Hormonal contraceptives : effectiveness and adverse effects

Vliet, H.A.A.M. van

Chapter 12

Summary, Discussion and Recommendations

SUMMARY AND DISCUSSION

Objectives of the thesis

Since the introduction of the first contraceptive pill in 1959, the development of new hormonal contraceptives has focused on maintaining the benefits of oral contraceptives while reducing their adverse effects. Four approaches have been used to optimize the risk-benefit profile: (i) lowering of the steroid dose; (ii) development of new formulas and schedules of administration; (iii) development of new steroids and (iv) development of new routes of administration. The *first* objective of this thesis was to compare the multiphasic schedule of administration of oral contraceptives with the classic monophasic schedule of administration in terms of contraceptive effectiveness, bleeding pattern and discontinuation. The *second* objective was to predict the thrombotic risk of oral contraceptives containing the new steroid drospirenone by comparing the thrombin generation-based APC-resistance in users of pills containing drospirenone with the APC-resistance in users of pills containing other progestogens. We also focused on the biological basis of acquired APC-resistance in oral contraceptive users by studying the two main determinants of the thrombin generation-based APC-resistance test, free protein S and tissue factor pathway inhibitor free antigen. In addition, we tested the usefulness of sex hormone binding globulin as a new marker for the thrombotic risk of a hormonal contraceptive. The *third* objective was to estimate the thrombotic risk of contraceptives which administer steroids vaginally, transdermally or intrauterine by assessing the effect of these contraceptives on thrombin generation-based APC-resistance. *At last* we evaluated whether varying levels of estradiol and progesterone during a natural menstrual cycle are associated with differences in APC-resistance.

New formulas and schedules of administration

In **chapter 2** and **chapter 3** we systematically reviewed the literature for randomized controlled trials comparing biphasic or triphasic oral contraceptives with monophasic oral contraceptives and planned to perform a meta-analysis for the outcomes contraceptive effectiveness, bleeding pattern and discontinuation. In **chapter 4** we conducted a systematic review of trials comparing biphasic and triphasic oral contraceptives. The literature search yielded twenty-one trials comparing triphasic regimens with monophasic regimens, one trial comparing biphasic regimens with monophasic regimens and two trials comparing triphasic regimens with biphasic regimens. These studies provided insufficient evidence to determine whether the multiphasic approach differs from the monophasic approach in contraceptive effectiveness, bleeding patterns and discontinuation rates. Nor was it possible to adequately compare the triphasic approach with the biphasic approach. Overall, the reporting of the study methods was limited and the methodological quality of the studies were poor [1-10]. Pooling of the data on contraceptive effectiveness or bleeding in a meta-analysis was generally not possible due to (i) differences in progestogen type, progestogen dosage and estrogen

dosage of the studied contraceptive pills and (ii) differences between the trials in measuring, analyzing and reporting the data on cycle disturbances. The sample sizes of the individual trials were too small to detect differences in contraceptive effectiveness. Some trials included in the reviews reported favorable bleeding patterns, i.e. less spotting, breakthrough bleeding or amenorrhea, in users of triphasic oral contraceptives than in users of monophasic or biphasic oral contraceptives. However, in most of these trials, the progestogen type differed between the two studied oral contraceptives. Since the type of progestogen is thought to affect cycle control, the observed differences in bleeding pattern might be explained by the differences in progestogen content rather than the phasic approach [11,12]. Further, several trials used the proportion of all cycles with spotting, breakthrough bleeding or amenorrhea as effect measure. This measure might give a distorted impression as one does not know whether a few women had all the cycles with bleeding problems or many women had a few cycles with bleeding problems. No differences in intermenstrual bleeding and amenorrhea were observed between biphasic and monophasic oral contraceptives. The three approaches did not differ significantly in the number of women who discontinued due to bleeding disturbances and other minor side effects which can be considered as an indicator of how women tolerated the contraceptive method.

In the absence of proven advantages of multiphasic oral contraceptives, the greater complexity of the multiphasic approach, and the higher costs of multiphasic oral contraceptives we recommend monophasic pills as the first choice for women starting oral contraceptive use. At first prescription, monophasic pills containing 30 µg estrogen are preferred over monophasic pills containing 20 µg estrogen since the latter cause more bleeding problems and discontinuation [13]. Large, adequately reported, high-quality, randomized controlled trials that compare triphasic and monophasic oral contraceptives with identical progestogens are needed to determine whether triphasic pills differ from monophasic pills in contraceptive effectiveness, bleeding patterns and continuation rates. Future studies should follow the recommendations of the Hormonal Contraceptives Trial Methodology Consensus Conference on recording of bleeding patterns [14] and the CONSORT guidelines on reporting of randomized controlled trials [2,3]. Given the limited availability and use of biphasic oral contraceptives, additional trials comparing the biphasic approach with the monophasic approach are of low-priority. Nevertheless, all new contraceptive preparations require comparison of effectiveness, frequent side effects and beneficial effects with the gold standard in adequately reported, high-quality randomized controlled trials. The presence of only two studies which were also of low methodological quality made it impossible to adequately compare triphasic pills with biphasic pills and to recommend one of the two approaches. Considering the sparse use of biphasic oral contraceptives, additional comparative trials are of low importance.

In **chapter 5** we investigated the frequency of quality assessment of randomized controlled trials within systematic reviews and the incorporation of the quality assessment in the

analysis. We included new systematic reviews of at least five trials of therapeutic or preventive interventions that appeared in issue 2, 2003, of the Cochrane Database of Systematic Reviews and all systematic reviews in the 2002 issues of the general and internal medicine journals Annals of Internal Medicine, BMJ, JAMA, and Lancet. Trial quality was assessed in all Cochrane reviews and most of the paper reviews. However, only half of the reviews incorporated the results of the quality assessment in the analysis, with no substantial difference between Cochrane and paper reviews. These findings were confirmed by a similar literature study [15].

Since isolated quality assessments without incorporation of its results in the analysis are futile, we advise authors, peer-reviewers, and editors not to exclusively focus on whether quality assessment has been performed, but also to concentrate on incorporation of quality assessments in the analysis of systematic reviews.

New steroids

In **chapter 6** we assessed the thrombotic profile of a new combined oral contraceptive containing drospirenone by comparing the resistance to APC in users of drospirenone-pills with the resistance to APC in users of pills containing second- or third-generation progestogens or cyproterone acetate. In addition, we measured the resistance to APC in women who switched from a drospirenone-containing pill to a second-generation oral contraceptive containing levonorgestrel or who switched from a second- or third-generation oral contraceptive to the drospirenone-containing pill. The resistance to APC was determined by quantifying the effect of APC on thrombin generation (thrombin generation-based or ETP-based APC-resistance test). The study demonstrated that women using oral contraceptives containing drospirenone were more resistant to APC than women using second-generation oral contraceptives containing levonorgestrel. The APC-resistance in users of drospirenone-containing pills was similar to the APC-resistance in users of pills with an increased risk of thrombosis, i.e. pills containing desogestrel or cyproterone acetate [16-19]. In the group of women who switched from oral contraceptive type the resistance to APC altered correspondingly, so it increased when switching from a levonorgestrel-pill to a drospirenone-pill and decreased when switching from drospironone-pill to a levonorgestrel-pill. This study confirmed that the thrombin generation-based APC-resistance test discriminates well between oral contraceptives with a high thrombotic risk (desogestrel and cyproterone acetate) and with a lower risk of thrombosis (levonorgestrel) [16-20]. The higher resistance to APC in users of drospirenone-containing pills compared to users of levonorgestrel-containing pills suggests an increased risk of thrombosis for drospirenone-containing oral contraceptives.

The predicted increased risk of thrombosis associated with oral contraceptives containing drospirenone was confirmed in two independent studies and contradicted in two studies sponsored by the manufacturer of the drospirenone-containing pill. A population based case-control study including 1524 patients with a venous thrombosis of the leg or pulmonary

embolism and 1760 controls observed an 1.7-fold increased risk of thrombosis (95% CI 0.7 to 3.9) for oral contraceptives containing drospirenone compared to oral contraceptives containing levonorgestrel [21]. A national cohort study comprising 10.4 million women-years of observation found the risk of thrombosis associated with contraceptive pills containing drospirenone to be increased by a factor 1.64 (95% CI 1.27 to 2.10) compared to contraceptive pills containing levonorgestrel [22]. No difference in the risk of thrombosis between oral contraceptives containing drospirenone and oral contraceptives containing levonorgestrel was observed in a cohort study of 16.534 women using drospirenone-containing pills followed for 28.621 women-years of observation and 15.428 women using levonorgestrel-containing pills followed for 31.415 years of observation (Hazard ratio drospirenone versus levonorgestrel 1.0; 95% CI 0.6 to 1.8) [23]. A cohort study of 22.429 users of drospirenone-containing oral contraceptives followed for 14.081 women-years and 44.858 users of oral contraceptives containing 'other progestogens' followed for 27.575 women-years observed no difference in thrombotic risk between the preparations (Hazard ratio drospirenone versus other progestogens 0.9; 95% CI 0.5 to 1.6) [24]. However, the sample size of the study by Dinger et al. was too small to detect a difference in thrombotic risk between drospirenone-containing and levonorgestrel-containing pills and the confidence interval did not exclude the 1.6 to 1.7-fold increased risk observed in the two studies mentioned above. In order to demonstrate a doubling of risk of venous thrombosis between two different oral contraceptives about 600.000 women should be followed for 1 year [18]. In the study by Seeger et al. the thrombotic risk of drospirenone-containing pills was compared with the thrombotic risk of pills containing 'other progestogens' which most likely also include progestogens with an increased risk of thrombosis. The primary comparator should have been an oral contraceptive with a low risk of thrombosis, i.e. containing the second-generation progestogen levonorgestrel. In addition, both studies where funded by the manufacturer of the drospirenone-pill [23,24]. Studies sponsored by pharmaceutical companies are more likely to have outcomes favoring the sponsor than studies funded by other sources [9,10]. An example of the impact of sponsorship on contraceptive research outcomes emerges from a meta-analyses by Kemmeren et al. which observed that the difference in thrombotic risk between second- and third-generation oral contraceptives was less in trials sponsored by the pharmaceutical industry compared to studies that were financed with public funds or through charities [19].

No differences in contraceptive effectiveness, bleeding pattern and minor adverse effects have been observed between oral contraceptives containing drospirenone or levonorgestrel [25]. Suggested beneficial effects on weight, acne, hirsutism and premenstrual syndrome of the drospirenone-pill in comparison with a levonorgestrel-pill were not confirmed by Cochrane reviews [26-29]. In the absence of proven advantages of the drospirenone-containing oral contraceptive, an increased risk of thrombosis, and the higher costs of the drospirenone-pill we advise not to prescribe this oral contraceptive as a first choice for women starting oral contraceptives.

Due to the low incidence of venous thrombosis in users of hormonal contraceptives, assessment of the thrombotic risk necessitates studies including hundreds of thousands of women or case-control studies. In a development program for market authorization of a new hormonal contraceptive evaluation of the thrombotic risk in a clinical trial is virtually impossible. We confirmed that the thrombin generation-based APC-resistance test discriminates well between oral contraceptives with a high and with a low risk of thrombosis. In agreement with the European Medicines Agency Guideline on Clinical Investigation of Steroid Contraceptives in Women we advocate that before a new hormonal contraceptive is marketed the effect of the preparation on the resistance to APC measured with the thrombin generation-based APC-resistance test is determined to estimate the thrombotic risk [30]. Preferably, the new hormonal contraceptive is compared with a combined oral contraceptive with the lowest thrombotic risk, i.e. a pill containing the second-generation progestogen levonorgestrel.

In **chapter 7** we focused on the biological basis of acquired APC-resistance in oral contraceptive users and the different effects of various types of oral contraceptives on the resistance to APC by studying the two main determinants of the thrombin generation-based APC-resistance test, free protein S and tissue factor pathway inhibitor (TFPI) free antigen [31]. In this study we observed that oral contraceptives containing different progestogens had different effects on free protein S and TFPI free antigen. Women using third-generation oral contraceptives containing desogestrel, which are known to double the risk of thrombosis compared to second-generation oral contraceptives containing levonorgestrel, had lower free protein S and TFPI free antigen levels than women using oral contraceptives containing levonorgestrel [16-19]. Free protein S and TFPI antigen levels in users of drospirenone-containing oral contraceptives were lower than in users of levonorgestrel-containing oral contraceptives and similar to those in users of third-generation oral contraceptives. Women using oral contraceptives containing cyproterone acetate, which are known to increase the thrombotic risk 4-fold compared to oral contraceptives containing levonorgestrel, had the lowest free protein S and TFPI free antigen levels [20]. Low free protein S and low TFPI free antigen levels were associated with an increased resistance to APC. These results indicate that the observed differences in APC-resistance induced by oral contraceptives containing different progestogens and the differences in thrombotic risk can at least in part be explained by different effects of oral contraceptives on free protein S and TFPI free antigen.

The low free protein S and TFPI free antigen levels in women using oral contraceptives with a high thrombotic risk is in concordance with other studies on the effect of oral contraceptives on protein S and TFPI [32-36]. We did not investigate the mechanism for the decrease in free protein S and TFPI free antigen caused by oral contraceptives. Kemmeren et al. explained the differences in free protein S induced by various oral contraceptives by the interaction between total protein S, free protein S and C4b binding protein [37]. C4b binding protein binds protein S. Kemmeren et al. observed that total protein S was decreased by pills containing desogestrel but was hardly affected by pills containing levonorgestrel and that

both pills equally lowered C4b binding protein. As a result free protein S levels are lower in users of desogestrel-pills compared to users of levonorgestrel-pills.

The decrease in free TFPI induced by oral contraceptives might be caused by changes in synthesis, clearance or lipoprotein profile [36,38]. TFPI is mainly synthesized by the vascular endothelium and a large amount of TFPI remains associated with the vessel wall [39,40]. A small pool of TFPI circulates in blood, primarily in complex form with lipoproteins, in free form or in platelets [39,40]. TFPI is cleared from the circulation by the liver and kidneys [39]. Future studies are indicated to elucidate the mechanism of the reduction of free protein S and TFPI by oral contraceptives.

In **chapter 8** we tested the usefulness of sex hormone binding globulin (SHBG) as a marker for the thrombotic risk of an oral contraceptive by determining SHBG levels and APC-resistance in women using various types of oral contraceptives. Some of the women switched to an oral contraceptive containing a different type of progestogen and in these women SHBG and APC-resistance were measured before and after the switch. Users of oral contraceptives with a moderately increased risk of thrombosis, i.e. third-generation pills containing gestodene or desogestrel, had SHBG plasma levels that were higher than for users of low-risk, second-generation oral contraceptives containing levonorgestrel [16-19]. For higher doses of estrogen in oral contraceptives we found higher SHBG levels. Users of oral contraceptives with the highest risk of thrombosis, i.e. cyproterone acetate-containing pills, also had the highest SHBG levels [20]. In the group of women who switched from oral contraceptive type SHBG levels altered correspondingly, e.g. switching from a highly thrombogenic pill containing cyproterone acetate to a less thrombogenic pill containing levonorgestrel resulted in a decrease of SHBG levels [20]. SHBG plasma levels were positively associated with the resistance to the anticoagulant action of APC so high SHBG levels were related to a high resistance to APC. These findings support the hypothesis that the effect of an oral contraceptive on SHBG levels is a marker for the thrombotic risk. We observed a positive association between the effect of an oral contraceptive on SHBG levels and the thrombotic risk of a formulation as reported in the literature. The relationship between SHBG plasma levels and the resistance to the anticoagulant action of APC provides additional support for the hypothesis that SHBG is a marker for the thrombotic risk of an oral contraceptive, since the thrombin generation-based APC-resistance test that we used in this study predicts the risk of thrombosis in users of oral contraceptives as well as in non-users and men [41].

In concordance with the European Medicines Agency Guideline on Clinical Investigation of Steroid Contraceptives in Women we advocate that before a new hormonal contraceptive is marketed the effect of the preparation on SHBG levels is determined to estimate the thrombotic risk [30]. We acknowledge that clinical data on the risk of thrombosis of new hormonal contraceptives are preferred to data on the effect of hormonal contraceptives on markers for the thrombotic risk. However, as mentioned above, due to the low incidence of venous thrombosis in users of hormonal contraceptives, a clinical study assessing the

thrombotic risk of a new preparation requires hundreds of thousands of participants [18]. The sample size needed renders a clinical study before market authorization nearly unfeasible. During the developmental phase of a new hormonal contraceptive the effect of the preparation on SHBG levels can be useful to evaluate the thrombotic safety of the preparation. Preferably, the effect of a new hormonal contraceptive on SHBG levels should be compared with the effect of a combined oral contraceptive with the lowest thrombotic risk, i.e. a preparation containing the second-generation progestogen levonorgestrel [16-19].

Recently a four-phasic oral contraceptive composed of the progestogen dienogest and the estrogen estradiol valerate (Qlaira®, Bayer Schering Pharma, Berlin, Germany) has been introduced on the market. Dienogest is derived from the estrane structure and has anti-androgenic and no androgenic activity [42,43]. Estradiol valerate is an esterified form of natural 17ß-estradiol [43]. Benefits claimed by the manufacturer include that Qlaira® is the first oral contraceptive containing 'natural' estrogen and that due to the four-phasic approach the right hormonal effect is given at the right time just as in the natural cycle [44]. Yet, limited data are available on the effectiveness, frequent side effects and thrombotic safety of the contraceptive composed of dienogest and estradiol valerate. Laboratory studies evaluating the effect of this new oral contraceptive on thrombin generation-based APC-resistance and SHBG levels and clinical studies assessing the absolute and relative risk of thrombosis associated with this new oral contraceptive are indicated.

New routes of administration

Contraceptive vaginal ring and transdermal patch

In order to assess the thrombotic profile of the contraceptive vaginal ring and contraceptive transdermal patch, we determined in **chapter 9** APC-resistance and SHBG levels in women randomly assigned to either the contraceptive patch followed by a second-generation, levonorgestrel-pill or vice versa, or the contraceptive ring followed by a second-generation, levonorgestrel-pill or vice versa. The resistance to APC was measured with the thrombin generation-based APC-resistance test. During use of the contraceptive patch and contraceptive ring APC-resistance and SHBG levels were higher than during use of the levonorgestrel-contraceptive pill. The contraceptive patch caused higher APC-resistance and SHBG levels than the contraceptive ring. These results suggest that the contraceptive patch and the contraceptive ring have an increased risk of thrombosis compared to second-generation, levonorgestrel-containing oral contraceptives.

The observed higher APC-resistance and SHBG levels during use of the contraceptive patch compared to use of the contraceptive pill is in agreement with other studies on the effect of the contraceptive patch and pill on the resistance to APC [45,46] and SHBG levels [45,47,48].

Our results on the contraceptive ring are in contrast with a study by Jensen et al. which reported no changes in APC-resistance and SHBG levels when switching from an

oral contraceptive to the contraceptive ring [48]. However, Jensen et al. primarily used as comparator third-generation oral contraceptives which are known to double the risk of thrombosis and to have disadvantageous effects on markers of thrombosis compared to second-generation oral contraceptives [16-19,33,34,49-52]. Further, the APC-resistance test used in the study by Jensen et al. is not capable to identify differences in resistance to APC caused by different hormonal contraceptive formulations since this test is only sensitive to APC-resistance caused by the factor V Leiden mutation [52]. Higher APC-resistance and SHBG levels in users of a contraceptive ring compared to users of an oral contraceptive were also observed in a study comparing a contraceptive ring developed by the Population Council containing 150 µg nestorone and 15 µg ethinylestradiol and a contraceptive pill containing 150 µg levonorgestrel and 30 µg ethinylestradiol [53,54].

Overall, a Cochrane review comparing the contraceptive ring and contraceptive patch with oral contraceptives found a similar effectiveness and acceptability for all three methods [55]. No differences in cycle control were observed for most trials comparing the contraceptive patch and pill. Users of the patch experienced more minor adverse effects like nausea, vomiting, dysmenorrhea and breast discomfort than contraceptive pill users and discontinuation was higher for patch users compared to pill users. Bleeding problems were generally similar or less common in ring users compared to pill users. Less nausea, irritability and depression but more vaginal irritation and discharge was observed for users of the ring compared to users of the pill. No differences in discontinuation between ring users and pill users were found.

Since the contraceptive ring and contraceptive patch are comparable in terms of effectiveness and acceptability with a second-generation oral contraceptive but have an unfavourable prothrombotic profile compared to a second-generation oral contraceptive we advise not to prescribe the contraceptive ring and contraceptive patch as a first-choice in women starting contraceptive use. Clinical studies to assess the absolute and relative risk of thrombosis in women using the contraceptive ring and contraceptive patch are needed.

Levonorgestrel-releasing intrauterine system

In **chapter 10** we compared the resistance to APC before and three months after insertion of the levonorgestrel-releasing or copper-containing intrauterine device in order to evaluate the thrombotic profile of the levonorgestrel-intrauterine device. The resistance to APC was determined by quantifying the effect of APC on thrombin generation. APC-resistance was lower thre months after the insertion of the levonorgestrel-intrauterine system than before the insertion. After insertion of the copper-intrauterine device the APC-resistance hardly changed. In women who switched from a combined oral contraceptive to the levonorgestrel-intrauterine device the decrease in APC-resistance after insertion of the levonorgestrel-intrauterine device was more pronounced. These results indicate that the levonorgestrel-

releasing intrauterine system does not have a prothrombotic effect and suggests that the levonorgestrel-intrauterine system does not increase the risk of venous thrombosis.

The decrease in APC-resistance after insertion of the levonorgestrel-releasing intrauterine system is in concordance with studies on the effect of other types of progestogen-only contraceptives on the resistance to APC [56,57]. Recently the first clinical study reporting the risk of thrombosis associated with use of the levonorgestrel-intrauterine system study was published. In this national cohort study including 101 thousand women-years of levonorgestrel-releasing intrauterine device use the risk of thrombosis associated with the levonorgestrel-intrauterine device was not increased (rate ratio 0.89; 95% CI 0.64 to 1.26) as compared with non-use of oral contraceptives [22]. The study also observed that the thrombotic risk of progestogen-only contraceptive pills was not increased [22]. Older observational studies did not find an evident increased risk of venous thrombosis in women using progestogen-only contraceptive methods as well but these studies are limited by the small number of women using progestogen-only contraceptives that were included [58-61]. Another limitation of the older studies is that the preparations used by the study subjects varied in doses and methods of administration. Clinical studies assessing the absolute and relative risk of thrombosis in women using the levonorgestrel-intrauterine system are required to confirm that the levonorgestrel-intrauterine system does not increase the risk of thrombosis. Of special interest would be the thrombotic risk associated with the use of the levonorgestrel-intrauterine system in women with thrombogenic mutations or a history of venous thrombosis.

The World Health Organization advises women with inherited coagulation defects, e.g. factor V Leiden, prothrombin mutation, deficiencies of protein S, protein C or antithrombin, or women with a history of deep venous thrombosis not to use combined oral contraceptives [62]. The same guidelines state that in women with thrombogenic mutations or with a history of thrombosis the advantages of using the levonorgestrel-intrauterine system generally outweigh the theoretical or proven risks. In our study the number of women with the factor V Leiden mutation or with a history of thrombosis was too small to assess the effect of the levonorgestrel-intrauterine device on the resistance to APC in these women. Caution is required in extrapolating the observed effect of the levonorgestrel-intrauterine device in women without thrombophilia or a history of thrombosis to women with thrombogenic mutations or with a history of thrombosis since the latter have a higher baseline resistance to APC [63] and a higher thrombotic risk [64-67]. However, based on a study by Kemmeren et al. who observed that levonorgestrel-only contraceptive pills cause a similar decrease in resistance to APC in non-carriers and in heterozygous carriers of the factor V Leiden mutation [57], we do not expect that the levonorgestrel-intrauterine system will have a different effect on the resistance to APC in women with and without the factor V Leiden mutation. Studies evaluating the effect of the levonorgestrel-intrauterine system on the resistance to APC in women with thrombogenic mutations are warranted.

Natural cycle

In order to evaluate whether varying levels of estradiol and progesterone during the natural cycle are associated with differences in sensitivity to APC, we determined in **chapter 11** the resistance to APC at six different time points during the menstrual cycle in wildtype women and women with the factor V Leiden mutation. The resistance to APC was determined with the thrombin generation-based APC-resistance test. In wildtype women the sensitivity to APC varied slightly throughout the cycle with an increased resistance to APC after the rise of estradiol through the follicular phase. Friedman analysis showed that it was more likely that women had a reduced resistance to APC at the beginning of the cycle than later on in the cycle.

The observed cyclic variability in APC-resistance is not consistent with three other studies evaluating the sensitivity to APC during the menstrual cycle. Wramsby et al. measured the resistance to APC at four different time points in 35 women in one study and 18 women in another study [68,69]. Ricci et al. determined the sensitivity to APC at five different time points in 41 women [70]. In these studies no changes in APC-resistance were observed during the menstrual cycle. An explanation for the contrasting results may lie in the APC-resistance tests which were used. Wramsby et al. and Ricci et al. measured the sensitivity to APC with the classic aPTT-based APC-resistance test. Studies have shown that the classic aPTT-based APC-resistance test is less sensitive to changes in APC-resistance caused by female hormones than the thrombin generation-based APC-resistance test which we used in our study [41,71]. Although using the thrombin generation-based APC-resistance test, van Rooijen et al. did not observe differences in sensitivity to APC between two phases of the menstrual cycle, the early follicular phase and the mid-luteal phase, in 72 women [72]. The difference in outcome with our study might be explained by the statistical methods used. In the study by van Rooijen et al., an one-way repeated measures ANOVA was performed with the menstrual phase as the within-subjects variable. Because of the small number of participants and the slight differences in APC-resistance between the distinct phases of the menstrual cycle we compared the sensitivity to APC during the menstrual cycle with the Friedman ranking test. For each phase of the menstrual cycle we assessed per participant whether the result of the nAPCsr was the highest, the middle or the lowest value and numbered the highest nAPCsr as one, the middle nAPCsr as two and the lowest nAPCsr as three. Using the Friedman ranking test we observed that it was more likely that women had the lowest resistance to APC at the beginning of the cycle than later on in the cycle.

The observed small differences in the sensitivity to APC between the different phases of the menstrual cycle could be useful in improving studies on APC-resistance. When evaluating the sensitivity to APC in women, it may be useful to take the phase of the menstrual cycle into account. Future studies to assess whether the small differences in APC-resistance have clinical significance are indicated.

RECOMMENDATIONS

Clinical

- Monophasic oral contraceptives are preferred over biphasic and triphasic oral contraceptives.
- Combined oral contraceptives containing the second-generation progestogen levonorgestrel are recommended over combined oral contraceptives containing drospirenone.
- The contraceptive ring and contraceptive patch are not recommended as a first-choice in women starting contraceptive use.

Research

- Large, adequately reported, high-quality, randomized controlled trials comparing triphasic and monophasic oral contraceptives with identical progestogens are needed to determine whether triphasic pills differ from monophasic pills in contraceptive effectiveness and acceptability. These studies should follow the recommendations of the Hormonal Contraceptives Trial Methodology Consensus Conference on recording of bleeding patterns and should adhere to the CONSORT guidelines on reporting of randomized controlled trials.
- Authors, peer-reviewers and editors should not exclusively focus on whether quality assessment has been performed in a systematic review, but also concentrate on incorporation of quality assessments in the analysis of a systematic review.
- Before a new hormonal contraceptive is marketed the effect of the preparation on the resistance to activated protein C measured with the thrombin-generation based APC-resistance test and the effect on SHBG levels should have been determined to evaluate the thrombotic risk of the new preparation. The effect of the new hormonal contraceptive on these markers of thrombosis should be compared with the effect of a combined oral contraceptive containing the second-generation progestogen levonorgestrel.
- Studies examining the mechanism of the reduction of free protein S and free TFPI by oral contraceptives are indicated to gain more insight in the biological basis of the increased risk of venous thrombosis in oral contraceptive users.
- Laboratory studies evaluating the effect of oral contraceptives containing dienogest and estradiol valerate on thrombin generation-based APC-resistance and on SHBG levels and clinical studies assessing the absolute and relative risk of thrombosis associated with oral contraceptives containing dienogest and estradiol valerate are indicated.
- Independent, clinical studies assessing the absolute and relative risk of thrombosis associated with use of the contraceptive vaginal ring and contraceptive transdermal patch are needed.
- Clinical studies assessing the absolute and relative risk of thrombosis in women using the levonorgestrel-releasing intrauterine system are required to confirm that the

levonorgestrel-intrauterine system does not increase the risk of thrombosis. Of special interest would be the thrombotic risk associated with use of the levonorgestrel-intrauterine system in women with thrombogenic mutations or a history of venous thrombosis. Studies evaluating the effect of the levonorgestrel-intrauterine system on thrombin generation-based APC-resistance in women with thrombogenic mutations are needed.

- Studies assessing whether the small variations in APC-resistance between the different phases of the menstrual cycle have clinical significance are indicated.

REFERENCES

1. DerSimonian R, Charette LJ, McPeek B, Mosteller F. Reporting on methods in clinical trials. N Engl J Med 1982;306:1332-7.
2. Moher D, Schulz KF, Altman DG. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. Lancet 2001;357:1191-4.
3. Altman DG, Schulz KF, Moher D, Egger M, Davidoff F, Elbourne D, Gøtzsche PC, Lang T. The revised CONSORT statement for reporting randomized trials: explanation and elaboration. Ann Intern Med 2001;134:663-94.
4. Schulz KF, Chalmers I, Hayes RJ, Altman DG. Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. JAMA 1995;273:408-12.
5. Schulz KF, Grimes DA. Generation of allocation sequences in randomised trials: chance, not choice. Lancet 2002;359:515-9.
6. Schulz KF, Grimes DA. Allocation concealment in randomised trials: defending against deciphering. Lancet 2002;359:614-8.
7. Schulz KF, Grimes DA. Blinding in randomised trials: hiding who got what. Lancet 2002;359:696-700.
8. Schulz KF, Grimes DA. Sample size slippages in randomised trials: exclusions and the lost and wayward. Lancet 2002;359:781-5.
9. Lexchin J, Bero LA, Djulbegovic B, Clark O. Pharmaceutical industry sponsorship and research outcome and quality: systematic review. BMJ 2003;326:1167-70.
10. Als-Nielsen B, Chen W, Gluud C, Kjaergard LL. Association of funding and conclusions in randomized drug trials: a reflection of treatment effect or adverse events? JAMA 2003;290:921-8.
11. Maitra N, Kulier R, Bloemenkamp KW, Helmerhorst FM, Gulmezoglu AM. Progestogens in combined oral contraceptives for contraception. Cochrane Database Syst Rev 2004;3:CD004861.
12. Rosenberg MJ, Long SC. Oral contraceptives and cycle control: a critical review of the literature. Adv Contracept 1992;8:35-45.
13. Gallo MF, Nanda K, Grimes DA, Lopez LM, Schulz KF. 20 microg versus >20 microg estrogen combined oral contraceptives for contraception. Cochrane Database Syst Rev 2008;4:CD003989.
14. Mishell DR, Guillebaud J, Westhoff C, Nelson AL, Kaunitz AM, Trussell J, Davis AJ. Recommendations for standardization of data collection and analysis of bleeding in combined hormone contraceptive trials. Contraception 2007;75:11-5.
15. Moja LP, Telaro E, D'Amico R, Moschetti I, Coe L, Liberati A. Assessment of methodological quality of primary studies by systematic reviews: results of the metaquality cross sectional study. BMJ 2005;330:1053.
16. Vandenbroucke JP, Rosing J, Bloemenkamp KW, Middeldorp S, Helmerhorst FM, Bouma BN, Rosendaal FR. Oral contraceptives and the risk of venous thrombosis. N Engl J Med 2001;344:1527-35.
17. Rosendaal FR, van Hylckama Vlieg A, Tanis BC, Helmerhorst FM. Estrogens, progestogens and thrombosis. J Thromb Haemost 2003;1:1371-80.
18. World Health Organization. Cardiovascular disease and steroid hormone contraception: report of a WHO scientific group. Geneva: World Health Organization; 1998.
19. Kemmeren JM, Algra A, Grobbee DE. Third generation oral contraceptives and risk of venous thrombosis: meta-analysis. BMJ 2001;323:131-4.
20. Vasilakis-Scaramozza C, Jick H. Risk of venous thromboembolism with cyproterone or levonorgestrel contraceptives. Lancet 2001;358:1427-9.
21. 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.

22. Lidegaard Ø, Løkkegaard E, Svendsen AL, Agger C. Hormonal contraception and risk of venous thromboembolism: national follow-up study. BMJ 2009;339:b2890.
23. Dinger JC, Heinemann LA, Kuhl-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;75:344-54.
24. Seeger JD, Loughlin J, Eng PM, Clifford CR, Cutone J, Walker AM. Risk of thromboembolism in women taking ethinylestradiol/drospirenone and other oral contraceptives. Obstet Gynecol 2007;110:587-93.
25. Suthipongse W, Taneepanichskul S. An open-label randomized comparative study of oral contraceptives between medications containing 3 mg drospirenone/30 microg ethinylestradiol and 150 microg levonogestrel/30 microg ethinylestradiol in Thai women. Contraception 2004;69:23-6.
26. Gallo MF, Lopez LM, Grimes DA, Schulz KF, Helmerhorst FM. Combination contraceptives: effects on weight. Cochrane Database Syst Rev 2008;4:CD003987.
27. Arowojolu AO, Gallo MF, Lopez LM, Grimes DA, Garner SE. Combined oral contraceptive pills for treatment of acne. Cochrane Database Syst Rev 2009;3:CD004425.
28. Lopez LM, Kaptein AA, Helmerhorst FM. Oral contraceptives containing drospirenone for premenstrual syndrome. Cochrane Database Syst Rev 2009;2:CD006586.
29. Helmerhorst FM, Lopez LM, Kaptein AA. Premenstrual syndrome. Lancet 2008;372:446-7.
30. European Medicines Agency: Committee for Medicinal Products for Human Use. Guideline on clinical investigation of steroid contraceptives in women. London: 2005. http://www.emea.europa.eu/pdfs/human/ewp/051998en.pdf.
31. 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.
32. Alhenc-Gelas M, Plu-Bureau, Guillonneau S, Kirzin JM, Aiach M, Ochat N, Scarabin PY. Impact of progestagens on activated protein C (APC) resistance among users of oral contraceptives. J Thromb Haemost 2004;2:1594-600.
33. Kemmeren JM, Algra A, Meijers JC, Tans G, Bouma BN, Curvers J, Rosing J, Grobbee DE. Effect of second- and third-generation oral contraceptives on the protein C system in the absence or presence of the factor VLeiden mutation: a randomized trial. Blood 2004;103:927-33.
34. Tans G, Curvers J, Middeldorp S, Thomassen MC, Meijers JC, Prins MH, Bouma BN, Büller HR, Rosing J. A randomized cross-over study on the effects of levonorgestrel- and desogestrel-containing oral contraceptives on the anticoagulant pathways. Thromb Haemost 2000;84:15-21.
35. Oral Contraceptive and Hemostasis Study Group. The effects of seven monophasic oral contraceptive regimens on hemostatic variables: conclusions from a large randomized multicenter study. Contraception 2003;67:173-85.
36. Harris GM, Stendt CL, Vollenhoven BJ, Gan TE, Tipping PG. Decreased plasma tissue factor pathway inhibitor in women taking combined oral contraceptives. Am J Hematol 1999;60:175-80.
37. Kemmeren JM, Algra A, Meijers JC, Bouma BN, Grobbee DE. Effects of second and third generation oral contraceptives and their respective progestagens on the coagulation system in the absence or presence of the factor V Leiden mutation. Thromb Haemost 2002;87:199-205.
38. Dahm AE, Iversen N, Birkenes B, Ree AH, Sandset PM. Estrogens, selective estrogen receptor modulators, and a selective estrogen receptor down-regulator inhibit endothelial production of tissue factor pathway inhibitor 1. BMC Cardiovasc Disord 2006;6:40.
39. Bajaj MS, Birktoft JJ, Steer SA, Bajaj SP. Structure and biology of tissue factor pathway inhibitor. Thromb Haemost 2001;86:959-72.
40. Sandset PM, Bendz B. Tissue factor pathway inhibitor: clinical deficiency states. Thromb Haemost 1997;78:467-70.

41. 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.
42. Henzl MR, Edwards JA. Pharmacology of progestins: 17alpha-hydroxyprogesterone derivatives and progestins of the first and second generation. In: Sitruk-Ware R and Mishell DR, editors. Progestins and antiprogestins in clinical practice. New York: Marcel Dekker; 2000. p.101-32.
43. Wellington K, Perry CM. Estradiol valerate/dienogest. Drugs 2002;62:491-504.
44. Bayer HealthCare Pharmaceuticals. Website Qlaira®. 2009. http://www.qlaira.nl.
45. Kluft C, Meijer P, LaGuardia KD, Fisher AC. Comparison of a transdermal contraceptive patch vs. oral contraceptives on hemostasis variables. Contraception 2008;77:77-83.
46. Johnson JV, Lowell J, Badger GJ, Rosing J, Tchaikovski S, Cushman M. Effects of oral and transdermal hormonal contraception on vascular risk markers: a randomized controlled trial. Obstet Gynecol 2008;111:278-84.
47. White T, Ozel B, Jain JK, Stanczyk FZ. Effects of transdermal and oral contraceptives on estrogen-sensitive hepatic proteins. Contraception 2006;74:293-6.
48. Jensen JT, Burke AE, Barnhart KT, Tillotson C, Messerle-Forbes M, Peters D. Effects of switching from oral to transdermal or transvaginal contraception on markers of thrombosis. Contraception 2008;78:451-8.
49. Rosing J, Middeldorp S, Curvers J, Thomassen MC, Nicolaes GA, Meijers JC, Bouma BN, Büller 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.
50. Middeldorp S, Meijers JC, van den Ende AE, van Enk A, Bouma BN, Tans G, Rosing J, Prins MH, Büller HR. Effects on coagulation of levonorgestrel- and desogestrel-containing low dose oral contraceptives: a cross-over study. Thromb Haemost 2000;84:4-8.
51. van Vliet HA, Winkel TA, Noort I, Rosing J, Rosendaal FR. Prothrombotic changes in users of combined oral contraceptives containing drospirenone and cyproterone acetate. J Thromb Haemost 2004;2:2060-2.
52. van Vliet HAAM, Rosendaal FR, Fleischer K, Rosing J, Helmerhorst FM. Effects of the contraceptive vaginal ring, the contraceptive transdermal patch and combined oral contraceptives on markers of hemostasis. Contraception 2010;81:88-9.
53. Rad M, Kluft C, Menard J, Burggraaf J, de Kam ML, Meijer P, Sivin I, Sitruk-Ware RL. Comparative effects of a contraceptive vaginal ring delivering a nonandrogenic progestin and continuous ethinyl estradiol and a combined oral contraceptive containing levonorgestrel on hemostasis variables. Am J Obstet Gynecol 2006;195:72-7.
54. Sitruk-Ware RL, Menard J, Rad M, Burggraaf J, de Kam ML, Tokay BA, Sivin I, Kluft C. Comparison of the impact of vaginal and oral administration of combined hormonal contraceptives on hepatic proteins sensitive to estrogen. Contraception 2007;75:430-7.
55. Lopez LM, Grimes DA, Gallo MF, Schulz KF. Skin patch and vaginal ring versus combined oral contraceptives for contraception. Cochrane Database Syst Rev 2008;1:CD003552.
56. Lindqvist PG, Rosing J, Malmquist A, Hillarp A. Etonogestrel implant use is not related to hypercoagulable changes in anticoagulant system. J Thromb Haemost 2003;1:601-2.
57. Kemmeren JM, Algra A, Meijers JC, Bouma BN, Grobbee DE. Effects of second and third generation oral contraceptives and their respective progestagens on the coagulation system in the absence or presence of the factor V Leiden mutation. Thromb Haemost 2002;87:199-205.
58. Bergendal A, Odlind V, Persson I, Kieler H. Limited knowledge on progestogen-only contraception and risk of venous thromboembolism. Acta Obstet Gynecol Scand 2009;88:261-6.
59. Vasilakis C, Jick H, del Mar Melero-Montes M. Risk of idiopathic venous thromboembolism in users of progestagens alone. Lancet 1999;354:1610-1.
60. Cardiovascular disease and use of oral and injectable progestogen-only contraceptives and combined injectable contraceptives. Results of an international, multicenter, case-control study.

World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Contraception 1998;57:315-24.
61. Heinemann LA, Assmann A, DoMinh T, Garbe E. Oral progestogen-only contraceptives and cardiovascular risk: results from the Transnational Study on Oral Contraceptives and the Health of Young Women. Eur J Contracept Reprod Health Care 1999;4:67-73.
62. World Health Organization. Improving access to quality care in family planning. Medical eligibility criteria for contraceptive use. Geneva: World Health Organization; 2004. http://whqlibdoc.who.int/publications/2004/9241562668.pdf.
63. Curvers J, Thomassen MC, Rimmer J, Hamulyak K, van der Meer J, Tans G, Preston FE, Rosing J. Effects of hereditary and acquired risk factors of venous thrombosis on a thrombin generation-based APC resistance test. Thromb Haemost 2002;88:5-11.
64. Bezemer ID, van der Meer FJ, Eikenboom JC, Rosendaal FR, Doggen CJ. The value of family history as a risk indicator for venous thrombosis. Arch Intern Med 2009;169:610-5.
65. Seligsohn U, Lubetsky A. Genetic susceptibility to venous thrombosis. N Engl J Med 2001;344:1222-31.
66. Rosendaal FR. Venous thrombosis: a multicausal disease. Lancet 1999;353:1167-73.
67. Blann AD, Lip GY. Venous thromboembolism. BMJ 2006;332:215-9.
68. Wramsby ML, Bokarewa MI, Blombäck M, Bremme AK. Response to activated protein C during normal menstrual cycle and ovarian stimulation. Hum Reprod 2000;15:795-7.
69. Wramsby ML, Bremme K, Blombäck M. Measurement of activated protein C resistance during menstrual cycle in women with and without the Leiden mutation. Thromb Haemost 2001;85:614-8.
70. Ricci G, Cerneca F, Simeone R, Pozzobon C, Guarnieri S, Sartore A, Pregazzi R, Guaschino S. Impact of highly purified urinary FSH and recombinant FSH on haemostasis: an open-label, randomized, controlled trial. Hum Reprod 2004;19:838-48.
71. 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.
72. van Rooijen M, Silveira A, Thomassen MC, Odeberg J, Hamsten A, Rosing J, Bremme K. APC resistance during the normal menstrual cycle. Thromb Haemost 2007;98:1246-51.