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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 17 α -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).

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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 17 β -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 cm² 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)

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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).

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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).

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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.

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