants that use the contraceptives containing DRSP/20EE and LNG/30EE because in Brazil, the contraceptive containing DRSP/20EE has a very high cost and is a new drug in comparison to the other available drugs, and the contraceptive containing LNG/30EE is usually used by women over 30 years of age. Furthermore, it was difficult to find women with a profile within the inclusion criteria and in a predetermined period of menstrual cycle to collect blood samples. For these reasons, we have a small sample size what conducted us to consider this work a preliminary study.

ACKNOWLEDGMENTS

The authors would like to thank the São Paulo Research Foundation (FAPESP) for the financial support of the study as well as Marlise Montes, Lilian Cataldi Rodrigues, and Francine Bianchini for the technical support. The authors would also like to thank Márcia Sueli Baggio and the Hemostasis Laboratory (FMRP-USP).

REFERENCES

1. Goldhaber MD, Samuel Z. Risk factors for venous thromboembolism. *JACC.* 2010;56:1–7.
2. Trussell J, Stewart F, Guest F, Hatcher RA. Emergency contraceptive pills: a simple proposal to reduce unintended pregnancies. *Fam Plann Perspect.* 1992;24 (6):269–273.
3. Conard J. Biological coagulation findings in third-generation oral contraceptives. *Hum Reprod Update.* 1999;5:672–680.
4. Rosendaal FR. Venous thrombosis: the role of genes, environment, and behavior. *Hematology.* 2005;1:1–12.
5. Poulter NR, Chang CL, Farley TMM, et al. Venous thromboembolic disease and combined oral contraceptives: results of international multicentre case-control study. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. *Lancet.* 1995;346:1575–1582.
6. Spitzer WO, Lewis MA, Heinenmann LAJ, et al. Third generation oral contraceptives and risk of venous thromboembolic disorders: an international case-control study. *BMJ.* 1996;312:83–88.
7. Bloemenkamp KW, Rosendaal FR, Büller HR, et al. Risk of venous thrombosis with use of current low-dose oral contraceptives is not explained by diagnostic suspicion and referral bias. *Arch Intern Med.* 1999;159:65–70.
8. Farmer RDT, Lawrenson RA, Todd J-C, et al. A comparison of the risks of venous thromboembolic disease in association with different combined oral contraceptives. *Br J Clin Pharmacol.* 2000;49:580–590.
9. Lidegaard Ø, Lokkegaard E, Svendsen AL. Hormonal contraception and risk of venous thromboembolism: national follow-up study. *BMJ.* 2009;339:b2890.
10. Van Hylckama V, Helmerhost FM, Vandenbroucke JP, et al. The venous thrombotic risk of oral contraceptives, effects of oestrogen dose and progestogen type: results of the MEGA case-control study. *BMJ.* 2009;339:b2921.
11. Naess IA, Christiansen SC, Romundstad P, et al. Incidence and mortality of venous thrombosis: a population-based study. *J Thromb Haemost.* 2007;5:692–699.
12. Gerstman BB, Piper JM, Tomita DK. Oral contraceptive estrogen dose and the risk of deep venous thromboembolic disease. *Am J Epidemiol.* 1991;133:32–37.
13. Odlin V, Milsom I, Persson I, et al. Can changes in sex hormone binding globulin predict the risk of venous thromboembolism with combined oral contraceptive pills? *Acta Obstet Gyn Scan.* 2002;81:482–490.
14. Farmer RDT, Lawrenson RA, Thompson CR, et al. Population-based study of risk of venous thromboembolism associated with various oral contraceptives. *Lancet.* 1997;349:83–88.
15. Kemmeren JM, Algra A, Grobbee DE. Third generation oral contraceptives and risk of venous thrombosis: meta-analysis. *Br Med J.* 2001;323:131–134.
16. Petitti DB. Clinical practice. Combination estrogen-progestin oral contraceptives. *N Engl J Med.* 2003;349:1443–1450.
17. Jick SS, Hernandez RK. Risk of non-fatal venous thrombo - embolism in women using oral contraceptives containing drospirenone compared with women using oral contraceptives containing levonorgestrel: case–control study using United States claims data. *BMJ.* 2011;342:d2151.
18. Parkin L, Sharples K, Hernandez RK, et al. Risk of venous thromboembolism in users of oral contraceptives containing drospirenone or levonorgestrel: nested case–control study based on UK General Practice Research Database. *BMJ.* 2011;342:d2139.
19. Dinger JC, Heinemann LA, Kühl-Habich D. The safety of a drospirenone-containing oral contraceptive: final results from the European Active Surveillance Study on oral contraceptives based on 142 475 women-years of observation. *Contraception.* 2007;75:344–354.
20. Seeger JD, Loughlin J, Eng PM, et al. Risk of thromboembolism in women taking ethinylestradiol/drospirenone and other oral contraceptives. *Obstet Gynecol.* 2007;110:587–593.
21. Lawrie TA, Helmerhorst FM, Maitra NK, et al. Types of progestogens in combined oral contraception: effectiveness and side-effects. *Cochrane Database Syst Rev.* 2011;5:CD004861.
22. Stocco B, Fumagalli HF, Franceschini SA, et al. Drospirenone and levonorgestrel in combination with either 30 or 20 mcg ethinylestradiol reduce soluble adhesion molecules in Brazilian women; cross-sectional study. *Contraception.* 2012;86:506–510.
23. Kluft C, Endrikat J, Mulder MS, et al. A prospective study on the effects on hemostasis of two oral contraceptives containing drospirenone in combination with either 30 or 20 (g ethinylestradiol and a reference containing desogestrel and 30 (g ethinylestradiol. *Contraception.* 2006;73:336–343.

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

24. Guder WG, Ehret W, da Fonseca-Wollheim F, et al. The quality of diagnostic samples. *J Lab Med.* 2002;26:267–283.

25. Kluft C. Renewed interest in haemostasis changes induced by oral contraceptives (OCs). *Thromb Haemostasis.* 2000;84:1–3.

26. Klipping C, Marr J. Effects of two combined oral contraceptives containing ethinyl estradiol 20 (g combined with either drospirenone or desogestrel on lipids, hemostatic parameters and carbohydrate metabolism. *Contraception.* 2005;71:409–416.

27. Tchaikovski NS, Rosing J. Mechanisms of estrogen-induced venous thromboembolism. *Thromb Res.* 2010;126:5–11.

28. The writing group for the pepi trial. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: the postmenopausal estrogen/progestin intervations (PEPI) trial. *JAMA.* 1995;203:199–208.

29. Crosignani PG. ESHRE Capri Workshop Group. Venous thromboembolism in women: a specific reproductive health risk. *Hum Reprod Update.* 2013;19:471–482.

30. Lidegaard O, Edström B, Kreiner S. Oral contraceptives and venous thromboembolism. A five-year national case-control study. *Contraception.* 2002;65:187–196.

31. JM, Algra A, Meijers JC, et al. Effect of second- and third-generation oral contraceptives on the protein C system in the absence or presence of the factor V Leiden mutation: a randomized trial. *Blood.* 2004;103:927–933.

32. Boucheix C. Monoclonal antibodies for domains which are differentially expressed in fibrinogen, fibrinogen degradation products or in fibrin degradation products. In: Pesters H, ed. *Protides of biological fluids.* 30 Pergamon Press; 1982:399–452.

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.