CHAPTER 8

General discussion and summary

Since the introduction of the first oral contraceptive pill in 1959, the development of contraceptives with new compounds and routes of administration was focused on reducing side effects, while maintaining the benefits. This thesis focuses on venous thrombosis as a rare, but serious side effect of hormonal contraceptive use. Nowadays it is known that use of different hormonal contraceptives is associated with different thrombotic risks, dependent on the estrogen dose and progestogen type of the hormonal contraceptive.

The first objective of this thesis was to investigate effectiveness, bleeding-pattern, minor side effects and acceptability during use of quadriphasic oral contraceptives compared with monophasic oral contraceptives (chapter 2). The second objective was to evaluate the levels of free TFPI and free Protein S as the main determinants of the thrombin generation-based APC resistance test, during use of different hormonal contraceptives (chapter 3). The third objective was to evaluate whether SHBG acts as a marker for venous thrombosis during use of different hormonal contraceptives, and to assess if SHBG reflects the ethinylestradiol levels of hormonal contraceptives (chapter 4, 5). The fourth objective was to investigate the thrombotic risk of a new combined oral contraceptive containing dienogest and estradiol valerate administered in a quadriphasic schedule (chapter 6). The last objective was to assess the association between the levels of TBG, FT4 and TSH during use of different hormonal contraceptives and their risk of venous thrombosis (chapter 7).

Hormonal contraceptives and venous thrombosis

New formulas and schedules of administration

In **chapter 2** we systematically reviewed the literature for randomized controlled trials comparing monophasic oral contraceptives with quadriphasic oral contraceptives, and planned to perform a meta-analysis for the outcomes on contraceptive effectiveness, bleeding pattern, minor side effects and acceptability. One double-blind, double-dummy randomized controlled trial comparing a quadriphasic oral contraceptive containing dienogest and estradiol valerate with a monophasic oral contraceptive containing 100 µg levonorgestrel and 20 µg ethinylestradiol was included. Contraceptive effectiveness, intracyclic bleeding and discontinuation due to side effects were similar for quadriphasic and monophasic pills. The number of women experiencing amenorrhea was higher in the quadriphasic group than in the monophasic group. Users of quadriphasic pills reported fewer bleeding and spotting days and fewer bleeding and spotting episodes than users of monophasic pills but it was unclear whether this was scheduled by the user or unscheduled. More women using quadriphasic oral contraceptives reported breast pain than women using monophasic oral contraceptives.

We concluded that the available evidence is insufficient to determine whether quadriphasic oral contraceptives differ from monophasic oral contraceptives in contraceptive effectiveness, bleeding pattern, minor side effects and acceptability, since only one randomized controlled trial could be included. Studies that compare quadriphasic and monophasic oral contraceptives with an

identical progestogen and estrogen type are needed to determine whether the quadriphasic approach differs from the monophasic approach. Studies that compare quadriphasic pills with monophasic pills containing 30µg ethinylestradiol are indicated to determine whether quadriphasic oral contraceptives have an advantage over the current, first choice oral contraceptive.

In the absence of proven advantages of multiphasic oral contraceptives, and because of the greater complexity of the multiphasic approach and the higher costs of multiphasic oral contraceptives, we recommend monophasic pills as first choice for women starting oral contraceptive use. Monophasic pills containing 30 µg estrogen are preferred over 20 µg estrogen since the latter causes more bleeding problems and more often lead to discontinuation of use than those containing 30 µg (5).

Biological explanation

TFPI and Protein S

In **chapter 3** we assessed the effect of hormonal and non-hormonal contraceptives with different routes of administration on free TFPI and free Protein S levels, as determinants of the thrombin generation-based APC resistance test. In addition, we measured APC sensitivity ratios (nAPCsr) by using the thrombin generation-based APC resistance test. We observed that users of contraceptives with the highest risk of venous thrombosis had the lowest free TFPI and free Protein S levels, and vice versa, women who used contraceptives with the lowest risk of venous thrombosis had the highest free TFPI and free Protein S levels. Furthermore, a negative association was observed between levels of free TFPI and APC resistance, and between free Protein S and APC resistance. Our study confirms that the different thrombotic risks associated with use of different hormonal contraceptives are reflected in the levels of free TFPI and free Protein S. Besides, our results confirm the hypothesis that the differences in APC resistance induced by hormonal contraceptives can at least be partially explained by different effects on free TFPI and free Protein S levels.

The lower free TFPI and free Protein S levels during use of third generation combined oral contraceptives or combined oral contraceptives containing cyproterone acetate or drospirenone are in concordance with several other studies on the effect of combined oral contraceptives on levels of free TFPI and free Protein S (6-11). Unfortunately, in our study the sample sizes of the groups of users of the vaginal ring and users of the transdermal patch were too small to draw conclusions. Since use of the transdermal patch and vaginal ring is associated with an increased risk of venous thrombosis with ORs of 7.9 and 6.5 respectively compared with non-use (12), decreased levels of both free TFPI and free Protein S are expected. Two studies have been performed on the levels of protein S in users of the transdermal patch or vaginal ring and the results were conflicting (13;14). A study with an adequate sample size is indicated to investigate the free Protein S and TFPI levels during use of the transdermal patch and vaginal ring.

The mechanism for the decrease in free Protein S and free TFPI has been studied, but not yet fully unraveled. Kemmeren *et al*. explained the differences in free Protein S induced by various

oral contraceptives by the interaction between Protein S and C4BP. C4BP binds Protein S in a high-affinity complex (7). They observed that total Protein S was decreased by third generation combined oral contraceptives containing desogestrel but was hardly affected by second generation combined oral contraceptives containing levonorgestrel and that both oral contraceptives equally lowered C4BP. As a result, free Protein S levels increased in users of the combined oral contraceptive with levonorgestrel and decreased in users of the combined oral contraceptive with desogestrel. Free Protein S forms a complex with free TFPI and acts as cofactor of TFPI through the extrinsic pathway (15-19). A possible explanation of the concomitant decrease in free TFPI levels could be that binding of free TFPI to free Protein S protects free TFPI from proteolytic degradation or slows down the clearance of free TFPI. A decrease of free Protein S will therefore be accompanied by a decrease in free TFPI. Further, since free Protein S and free TFPI are both produced by the endothelium it is likely that TFPI production or release is also influenced by hormonal contraceptives; both proteins share the endothelium as common production site. In addition, the secretion of TFPI from endothelial cells might be coupled to Protein S secretion, as has recently been discovered for Protein S and the beta-chain of C4BP by Carlsson *et al*. (18;20). Future studies are indicated to unravel the mechanism of the decreased free TFPI and free Protein S levels during use of hormonal contraceptives and the association of their relationship with the risk of venous thrombosis.

Predicting thrombotic risk by use of surrogate markers

In **chapter 4** we focused on SHBG as potential marker for venous thrombosis during use of hormonal contraceptives. We investigated whether a positive association could be found between levels of SHBG and APC resistance and between levels of SHBG and relative risks of venous thrombosis as reported in the literature during use of different combined oral contraceptives, LNG-IUD and copper-IUD, transdermal patch and vaginal ring. APC resistance was determined using the thrombin generation-based APC resistance test. The study demonstrates that users of contraceptives with a higher risk of venous thrombosis, i.e. third generation combined oral contraceptives, oral contraceptives containing cyproterone acetate or drospirenone, the transdermal patch and vaginal ring had higher SHBG levels than users of a second generation levonorgestrel combined oral contraceptive, which carry a lower thrombotic risk. The lowest SHBG levels were found during use of the LNG-IUD, which is not associated with an increased risk of venous thrombosis. A positive association was found between SHBG levels and APC resistance and between SHBG levels and the relative risks as reported in the literature. These results suggest that SHBG is a useful marker to estimate the risk of venous thrombosis during use of hormonal contraceptives. We recommend that the effect of a new hormonal contraceptive on SHBG should be measured before licensing, and compared with the effect on the combined oral contraceptive with the lowest risk of venous thrombosis, i.e. containing 30 µg ethinylestradiol and 150 µg levonorgestrel.

In **chapter 5** we determined the effect of the ethinylestradiol dose of combined oral contraceptives on SHBG levels, since the estrogen dose is thought to be the most important factor in

hormonal contraceptives in causing the increased risk of venous thrombosis. In this study we observed that women using a combined oral contraceptive with ≥ 35 µg ethinylestradiol had higher SHBG levels than women using a combined oral contraceptive with 20 µg ethinylestradiol. However, SHBG levels were only slightly higher in users of a combined oral contraceptive with 30 µg ethinylestradiol than 20 µg ethinylestradiol.

Our results of SHBG during use of combined oral contraceptives, the transdermal patch and the vaginal ring are in concordance with other studies on the effect of hormonal contraceptives on levels of SHBG (3;21-26). We are not aware of studies published about SHBG during use of the LNG-IUD, or studies about the effect of different ethinylestradiol levels on SHBG.

Stegeman *et al*. (27) investigated whether increased SHBG levels are causally related to venous thrombosis in women not using hormonal contraceptives. They used a Mendelian randomization approach and showed that SHBG is only a marker for venous thrombosis during hormonal contraceptive use, and not a cause for an increased risk of venous thrombosis per se.

Some researchers have questioned whether SHBG can act as a marker for venous thrombosis. They stated that SHBG has no relation with coagulation, is not validated as a marker and affected by many factors not involved by coagulation (28). Currently, a biological explanation for the association between the changes in SHBG levels and APC resistance induced by hormonal contraceptives indeed is lacking. Our study supports the hypothesis of Odlind *et al*. (21) that SHBG reflects the overall estrogenicity of a hormonal contraceptive, and thereby the risk of venous thrombosis. SHBG and several coagulation factors and anticoagulant proteins are synthesized in the liver, and hormonal contraceptives, which are metabolized in the liver, might interfere with the synthesis of both SHBG and coagulation factors. Further research is needed to understand the association between increased SHBG levels and the increased risk of venous thrombosis during use of hormonal contraceptives, which, however, is not a prerequisite for its use as a marker of thrombogenicity.

We acknowledge that clinical data on the risk of venous thrombosis of new hormonal contraceptives are in theory to be preferred over markers such as SHBG. However, a non-clinical marker has the advantage that fewer women need to suffer venous thrombosis before a decision can be made. In addition, a marker should be validated in a prospective trial in which both the marker and the clinical endpoint are assessed (28). Given the low incidence of venous thrombosis during oral contraceptive use, a high number of participants is necessary in clinical studies to provide absolute and relative risks, which would be almost impossible before market authorization (29). Hence, during the developmental phase of a new contraceptive, the thrombotic risk can be estimated by using SHBG as marker and by comparing the SHBG levels of the new contraceptive with the combined oral contraceptive with the lowest risk of venous thrombosis, i.e. containing levonorgestrel. Thrombin generation-based APC resistance is a validated marker for venous thrombosis (8), but a complex measurement which cannot be performed in every laboratory. SHBG, however, can easily be measured in every routine laboratory. The EMA therefore now recommends SHBG measurement in guidelines applying to the clinical development of a new combined hormonal contraceptive (30).

In **chapter 6** we investigated the thrombogenicity of a new oral contraceptive containing dienogest and estradiol valerate administered in a quadriphasic schedule. APC resistance and SHBG levels were measured in participants using this new oral contraceptive and compared with APC resistance and SHBG levels during use of a monophasic oral contraceptive containing levonorgestrel and ethinylestradiol. We observed no clear differences in APC resistance and SHBG as markers for venous thrombosis between the oral contraceptives. During the pill cycle, SHBG levels increased gradually in both dienogest with estradiol valerate users as well as in and in levonorgestrel with ethinylestradiol users. No differences in APC resistance were observed during the pill cycle.

These results are in concordance with two studies sponsored by the manufacturer. Klipping *et al*. (1) conducted a randomized, open label, cross-over study of dienogest with estradiol valerate and monophasic levonorgestrel with ethinylestradiol and observed lower nAPCsr and lower SHBG levels in users of dienogest with estradiol valerate than in users of levonorgestrel with ethinylestradiol. Junge *et al*. (2) performed a randomized, open label study and also observed less pronounced SHBG levels in users of dienogest with estradiol valerate than in users of triphasic levonorgestrel withethinylestradiol. In our study, nAPCsr levels were overall lower in users of dienogest with estradiol valerate, but the differences were not pronounced and not statistically significant. This can be due to a smaller sample sizes in the studies of the manufacturer. Based on the results of these three studies, it can be stated, however, that dienogest with estradiol valerate does not lead to a more thrombogenic state than levonorgestrel with ethinylestradiol. However, clinical studies assessing the absolute and relative risk of thrombosis in women using dienogest with estradiol valerate are indicated to confirm this.

Dienogest has, in addition to estradiol valerate, also been combined with ethinylestradiol in a combined oral contraceptive pill (Valette®, Bayer Schering Pharma, Berlin, Germany; not available in the Netherlands). In a study by Wiegratz *et al*. (3) users of oral contraceptives containing 2mg dienogest and 20 µg ethinylestradiol had higher SHBG levels than users of oral contraceptives containing 100 µg levonorgestrel and 20 µg ethinylestradiol. This indicates that dienogest is a less anti-estrogenic progestogen than levonorgestrel. In our study no differences in SHBG were observed between users of dienogest with estradiol valerate and levonorgestrel with ethinylestradiol. No studies have been published yet on the effect of dienogest with ethinylestradiol on nAPCsr, or the risk of venous thrombosis of combined oral contraceptives containing estradiol valerate as estrogen content. Estradiol valerate seems to have a favorable effect on the risk of venous thrombosis, but has not been combined with other progestogens before. Therefore, the impact of estradiol valerate alone on venous thrombosis remains unknown. Future research should focus on the effect of estradiol valerate as the estrogen compound of a combined oral contraceptive on the risk of venous thrombosis compared with ethinylestradiol.

In 2011, a new monophasic combined oral contraceptive composed of the progestogen nomegestrol acetate and the estrogen 17ß-estradiol (Zoely®, Merck & Co., Inc., Whitehouse Station, New Jersey USA) was marketed. Gaussem *et al*. (4) compared APC resistance and SHBG levels during use of this new combined oral contraceptive with the monophasic combined oral contraceptive

containing 100 µg levonorgestrel and 20 µg ethinylestradiol. SHBG levels were similar for the two oral contraceptives, and APC resistance levels were more favorable during use of nomegestrol acetate with estradiol than during use of levonorgestrel with ethinylestradiol. Gaussem *et al*. observed no increase in markers for the risk of venous thrombosis during use of the new combined oral contraceptive containing nomegestrol actetate with estradiol compared with levonorgestrel with ethinylestradiol. Clinical data of independent studies are indicated to assess the absolute and relative risk of venous thrombosis during use of this new combined oral contraceptive.

Thyroid parameters and venous thrombosis

In **chapter 7** we investigated whether there is an association between the levels of thyroid parameters during use of hormonal contraceptives and the risk of venous thrombosis. We measured the thyroid parameters TBG, FT4 and TSH, as well as APC resistance determined by the thrombin generation-based APC resistance test. We observed that users of different hormonal contraceptives had different levels of TBG, TSH and FT4. A positive association was found between TBG levels and nAPCsr. Users of hormonal contraceptives associated with an increased risk of venous thrombosis had higher TBG levels than users of the LNG-IUD, which is not associated with an increased risk of venous thrombosis. TSH levels showed the same, but less pronounced, trend during hormonal contraceptive use: users of hormonal contraceptives with a high risk of venous thrombosis had higher TSH levels than users of low-risk hormonal contraceptives. FT4 levels stayed within the normal range, indicating that use of hormonal contraceptives does not lead to a hyperthyroid state. Therefore, the increased risk of venous thrombosis during use of hormonal contraceptives cannot be explained by hypercoagulability caused by a hyperthyroid state as a result of hormonal contraceptive use.

The increased levels of TBG in users of combined oral contraceptives are in concordance with other studies (3;22;31-34). We found a less pronounced increase in TSH levels and hardly any changes in FT4 levels, as was also observed by Ågren *et al*. and Sänger *et al*. (32;33).

Currently, a biological explanation of the increased risk of venous thrombosis during use of combined hormonal contraceptives is lacking. It seems likely that the liver plays a modulating role in the increased risk of venous thrombosis during use of combined hormonal contraceptives: the serum levels of hepatic binding globulins such as TBG and SHBG are increased during use of combined hormonal contraceptives, coagulation factors are produced in the liver and hormonal contraceptives are metabolized in the liver. Possibly, combined hormonal contraceptives interfere with the synthesis of both binding globulins and coagulation factors. Estrogens regulate levels of polymorphic glycoproteins, e.g. hepatic binding globulins, by affecting their sialylation (35). Sialic acid occupies the terminal ends of oligosaccharides of glycoproteins and prevents degradation of these molecules. A high sialic acid content leads to slower breakdown and higher circulating serum levels of glycoproteins (36). Besides binding globulins such as TBG and SHBG, coagulation factors (e.g. FV) and anticoagulation factors (e.g. APC) are all glycoproteins and therefore contain sialic acid. Hau *et al*. observed that desialylated APC was more active than normal APC, suggesting

that sialic acid inhibits the activity of APC (37). Fernández *et al*. studied the protective role of the sialic acid content of FV in its inactivation by APC and found that desialylation of FV increases its susceptibility for proteolytic inactivation by APC (38). Therefore, in the case of increased sialic acid, it is possible that FV becomes more resistant for the action of APC which increase the thrombotic risk. We hypothesize that the increased sialic acid compound of glycoproteins, as a result of estrogen administration during use of hormonal contraceptives, contributes to the increased risk of venous thrombosis associated with hormonal contraceptive use. Further research is indicated to assess the effect of different hormonal contraceptives on the sialic acid content of glycoproteins affected by hormonal contraceptive use, such as TBG, SHBG and FV.

Recommendations

Clinical

- The combined oral contraceptive with 30 µg ethinylestradiol and 150 µg levonorgestrel is recommended over other combined hormonal contraceptives.
- The new oral contraceptive containing dienogest with estradiol valerate has a similar thrombotic profile as levonorgestrel with ethinylestradiol.
- A quadriphasic regimen is not favorable compared with monophasic preparations regarding effectiveness, side effects and acceptability.
- Use of hormonal contraceptives does not lead to a subclinical hyperthyroid state.

Research

- Clinical epidemiological studies are necessary to assess the absolute and relative risk of the quadriphasic oral contraceptive containing dienogest with estradiol valerate, and to confirm the hypothesis that this quadriphasic oral contraceptive has a similar risk of venous thrombosis as the monophasic oral contraceptive containing levonorgestrel with ethinylestradiol.

- Independent laboratory studies are indicated to estimate the thrombotic risk of the new monophasic oral contraceptive containing nomegestrol acetate with estradiol by measuring nAPCsr and SHBG levels. Clinical epidemiologic studies are indicated to assess the absolute and relative risk of venous thrombosis during use of this new oral contraceptive.
- Future studies examining the mechanism of the reduction of free PS and free TFPI during use of hormonal contraceptives are indicated to gain more insight in the basis of the increased risk of venous thrombosis.
- Before a new hormonal contraceptive is licensed or used in practice, the effect of the preparation on nAPCsr and SHBG levels should be evaluated to estimate the risk of venous thrombosis. These measurements should be included in the general benefit-risk analysis of the new preparation. The new prescription should be compared with an oral contraceptive containing 30 µg ethinylestradiol and 150 µg levonorgestrel.
- Laboratory studies are indicated to assess the biological explanation for the association between the changes in SHBG levels and nAPCsr induced by hormonal contraceptives.
- Sialic acid should be measured in users of different hormonal contraceptives to investigate the role of sialic acid and its influence on glycoproteins in the etiology of venous thrombosis during use of hormonal contraceptives.

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