Effectiveness and side effects of hormonal contraceptives

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CHAPTER 1

General introduction

Effectiveness and side effects of hormonal contraceptives

Shortly after the introduction of the contraceptive pill in 1960, the first case of venous thrombosis during use of an oral contraceptive was reported (1;2). The incidence of venous thrombosis in the general population is low, approximately 3 per 10 000 women-years among women in reproductive age. Despite the low incidence, use of oral contraceptives frequently causes venous thrombosis since millions of women worldwide use an oral contraceptive (3;4). Overall, combined oral contraceptive use is associated with a two- to six-fold increased risk of venous thrombosis (5-7). The aim of this thesis is to study the risk of venous thrombosis during use of different hormonal contraceptives, focusing on the pathogenesis, the evaluation of a marker to estimate the risk of venous thrombosis, and the assessment of effectiveness, side effects including the risk of venous thrombosis and acceptability of a new hormonal contraceptive.

Hormonal contraceptives and venous thrombosis

After the introduction of the first oral contraceptive pill, new prescriptions were developed to lower side effects like venous thrombosis, while maintaining the benefits of contraception and cycle control. The attempts that have been made to achieve this can be classified in four categories:

1. Lowering the hormone dose,
2. New formulas and schedules,
3. New steroids and
4. New routes of administration.

Lowering the hormone dose

Because the estrogen compound in combined oral contraceptives was thought to cause the increased risk of venous thrombosis, the estrogen dose was stepwise reduced from 150 µg to 80 µg, 50 µg, 30 µg, to 20 µg currently (8-11). Lowering this dose reduced the risk of venous thrombosis (5;8;9;12). The currently most prescribed combined oral contraceptives contain 20-30 µg estrogen in the form of ethinylestradiol.

New formulas and schedules of administration

Furthermore, new formulas and schedules of administration were introduced. The first oral contraceptives contained a fixed dose of estrogen and progestogen in a 21-day schedule, the monophasic contraceptives. The multiphasic approach (biphasic and triphasic) was developed to mimic the natural cycle and improve acceptability (13). In these schedules, estrogens and progestogens are administered in varying dosages in two or three phases during the cycle. The manufacturer claimed that the multiphasic approach imitates the natural menstrual cycle resulting in a better bleeding profile (14). However, the biphasic and triphasic preparations were evaluated and

compared with monophasic contraceptives in three systematic reviews which showed that the multiphasic approach had no advantages over the monophasic approach in contraceptive effectiveness, side effects and cycle control and continuation rates (15-17). The thrombotic safety of multiphasic oral contraceptives compared with monophasic oral contraceptives was studied by Lidegaard *et al*. They compared monophasic with biphasic oral contraceptives containing levonorgestrel and ethinylestradiol and found no significant differences in thrombotic safety (adjusted rate ratio 1.07, 95% CI 0.75 to 1.52) (18).

New steroids

Another attempt to lower side effects of oral contraceptives was adjusting the chemical structure of progestogens. Progestogens were originally classified as first, second or third generation progestogens, based on the order of introduction and depending on their chemical structure. First generation progestogens (norethynodrel, norethisterone and lynestrenol) were developed in the 1960s and are derived from the estrane steroids. Second generation (norgestrel and levonorgestrel) and third generation (desogestrel and gestodene) progestogens were developed in the 1970s and 1980s and are derived from gonane steroids. Dienogest and drospirenone have been synthesized in the last 20 years and may be considered as fourth generation progestogens in a temporal fashion, although they do not share a common chemical structure (19). Drospirenone is derived from 17α-spironolactone, and was marketed internationally in 2000 (20). Dienogest is derived from the estrane steroids and was firstly marketed in 1995 in Germany, but only available in the Netherlands since 2008 (20). Cyproterone acetate is not included in the classification by generations. It was developed in the late 1980s and is derived from pregnane steroids. Since the characteristics of progestogens differ even within the generation groups, and the risk of venous thrombosis is dependent on the type of the progestogen instead of the generation, it is probably best to abstain from any classification of progestogens (21).

In 1995, three studies reported a two-fold increased risk of venous thrombosis in women using combined oral contraceptives containing the third generation progestogens gestodene and desogestrel compared with women using second generation oral contraceptives (5;7;22-26). Subsequently, cyproterone acetate containing oral contraceptives were also reported to have a two-fold increased risk compared with second generation combined oral contraceptives (23). In 2009, the combined oral contraceptive containing drospirenone was found to increase the risk of venous thrombosis 1.7-fold compared with second generation oral contraceptives (23). Both oral contraceptives showed altered concentrations of coagulation factors and increased APC resistance before the increased risk of venous thrombosis was identified in clinical trials. The ORs of different combined oral contraceptives known from recent literature are summarized in table 1.

Recently, a new combined oral contraceptive was marketed with a new estrogen: estradiol valerate (Qlaira ®, 2009, Bayer Schering Pharma, Berlin, Germany), which is an ester of the natural female hormone 17ß-estradiol. The progestogen compound is dienogest, a fourth generation progestogen. The oral contraceptive is administered in a four-phasic (quadriphasic) schedule (20;27), with

an estrogen step-down and a progestogen step-up scheme and contains 26 acting tablets and two placebos on days 27 and 28 of the cycle. The manufacturer claimed that estradiol valerate and the quadriphasic scheme is more physiological, mimics the natural cycle and should therefore lead to fewer side effects and better acceptability (28). In *chapter 2* the results of a Cochrane systematic review are presented in which the quadriphasic schedule of the combined oral contraceptive containing dienogest and estradiol valerate was compared with monophasic oral contraceptives, in terms of effectiveness, bleeding pattern, side effects and acceptability.

New routes of administration

A third attempt to lower side effects was to develop new routes of administration of hormones, such as the vaginal ring and the transdermal patch. The rationale behind non-oral administration was a continuous release of hormones which bypass the first-pass liver effect. This should allow a lower estrogen and progestogen dose, less loss of bioavailability and fewer peaks in serum levels resulting in increased acceptability (29-34).

The levonorgestrel-containing intra-uterine device (LNG-IUD, Mirena®, Bayer Schering Pharma, Berlin, Germany) is also a non-oral hormonal contraceptive, and available since 1990. It is inserted in the uterine cavity for a maximum of five years. It contains 52 mg levonorgestrel which is released continuously; the daily dose released in vivo is 14-20 µg per day and the circulating levonorgestrel plasma levels are 150-200 pg/mL. Use of levonorgestrel-only contraceptive pills results in higher levonorgestrel plasma levels of 800 pg/mL (35-37). The LNG-IUD showed no increased risk of venous thrombosis; reported odds ratios (ORs) vary from 0.3 to 0.57 (Table 1) (38;39).

The transdermal patch (Ortho-Evra ®/Evra®, Ortho-McNeil-Janssen Pharmaceuticals, Raritan, NJ, USA) was marketed in 2002. It is a thin adhesive square of 20 cm^2 which is applied on the skin (29;40) and continuously releases a daily dose of 203 µg norelgestromin (the active metabolite of norgestimate, a third generation progestogen (41)) and 33.9 µg ethinylestradiol for one week. According to the prescription, the patch is used in three consecutive weeks, followed by a patch-free interval of one week in which a withdrawal bleeding may occur (40).

The vaginal ring (NuvaRing®, Organon, Oss, The Netherlands) was marketed in 2001 and contains etonogestrel (a third generation progestogen) and ethinylestradiol. It is a flexible, soft, 4 mm thick ring of 5.4 cm in diameter which can easily be inserted into and removed from the vagina by the woman herself (31;42). The ring contains 11.7 mg etonogestrel and 2.7 mg ethinylestradiol and continuously releases a daily dose of 120 µg etonogestrel and 15 µg ethinylestradiol over a period of 3 weeks (42).

Despite attempts to reduce side effects such as venous thrombosis, the transdermal patch and vaginal ring did not lead to a lower thrombotic risk than with use of oral contraceptives containing levonorgestrel: the Food and Drug Administration (FDA) reported an OR of 7.9 (95% CI 3.5 to 17.7) during use of the transdermal patch and an OR of 6.5 (95% CI 4.7 to 8.9) during use of

the vaginal ring (Table 1) (43) compared with four low-estrogen combined hormonal contraceptives. These increased risks were later confirmed by Lidegaard *et al.*, in a large Danish cohort (39). The high thrombotic risk of the vaginal ring may be caused by its release of a third generation progestogen which is associated with an increased thrombotic risk, as described above. During use of the transdermal patch, 60% higher estrogen levels were measured than with a combined oral contraceptive containing the same steroids, which is associated with a higher risk of venous thrombosis (40;44).

Table 1: *Odds ratios and 95% confidence intervals of hormonal contraceptives. All oral contraceptives All for preparations that contain 30-37.5 µg ethinylestradiol.*

Contraceptive		OR	95% CI	Reference
Non use (reference)		1.0	-	-
Combined oral contraceptive	Lynestrenol	5.6	3.0 – 10.2	(23)
	Norethisterone	3.9	1.4 – 10.6	(23)
	Levonorgestrel	3.2	2.5 – 3.9	(21)
	Desogestrel	5.5	4.4 – 6.8	(21)
	Gestodene	4.6	3.6 – 5.9	(21)
	Norgestimate	3.9	2.9 – 5.4	(21)
	Cyproterone Acetate	5.5	3.9 – 7.7	(21)
	Drospirenone	6.0	4.1 – 8.9	(21)
Transdermal Patch	Norelgestromin	7.9	3.5 – 17.7	(43)
Vaginal ring	Etonogestrel	6.5	4.7 – 8.9	(43)
Levonorgestel - IUD	Levonorgestrel	0.6	0.4 – 0.8	(38;39)

Biological explanation

The exact biological mechanism of the increased risk of venous thrombosis during oral contraceptive use and the differences between the different oral contraceptives is unknown. Various studies showed altered levels of clotting factors during use of combined oral contraceptives. Increased levels of the procoagulant factors prothrombin, factor VII, VIII, IX, X, XI, XII, von Willebrand factor and fibrinogen, and decreased levels of the anticoagulant factors protein S and antithrombin were observed (45-48). The interpretation of the changes in clotting factors differed between researchers, since the net effect of changes of the haemostatic system was not known.

APC resistance

A major step forward in the biological understanding of the increased risk of venous thrombosis during use of oral contraceptives was the observation that oral contraceptives induce an acquired form of resistance to Activated Protein C (APC) (49;50). APC is a physiological anticoagulant which inactivates factor V and thereby inhibits coagulation. APC resistance is the relative inability of activated protein C to cleave activated factor V or factor VIII, leading to a prothrombotic state.

It was first described by Dählback *et al*. in a family with a hereditary tendency for venous thrombosis (51). A year later, Bertina *et al*. identified the factor V Leiden mutation (replacement of the amino acid Arg506 by Gln), which is the most common form of hereditary APC resistance (52). APC resistance not caused by the factor V Leiden mutation is also an important risk factor for venous thrombosis (53;54).

APC resistance was originally measured by the aPTT-based APC resistance test, which measures the effect of APC on the clotting time of plasma in which coagulation is initiated via the intrinsic pathway (55). In 1995 the thrombin generation-based APC resistance test was developed by Rosing and Hemker (56;57). The test quantifies the effect of APC on the time integral of thrombin formation in plasma in which coagulation is initiated via the extrinsic coagulation pathway. The test is a global assay, which includes the effects on individual clotting factors (procoagulant and anticoagulant factors) and combines them into a net effect (56;57). Women using combined oral contraceptives were found to be resistant to APC by this thrombin generation-based APC resistant test. More importantly, differences in APC resistance between users of second and third generation combined oral contraceptives were shown, and the test provided an explanation for the differences in venous thrombosis risk between the oral contraceptives (58;59). The thrombin generation-based APC resistance test was validated in the Leiden Thrombophilia Study and predicts the risk of venous thrombosis in users of combined oral contraceptives as well as in non-users and men (60).

TFPI and Protein S

The two main determinants of the thrombin generation-based APC resistance test are free Protein S and free Tissue Factor Pathway Inhibitor (TFPI) (61-63). Hereditary and acquired deficiencies of Protein S and low plasma levels of TFPI are associated with an increased risk of venous thrombosis (64-67). Moreover, Protein S-deficient individuals also have decreased TFPI levels, probably due to common mechanisms regulating biosynthesis of both proteins (64). Van Vliet *et al*. observed that women using combined oral contraceptives with the highest risk of venous thrombosis (i.e. containing desogestrel, cyproterone acetate or drospirenone) had lower free Protein S and free TFPI levels compared with women using the combined oral contraceptive with the lowest risk of venous thrombosis (i.e. containing levonorgestrel). The study concluded that the differences in APC resistance, induced by combined oral contraceptives, can at least partially be explained by differences in free Protein S and free TFPI (61).

Chapter 3 describes a study which evaluates whether the different risks of venous thrombosis caused by different hormonal and non-hormonal methods of contraception are reflected in the levels of free TFPI and free Protein S. The association of these levels with APC resistance measured by the thrombin generation-based APC resistance test, and with relative risks as reported in the literature is examined.

Predicting thrombotic risk by use of markers

When a new hormonal contraceptive is introduced, an estimation of the thrombotic risk is required in order not to expose women to an unnecessary high risk of venous thrombosis by prescribing this new contraceptive. As the result of the high number of participants required for a clinical study due to the low incidence of venous thrombosis, assessment of the thrombotic risk of new contraceptives in clinical preregistration studies is unfeasible. In order to demonstrate a doubling of the risk of venous thrombosis between two different combined hormonal contraceptives with a power of 80% and a significance level of 5%, a cohort of approximately 500 000 women would need to be followed for 1 year (68).

To predict the risk of venous thrombosis of a new hormonal contraceptive before market authorization, one can revert to a study with markers. A marker is a proxy, usually a laboratory test, for a clinical outcome. They make it possible to perform a study with smaller sample sizes and shorter observation periods than necessary for true clinical outcomes. Preferably, a marker is validated in a study in which both the marker and the clinical endpoint are assessed (69). An important, and validated marker for venous thrombosis during use of hormonal contraceptives is APC resistance, measured by the thrombin generation-based APC resistance test (expressed in normalized APC sensitivity ratios, nAPCsr), since it differentiates well between "low-risk" and "high-risk" oral contraceptives (60).

In search of other markers, Odlind *et al*. suggested Sex Hormone-Binding Globulin (SHBG) as marker of "estrogenicity" and thereby venous thrombosis (70). SHBG is a carrier protein produced in the liver and transports testosterone and estrogen. The effect of an oral contraceptive on SHBG levels can be interpreted as the sum of the dose-related estrogenic effect of ethinylestradiol and the dose- and type-related anti-estrogenic effect of the progestogen, resulting in the total "estrogenicity" of the oral contraceptive (70). In two observational studies, an association between APC resistance and SHBG levels was found in users of different hormonal contraceptives and non-users which support the "estrogenicity" hypothesis (71;72).

To determine whether SHBG is a useful marker for the risk of venous thrombosis of combined oral contraceptives, we conducted an observational study assessing SHBG levels in non-users, users of the hormonal and non-hormonal IUD, and users of different oral contraceptives. The results of the comparison between the SHBG levels, nAPCsr, and the risks of venous thrombosis as reported in the literature are described in *chapter 4*. Besides, we investigated whether an ethinylestradiol-dose related increase in SHBG levels is present during use of different combined oral contraceptives, which is described in *chapter 5*.

The absolute and relative risk of venous thrombosis during use of the above mentioned recently introduced combined oral contraceptive is unknown. *Chapter 6* describes a randomized controlled trial in which APC resistance and SHBG levels during use of the combined oral contraceptives containing dienogest/estradiol valerate and levonorgestrel/ethinylestradiol were compared.

Thyroid parameters and venous thrombosis

There are many risk factors for venous thrombosis, which can be broadly defined in those related to immobilization and to changes in coagulability. The latter can be divided in genetic abnormalities in the coagulation system, and acquired factors, amongst which are cancer and use of oral contraceptives. Besides the use of hormonal contraceptives, hyperthyroidism is also associated with an increased risk of venous thrombosis (73-77) and causes a hypercoagulable state with increased levels of procoagulant and anticoagulant factors. Hyperthyroidism increases antifibrinolysis and induces changes in the inflammatory pathway through complement C3 which induces a hypercoagulable state (45;77). The hypercoagulable state is probably due to high free Thyroxine (T4) levels which influence the coagulation system (73-75).

During use of hormonal contraceptives, Thyroxine-Binding Globulin (TBG) levels are increased (70;78-83). TBG is a hepatic globulin which transports thyroid hormones. Higher TBG levels lead to higher total T4 and total Tri-iodothyronine (T3) levels. Like SHBG, TBG levels are associated with the increased risk of venous thrombosis during use of hormonal contraceptives (70;71). Whether the increased levels of thyroid parameters during use of hormonal contraceptives are associated with the increased risk of venous thrombosis during use of these contraceptives has not yet been studied.

In *chapter 7* we questioned whether there is an association between the levels of TBG, FT4 and TSH during use of hormonal contraceptives and the risk of venous thrombosis. The study examines whether an association can be found between thyroid parameters and APC resistance measured with the thrombin generation-based APC resistance test and the thrombotic risks as reported in the literature.

References

1 Christin-Maitre S. History of oral contraceptive drugs and their use worldwide. Best Pract Res Clin Endocrinol Metab 2013, 27:3-12.

2 Jordan W. Pulmonary embolism. Lancet 1961, 278:1146-7.

3 Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrom J. Incidence and mortality of venous thrombosis: a population-based study. J Thromb Haemost 2007, 5:692-9.

4 United Nations,Department of Economic and Social Affairs,Population Division, World Contraceptive Use 2011, www.unpopulation.org.

5 Cardiovascular disease and steroid hormone contraception. Report of a WHO Scientific Group. 877:1-89. 1998.

6 Rosendaal FR, Van Hylckama Vlieg A, Tanis BC, Helmerhorst FM. Estrogens, progestogens and thrombosis. J Thromb Haemost 2003 1:1371-80.

7 Vandenbroucke JP, Rosing J, Bloemenkamp KWM, Middeldorp S, Helmerhorst FM, Bouma BN, Rosendaal FR. Oral contraceptives and the risk of venous thrombosis. N Eng J Med 2001, 344:1527-34.

8 Inman WH, Vessey MP, Westerholm B, Engelund A. Thromboembolic disease and the steroidal content of oral contraceptives. A report to the Committee on Safety of Drugs. Br Med J 1970, 2:203-9.

9 Vessey M, Mant D, Smith A, Yeates D. Oral contraceptives and venous thromboembolism: findings in a large prospective study. Br Med J (Clin Res Ed) 1986, 292:526.

10 Thorogood M, Villard-Mackintosh L. Combined oral contraceptives: risks and benefits. Br Med Bull 1993, 49:124-39.

11 Wharton C. Lower dose pills. Population Rep 1988;16:1-31.

12 Lidegaard O, Edstrom B, Kreiner S. Oral contraceptives and venous thromboembolism: a five-year national case-control study. Contraception 2002, 65:187-96.

13 Speroff L, Darney PD. Oral contraception. In: Speroff L, Darney PD, editors. A clinical guide for contraception. Philadephia: Lippincott Williams&Wilkins; 2001. p. 21-138.

14 Upton GV. The phasic approach to oral contraception: the triphasic concept and its clinical application. Int J Fertil 1983, 28:121-40.

15 van Vliet HA, Grimes DA, Lopez LM, Schulz KF, Helmerhorst FM. Triphasic versus monophasic oral contraceptives for contraception. Cochrane Database Syst Rev 2006:CD003553.

16 van Vliet HA, Grimes DA, Helmerhorst FM, Schulz KF. Biphasic versus triphasic oral contraceptives for contraception. Cochrane Database Syst Rev 2006:CD003283.

17 van Vliet HA, Grimes DA, Helmerhorst FM, Schulz KF. Biphasic versus monophasic oral contraceptives for contraception. Cochrane Database Syst Rev 2006:CD002032.

18 Lidegaard O, Nielsen LH, Skovlund CW, Skjeldestad FE, Lokkegaard E. Risk of venous thromboembolism from use of oral contraceptives containing different progestogens and oestrogen doses: Danish cohort study, 2001-9. BMJ 2011, 343:d6423.

19 Sitruk-Ware R. Pharmacological profile of progestins. Maturitas 2004, 47:277-83.

20 Henzl MR, Edwards JA. 17alpha-hydroxyprogesterone derivates and progestins of the first and second generation. In: Sitruk-Ware R, Mishell DR, editors. Progestins and antiprogestins in clinical practice. New York: Marcel Dekker; 2000. p. 101-32.

21 Stegeman BH. Hormonal contraceptives and venous thrombosis. Thesis, 2013.

22 Lidegaard O, Lokkegaard E, Svendsen AL, Agger C. Hormonal contraception and risk of venous thromboembolism: national follow-up study. BMJ 2009, 339:b2890.

23 Van Hylckama Vlieg A, Helmerhorst FM, Vandenbroucke JP, Doggen CJ, Rosendaal FR. 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.

24 Bloemenkamp KW, Rosendaal FR, Helmerhorst FM, Buller HR, Vandenbroucke JP. Enhancement by factor V Leiden mutation of risk of deep-vein thrombosis associated with oral contraceptives containing a third-generation progestagen. Lancet 1995, 346:1593-6.

25 Jick H, Jick SS, Gurewich V, Myers MW, Vasilakis C. Risk of idiopathic cardiovascular death and nonfatal venous thromboembolism in women using oral contraceptives with differing progestagen components. Lancet 1995, 346:1589-93.

26 Kemmeren JM, Algra A, Grobbee DE. Third generation oral contraceptives and risk of venous thrombosis: meta-analysis. BMJ 2001, 323:131-4.

27 Wellington K, Perry CM. Estradiol valerate/dienogest. Drugs 2002, 62:491-504.

28 Qlaira website http://www.qlaira.com. 2013.

29 Burkman RT. The transdermal contraceptive system. Am J Obstet Gynecol 2004, 190:S49-S53.

30 Rowlands S. New technologies in contraception. BJOG 2009, 116:230-9.

31 Roumen FJ. The contraceptive vaginal ring compared with the combined oral contraceptive pill: a comprehensive review of randomized controlled trials. Contraception 2007, 75:420-9.

32 Lopez LM, Grimes DA, Gallo MF, Schulz KF. Skin patch and vaginal ring versus combined oral contraceptives for contraception. Cochrane Database Syst Rev 2008:CD003552.

33 Johansson ED, Sitruk-Ware R. New delivery systems in contraception: vaginal rings. Am J Obstet Gynecol 2004, 190:S54-S59.

34 van den Heuvel MW, van Bragt AJ, Alnabawy AK, Kaptein MC. Comparison of ethinylestradiol pharmacokinetics in three hormonal contraceptive formulations: the vaginal ring, the transdermal patch and an oral contraceptive. Contraception 200,5 72:168-74.

35 Shulman LP, Nelson AL, Darney PD. Recent developments in hormone delivery systems. Am J Obstet Gynecol 2004, 190:S39-S48.

36 Jensen JT. Contraceptive and therapeutic effects of the levonorgestrel intrauterine system: an overview. Obstet Gynecol Surv 2005, 60:604-12.

37 Mirena website, http://www.mirena-us.com. 2013.

38 Van Hylckama Vlieg A, Helmerhorst FM, Rosendaal FR. The risk of deep venous thrombosis associated with injectable depot-medroxyprogesterone acetate contraceptives or a levonorgestrel intrauterine device. Arterioscler Thromb Vasc Biol 2010, 30:2297-300.

39 Lidegaard O, Nielsen LH, Skovlund CW, Lokkegaard E. Venous thrombosis in users of non-oral hormonal contraception: follow-up study, Denmark 2001-10. BMJ 2012, 344:e2990.

40 Ortho-McNeil-Janssen Pharmaceuticals. US Product Information Ortho-Evra®. http://www.orthoevra.com. 2009.

41 Gaspard UJ. Progestogens in contraception: third generation pills. In: Sitruk-Ware R and Mishell DR, editors. Progestins and antiprogestins in clinical practice. New York, Marcel Dekker. 2000, p 179-216.

42 NuvaRing website, http://www.nuvaring.com. 2013.

43 Combined Hormonal Contraceptives (CHC's) and the Risk of Cardiovascular Disease Endpoints; Food and Drug Administration, http://www.fda.gov/downloads/Drugs/DrugSafety/UCM277384.pdf. 2012.

44 Devineni D, Skee D, Vaccaro N, Massarella J, Janssens L, LaGuardia KD, Leung AT. Pharmacokinetics and pharmacodynamics of a transdermal contraceptive patch and an oral contraceptive. J Clin Pharmacol 2007, 47:497-509.

45 Tanis BC, Rosendaal FR. Venous and arterial thrombosis during oral contraceptive use: risks and risk factors. Semin Vasc Med 2003, 3:69-84.

46 Kluft C, Lansink M. Effect of oral contraceptives on haemostasis variables. Thromb Haemost 1997, 78:315-26.

47 Winkler UH. Blood coagulation and oral contraceptives. A critical review. Contraception 1998, 57:203-9.

48 Rosing J. Mechanisms of OC related thrombosis. Thromb Res 2005, 115 Suppl 1:81-3.

49 Olivieri O, Friso S, Manzato F, Guella A, Bernardi F, Lunghi B, Girelli D, Azzini M, Brocco G, Russo C, . Resistance to activated protein C in healthy women taking oral contraceptives. Br J Haematol 1995, 91:465-70.

50 Henkens CM, Bom VJ, Seinen AJ, van der Meer J. Sensitivity to activated protein C; influence of oral contraceptives and sex. Thromb Haemost 1995, 73:402-4.

51 Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. Proc Natl Acad Sci U S A 1993, 90:1004-8.

52 Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, De Ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994, 369:64-7.

53 de Visser MC, Rosendaal FR, Bertina RM. A reduced sensitivity for activated protein C in the absence of factor V Leiden increases the risk of venous thrombosis. Blood 1999, 93:1271-6.

54 Rodeghiero F, Tosetto A. Activated protein C resistance and factor V Leiden mutation are independent risk factors for venous thromboembolism. Ann Intern Med 1999, 130:643-50.

55 Curvers J, Thomassen MC, Nicolaes GA, Van Oerle R, Hamulyak K, Hemker HC, Tans G, Rosing J. Acquired APC resistance and oral contraceptives: differences between two functional tests. Br J Haematol 1999, 105:88-94.

56 Nicolaes GA, Thomassen MC, Van Oerle R, Hamulyak K, Hemker HC, Tans G, Rosing J. A prothrombinase-based assay for detection of resistance to activated protein C. Thromb Haemost 1996, 76:404-10.

57 Hemker HC, Beguin S. Thrombin generation in plasma: its assessment via the endogenous thrombin potential. Thromb Haemost 1995, 74:134-8.

58 Rosing J, Tans G, Nicolaes GA, Thomassen MC, van Oerle R., van der Ploeg PM, Heijnen P, Hamulyak K, Hemker HC. Oral contraceptives and venous thrombosis: different sensitivities to activated protein C in women using second- and third-generation oral contraceptives. Br J Haematol 1997, 97:233-8.

59 Rosing J, Middeldorp S, Curvers J, Christella M, Thomassen LG, Nicolaes GA, Meijers JC, Bouma BN, Buller HR, Prins MH, Tans G. Low-dose oral contraceptives and acquired resistance to activated protein C: a randomised cross-over study. Lancet 1999, 354:2036-40.

60 Tans G, Van Hylckama Vlieg A, Thomassen MC, Curvers J, Bertina RM, Rosing J, Rosendaal FR. Activated protein C resistance determined with a thrombin generation-based test predicts for venous thrombosis in men and women. Br J Haematol 2003, 122:465-70.

61 van Vliet HA, Bertina RM, Dahm AE, Rosendaal FR, Rosing J, Sandset PM, Helmerhorst FM. Different effects of oral contraceptives containing different progestogens on protein S and tissue factor pathway inhibitor. J Thromb Haemost 2008, 6:346-51.

62 Hoibraaten E, Mowinckel MC, de Ronde H., Bertina RM, Sandset PM. Hormone replacement therapy and acquired resistance to activated protein C: results of a randomized, double-blind, placebo-controlled trial. Br J Haematol 2001, 115:415-20.

63 de Visser MC, Van Hylckama Vlieg A, Tans G, Rosing J, Dahm AE, Sandset PM, Rosendaal FR, Bertina RM. Determinants of the APTT- and ETP-based APC sensitivity tests. J Thromb Haemost 2005, 3:1488-94.

64 Castoldi E, Simioni P, Tormene D, Rosing J, Hackeng TM. Hereditary and acquired protein S deficiencies are associated with low TFPI levels in plasma. J Thromb Haemost 2010, 8:294-300.

65 Comp PC, Esmon CT. Recurrent venous thromboembolism in patients with a partial deficiency of protein S. N Engl J Med 1984, 311:1525-8.

66 Schwarz HP, Fischer M, Hopmeier P, Batard MA, Griffin JH. Plasma protein S deficiency in familial thrombotic disease. Blood 1984, 64:1297-300.

67 Dahm A, Van Hylckama Vlieg A, Bendz B, Rosendaal F, Bertina RM, Sandset PM. Low levels of tissue factor pathway inhibitor (TFPI) increase the risk of venous thrombosis. Blood 2003, 101:4387-92.

68 WHO. Medical eligibility criteria for contraceptive use. 4th ed. Geneva: WHO; 2009. Available at http://www.who.int/reproductivehealth/publications/family_planning/9789241563888/en/index.html.

69 Stanczyk FZ, Grimes DA. Sex hormone-binding globulin: not a surrogate marker for venous thromboembolism in women using oral contraceptives. Contraception 2008, 78:201-3.

70 Odlind V, Milsom I, Persson I, Victor A. Can changes in sex hormone binding globulin predict the risk of venous thromboembolism with combined oral contraceptive pills? Acta Obstet Gynecol Scand 2002, 81:482-90.

71 van Vliet HA, Frolich M, Christella M, Thomassen LG, Doggen CJ, Rosendaal FR, Rosing J, Helmerhorst FM. Association between sex hormone-binding globulin levels and activated protein C resistance in explaining the risk of thrombosis in users of oral contraceptives containing different progestogens. Hum Reprod 2005, 20:563-8.

72 Fleischer K, van Vliet HA, Rosendaal FR, Rosing J, Tchaikovski S, Helmerhorst FM. Effects of the contraceptive patch, the vaginal ring and an oral contraceptive on APC resistance and SHBG: a cross-over study. Thromb Res 2009, 123:429-35.

73 Debeij J, Cannegieter SC, Van Zaane B., Smit JW, Corssmit EP, Rosendaal FR, Romijn JA, Dekkers OM. The effect of changes in thyroxine and thyroid-stimulating hormone levels on the coagulation system. J Thromb Haemost 2010, 8:2823-6.

74 Debeij J, Dekkers OM, Asvold BO, Christiansen SC, Naess IA, Hammerstrom J, Rosendaal FR, Cannegieter SC. Increased levels of free thyroxine and risk of venous thrombosis in a large population-based prospective study. J Thromb Haemost 2012, 10:1539-46.

75 Van Zaane B., Squizzato A, Huijgen R, van Zanten AP, Fliers E, Cannegieter SC, Buller HR, Gerdes VE, Brandjes DP. Increasing levels of free thyroxine as a risk factor for a first venous thrombosis: a case-control study. Blood 2010, 115:4344-9.

76 Stuijver DJ, Van Zaane B., Romualdi E, Brandjes DP, Gerdes VE, Squizzato A. The effect of hyperthyroidism on procoagulant, anticoagulant and fibrinolytic factors. A systematic review and meta-analysis. Thromb Haemost 2012, 108:1077-88.

77 Van Zaane B., Squizzato A, Debeij J, Dekkers OM, Meijers JC, van Zanten AP, Buller HR, Gerdes VE, Cannegieter SC, Brandjes DP. Alterations in coagulation and fibrinolysis after levothyroxine exposure in healthy volunteers: a controlled randomized crossover study. J Thromb Haemost 2011, 9:1816-24.

78 Wiegratz I, Kutschera E, Lee JH, Moore C, Mellinger U, Winkler UH, Kuhl H. Effect of four different oral contraceptives on various sex hormones and serum-binding globulins. Contraception 2003, 67:25-32.

79 Sanger N, Stahlberg S, Manthey T, Mittmann K, Mellinger U, Lange E, Kuhl H, Wiegratz I. Effects of an oral contraceptive containing 30 mcg ethinyl estradiol and 2 mg dienogest on thyroid hormones and androgen parameters: conventional vs. extended-cycle use. Contraception 2008, 77:420-5.

80 Kuhl H, Jung-Hoffmann C, Weber J, Boehm BO. The effect of a biphasic desogestrel-containing oral contraceptive on carbohydrate metabolism and various hormonal parameters. Contraception 1993, 47:55-68.

81 Agren UM, Anttila M, Maenpaa-Liukko K, Rantala ML, Rautiainen H, Sommer WF, Mommers E. Effects of a monophasic combined oral contraceptive containing nomegestrol acetate and 17beta-oestradiol in comparison to one containing levonorgestrel and ethinylestradiol on markers of endocrine function. Eur J Contracept Reprod Health Care 2011, 16:458-67.

82 White T, Ozel B, Jain JK, Stanczyk FZ. Effects of transdermal and oral contraceptives on estrogen-sensitive hepatic proteins. Contraception 2006, 74:293-6.

83 Duijkers I, Killick S, Bigrigg A, Dieben TO. A comparative study on the effects of a contraceptive vaginal ring NuvaRing and an oral contraceptive on carbohydrate metabolism and adrenal and thyroid function. Eur J Contracept Reprod Health Care 2004, 9:131-40.