Effectiveness and side effects of hormonal contraceptives

Raps, M.

CHAPTER 4

Sex hormone-binding globulin as a marker for the thrombotic risk of hormonal contraceptives

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Abstract

Background:

It takes many years to obtain reliable values for the risk of venous thrombosis of hormonal contraceptive users from clinical data. Measurement of activated protein C (APC) resistance via thrombin generation is a validated test for determining the thrombogenicity of hormonal contraceptives. Sex hormone-binding globulin (SHBG) might serve as a marker for the risk of venous thrombosis, and can be easily and rapidly measured in routine laboratories.

Objective:

To determine whether SHBG is a useful marker for the thrombotic risk of hormonal contraceptive users by comparing plasma SHBG levels with normalized APC sensitivity ratio (nAPCsr) values and thrombosis risks reported in the recent literature.

Methods:

We conducted an observational study in 262 users of different contraceptives, and measured nAPCsr and SHBG levels.

Results:

Users of contraceptives with a higher risk of causing venous thrombosis, i.e. combined hormonal contraceptives containing desogestrel, cyproterone acetate or drospirenone, and the transdermal patch, had higher SHBG levels than users of combined hormonal contraceptives containing levonorgestrel, which carry a lower thrombosis risk. Users of the patch had the highest SHBG levels, with a mean difference of 246 nmol/L (95% confidence interval 179–349) from that in users of levonorgestrel-containing combined hormonal contraceptives. SHBG levels were positively associated with both the nAPCsr and the risks of venous thrombosis reported in the recent literature.

Conclusion:

SHBG is a useful marker with which to estimate the thrombotic safety of a preparation.

Introduction

The use of combined oral contraceptives is associated with a three-fold to six-fold increased risk of venous thrombosis (1). This increased risk depends on both the estrogen dose and the progestogen type of combined oral contraceptives (1). So-called 'high-dose' combined oral contraceptives containing 50 µg or more ethinylestradiol (EE) are associated with a two-fold higher risk of thrombosis than 'low-dose' combined oral contraceptives containing 20–30 µg of EE (2;3). Furthermore, combined oral contraceptives containing the progestogens gestodene (GTD), desogestrel (DSG), cyproterone acetate (CPA) or drospirenone (DRSP) increase the risk of venous thrombosis by a factor of two as compared with combined oral contraceptives containing levonorgestrel (LNG) (1–13).

The differences in the risk of venous thrombosis can be at least partially explained by the association of various combined oral contraceptives with differences in resistance to activated protein C (APC) as measured with the thrombin generation-based APC resistance test and quantified via a normalized APC sensitivity ratio (nAPCsr) (14–16). High nAPCsr indicates increased APC resistance, which is a risk factor for venous thrombosis. Thrombin generation-based APC resistance has been validated in a case–control study by Tans *et al.* (17), and predicts the risk of venous thrombosis in users of combined oral contraceptives, as well as in non-users and men, with or without the factor V Leiden mutation. The highest odds ratio (OR) of venous thrombosis in the absence of the FV Leiden mutation was observed in premenopausal women using combined oral contraceptives, lending support to the hypothesis that the prothrombotic effect of combined oral contraceptives is the result of acquired APC resistance in a thrombin generation-based test (17). Users of combined oral contraceptives with a higher risk of causing venous thrombosis, e.g. those containing DSG, CPA or DRSP, have been found to be more resistant to the anticoagulant action of APC than users of combined oral contraceptives with a lower risk of causing venous thrombosis, i.e. those containing LNG (3;6;9;10;14–16).

As the absolute risk of venous thrombosis in women using combined oral contraceptives is low, i.e. three to four per 10 000 woman-years (1), the assessment of differences in risk between an existing and a new preparation requires hundreds of thousands of users. This sample size makes a clinical study of a new hormonal contraceptive before market authorization almost impossible.

In a search for other markers that can predict the risk of venous thrombosis in users of hormonal contraceptives, Odlind *et al.* (18) postulated sex hormone-binding globulin (SHBG) as a marker for estrogenicity of a contraceptive preparation and possibly for the risk of venous thrombosis. SHBG is a carrier protein that is produced in the liver and binds estrogen and testosterone (19). The hypothesis is that estrogens cause a dose-related increase in SHBG levels, whereas progestogens induce a decrease in SHBG levels, dependent on both the dose and the type of progestogen (20–22). The type-related differences in the progestogen-induced decrease in SHBG levels can be interpreted as differences in the antiestrogenic properties of progestogens. Thus, the effect of a hormonal contraceptive on SHBG is the combined result of the estrogenic effect of EE and the antiestrogenic effect of the progestogen, yielding the total estrogenicity of that hormonal

contraceptive. This estrogenicity might serve as a marker for venous thrombosis. Several studies have shown an association between the risk of causing venous thrombosis of combined oral contraceptives, APC resistance, and SHBG levels (1–3;15;23).

To investigate whether SHBG is a useful marker for the risk of venous thrombosis of combined oral contraceptives, we determined SHBG levels in non-users and in users of different contraceptives, both hormonal and non-hormonal, and compared the SHBG levels with nAPCsr as determined via thrombin generation and with the risks of venous thrombosis as reported in the literature.

Materials and methods

Study design and participants

We conducted an observational study. In a series of four different studies, we included users of various hormonal and non-hormonal contraceptives (15;24–26). Users of different combined hormonal contraceptives, including oral, transdermal and vaginal combined hormonal contraceptives, users of LNG-releasing intrauterine devices (IUDs) (LNG-IUDs), users of copper-releasing IUDs (Cu-IUDs) and healthy female non-users with regular, ovulatory menstrual cycles were studied.

The inclusion criterion for all participants was as follows: healthy women using a hormonal contraceptive for at least three cycles. Exclusion criteria were age < 18 years, and contraindications for combined hormonal contraceptive use as stated by the World Health Organization (27). A more detailed description can be found in the original articles (15;24–26).

Participants who were carriers of the FV Leiden mutation were excluded from the analysis, because this mutation causes resistance to APC without affecting SHBG levels (n = 30). The following data were not used because of a small sample size: users of a combined oral contraceptive containing GTD, norgestimate and norethisterone (n = 3 for GTD, n = 1 for norgestimate, and n = 2 for norethisterone). Furthermore, we only used data from users of combined oral contraceptives containing 30–35 µg of EE; users of preparations with other amounts of EE were excluded (n = 24). For 26 participants, data were not complete, so they were excluded. In total, we excluded 86 participants.

In our final analysis, we used the samples of 262 participants: 159 users of a combined oral contraceptive (containing 30–35 µg of EE and LNG, DSG, CPA, or DRSP), 60 users of the LNG-IUD, 17 users of the Cu-IUD, seven users of the transdermal patch (containing EE and norelgestromine (NGM)), six users of the vaginal ring (containing EE and etonogestrel (ENG)), and 13 non-users (mid-cycle).

Written informed consent was given by all participants, and the studies were all approved by theMedical Ethics Committee of the Leiden University Medical Center, The Netherlands.

Laboratory methods

The plasma samples from the studies were taken, processed and stored identically. Blood samples were taken from the antecubital vein in the morning in a fasting state, and collected in 0.106 mol/L sodium citrate (pH 5.8). Cell-free, citrated plasma was prepared by centrifuging blood at 2.100 g for 10 min at 18 °C, coded, and centrally stored at - 80 °C.

SHBG (nmol/L) was measured with an immunometric assay (Immulite 2000 XPi; Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The sensitivity is 0.2 nmol/L, and has a long-term variation of 6%, at levels of both 5 and 80 nmol/L The within-assay variation is 3–4%, and the between-assay variation is 3.5–6%. APC resistance was measured with the thrombin generation-based APC resistance test, as described previously (14).

nAPCsr values of plasma samples from women using an LNG-IUD or a Cu-IUD were originally measured with a variant of the thrombin generation-based APC resistance assay, by the use of using calibrated automated thrombinography (24;28). As nAPCsr values determined with calibrated automated thrombinography are higher than those determined with the classical endpoint method (16;29), the plasma samples from IUD users were reanalyzed with the endpoint method.

SHBG levels and APC resistance in non-users during midcycle were used in the analysis. The different phases in the menstrual cycle were defined by repeated measurements of progesterone and estradiol levels; mid-cycle is defined as the time when estradiol levels are high and progesterone levels are low.

Statistical analysis

We used means, mean differences, 95% confidence intervals and ranges to describe variables. We constructed a scatterplot to describe the association between SHBG levels and nAPCsr; in this figure SHBG data were logarithmically transformed to create normality and a histogram analysis of the residuals was performed to check whether this assumption is valid. A regression analysis was performed to describe the association.

Table 1: *Body mass index (BMI) and age of the research population*

Contraceptive	n	BMI (kg m^{-2})		Age (years)	
		Mean	Range	Mean	Range
None	13	21.7	19–29	29.0	20–48
LNG-IUD	60	24.5	18–47	32.6	17–52
Cu-IUD	17	24.2	18–32	32.4	20–45
LNG/EE	72	22.2	17–38	25.7	18–51
DSG/EE	18	24.0	20–32	30.2	18–49
DRSP/E	22	22.1	19–26	27.5	19–44
CPA/EE	22	22.1	19–26	27.5	19–44
ENG/EE (ring)	6	24.2	21–28	26.4	20–36
NGM/EE (patch)	7	22. 4	20–26	31.1	25–43
All	262	23.5	18–47	28.8	17–52

CPA, cyproterone acetate; Cu-IUD, copper-releasing intrauterine device; EE, ethinylestradiol; ENG, etonogestrel; DRSP, drospirenone; DSG, desogestrel; LNG, levonorgestrel; LNG-IUD, levonorgestrelre-leasing intrauterine device; NGM, norelgestromine.

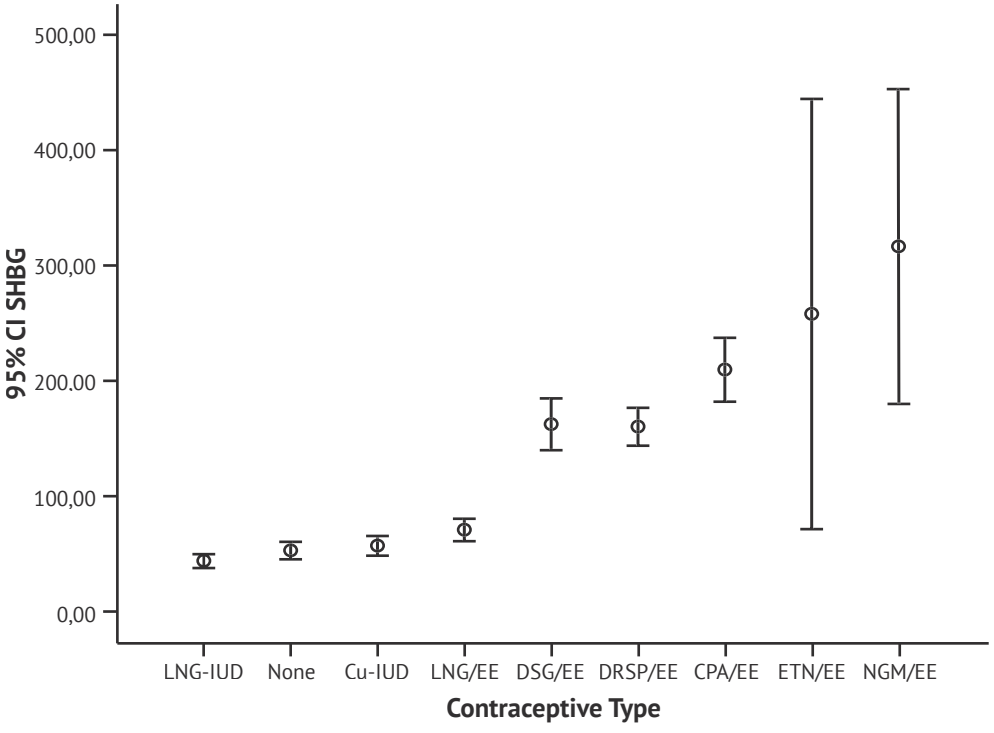

Fig. 1. *Sex hormone-binding globulin (SHBG) levels and their 95% confidence intervals (CIs) by contraceptive type. CPA, cyproterone acetate;Cu-IUD, copper-releasing intrauterine device; DRSP, drospirenone;DSG, desogestrel; EE, ethinylestradiol; ENG, etonogestrel; LNG, levonorgestrel; LNG-IUD, levonorgestrel-releasing intrauterine device; NGM, norelgestromine.*

Table 2: *Mean sex hormone-binding globulin (SHBG) and activated protein C (APC) resistance levels, mean differences (MDs) and 95% confidence intervals (CIs) for non-users as compared with levonorgestrel (LNG)/ethinylestradiol (EE) users*

Contraceptive	n	SHBG (nmol L^{-1})					APC-resistance (ratio)		
			Compared to non-use		Compared to LNG/EE		Compared to non-use		
		Mean	MD	95% CI	MD	95% CI	Mean	MD	95% CI
None	13	53.22	Ref		-17.78	-41.35 to 5.44	1.54	Ref	
LNG-IUD	60	43.77	-9.45	-22.08 to 3.17	-27.23	-39.03 to -15.44	0.85	-0.69	-1.03 to -0.36
Cu-IUD	17	57.52	4.29	-7.26 to 15.85	-13.48	-34.00 to 7.03	1.03	-0.51	-0.93 to -0.09
LNG/EE	72	71.00	17.78	-5.46 to 41.02	Ref		2.66	1.12	0.69 to 1.54
DSG/EE	18	162.78	109.55	82.98 to 136.13	91.78	69.60 to 113.96	3.94	2.40	1.93 to 2.86
DRSP/EE	47	161.04	107.82	7.10 to 139.54	90.04	72.23 to 107.85	3.53	1.98	1.49 to 2.48
CPA/EE	22	210.27	157.05	121.03 to 193.07	139.27	116.41 to 162.13	4.00	2.46	2.07 to 2.84
ENG/EE (Ring)	6	258.93	205.71	104.77 to 306.65	187.93	136.51 to 239.36	3.02	1.47	0.94 to 2.02
NGM/EE (Patch)	7	317.57	264.35	179.63 to 349.06	246.57	201.29 to 291.85	3.12	1.57	0.87 to 2.28

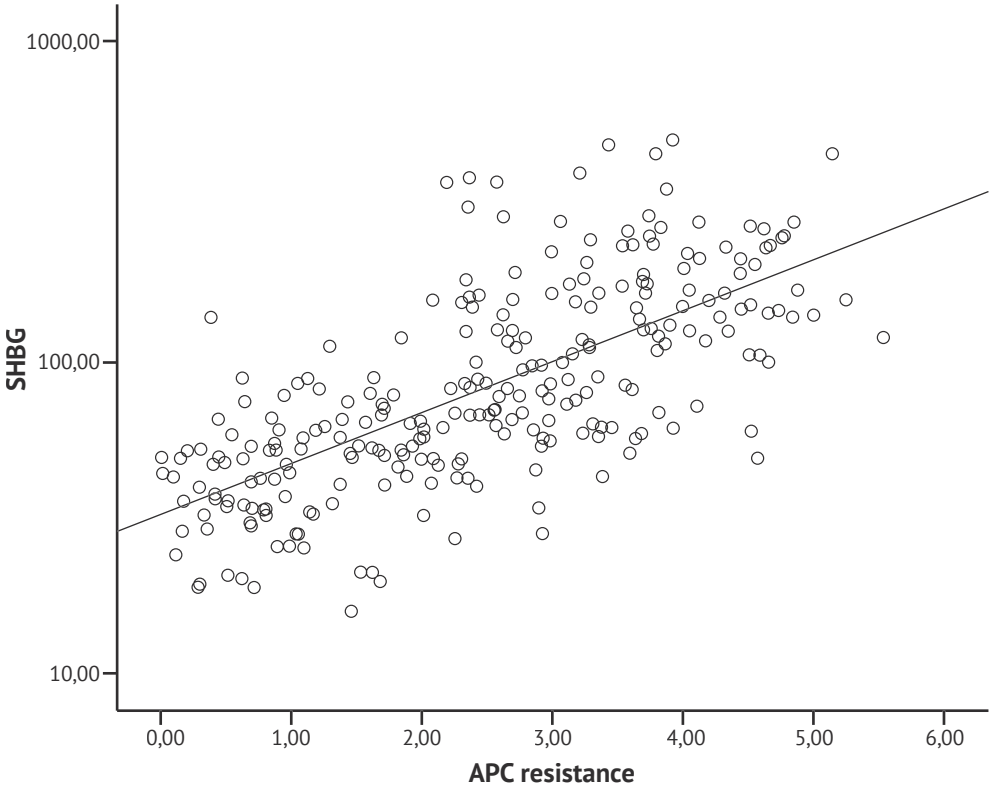

Fig. 2. *The association between sex hormone-binding globulin (SHBG) and activated protein C (APC) resistance. Equation: log10(SHBG) = 1.525 + (0.160 × nAPCsr).*

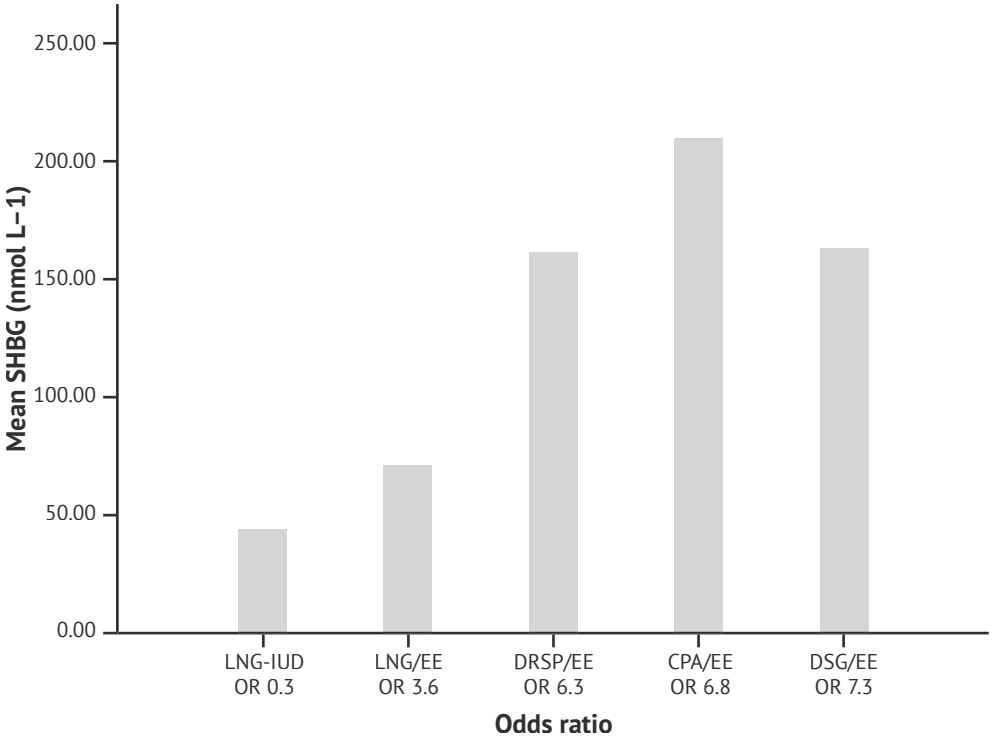

Fig. 3. *The association between odds ratios (ORs) of the risk of venous thrombosis of various contraceptives as published in the recent literature [3,31,32] and sex hormone-binding globulin (SHBG) levels of hormonal contraceptives. CPA, cyproterone acetate; DRSP, drospirenone; DSG, desogestrel; EE, ethinylestradiol; LNG, levonorgestrel; LNG-IUD, levonorgestrel-releasing intrauterine device.*

Table 3. *The odds ratios (ORs) of venous thrombosis during the use ofdifferent types of hormonal contraceptive as compared with non-users,according to the recent literature [3,31,32]*

Contraceptive	Risk		Reference
	OR	95% CI	
None	Ref		
LNG-IUD	0.3	0.1 to 1.1	(31)
Cu-IUD	-	-	
LNG/EE	3.6	2.9 to 4.6	(3)
DSG/EE	7.3	5.3 to 10.0	(3)
DRSP/EE	6.3	2.9 to 13.7	(3)
CPA/EE	6.8	4.6 to 10.0	(3)
ETN/EE	-	-	
NGM/EE	1.3 to 2.0	-	(32)

CI, confidence interval; CPA, cyproterone acetate; Cu-IUD, copperreleasing intrauterinedevice;DRSP, dros pirenone;DSG,desogestrel;EE, ethinylestradiol; ENG, etonogestrel; LNG, levonorgestrel; LNG-IUD, levonorgestrel-releasing intrauterine device; NGM, norelgestromine.

Results

There were no significant differences in BMI and age between the women using different kinds of hormonal contraceptives (Table 1).

SHBG levels during contraceptive use

SHBG levels of the studied contraceptives were compared to non-users and to users of the most used combined oral contraceptive containing LNG/EE. Users of contraceptives containing EE plus CPA, DRSP or DSG and users of the transdermal patch or vaginal ring had higher SHBG levels than users of the LNG/EE containing combined oral contraceptive. Users of the LNG-IUD or Cu-IUD had lower or comparable SHBG levels as non-users. (Fig. 1, Table 2).

Association between SHBG and APC resistance

SHBG plasma levels were positively associated with nAPCsr in users of different kinds of hormonal contraceptives (i.e. combined oral contraceptives and LNG-IUD) and non-users. An exponential association was observed according the equation: log10(SHBG) = 1,525 + 0,160 × nAPCsr. Thus, when the nAPCsr increases with 1 unit, SHBG levels increase with 45% (100.160 = 1.45) (Fig. 2).

Risk ranking per contraceptive

For risk ranking, we used recent publications by van Hylckama Vlieg *et al* (3) and Jick *et al* (30). (Table 3) The observed odds ratio for venous thrombosis during use of the LNG-IUD compared to non-users was 0.3 (95% CI 0.1 to 1.1) (3) and the observed odds ratio during use of the transdermal patch compared to use of the LNG containing combined oral contraceptives was variable and reported to be between 1.3 and 2.0 (30). The risk of venous thrombosis during use of a Cu-IUD is unknown, but expected not to be increased compared to non-users. There are no data on the contraceptive vaginal ring compared to non-users, but a study on the risk of venous thrombosis of the contraceptive ring showed an 1.56 fold increased risk compared to a group of combined oral contraceptives with low estrogen (13).

SHBG levels measured in this study are associated with the odds ratios reported in recent literature: higher SHBG levels are present in users of contraceptives with a higher risk of venous thrombosis (Table 3, Fig. 3).

Discussion

In this study we observed positive associations between the effects of hormonal contraceptives on SHBG levels, the nAPCsr and the thrombotic risk reported in recent literature. High nAPCsr in the thrombin-generation based test indicate increased resistance to APC and is reported to be a risk factor for venous thrombosis (11). Together, these observations support the hypothesis that not only the APCsr, but also SHBG levels are a marker for the risk of venous thrombosis during the use of hormonal contraceptives.

The use of the LNG-IUD did not increase SHBG levels, which is in concordance with recent clinical data. In a national cohort study by Lidegaard *et al* (12), users of the LNG-IUD had no increased risk of thrombosis compared to non-users (RR 0.83 and 95% CI 0.63 to 1.08). This was confirmed by van Hylckama Vlieg *et al* (31) who also did not find an increased risk in a recent case-control study (OR 0.3 and 95% CI 0.1 to 1.1).

Limited data are available on the thrombotic risk of the contraceptive transdermal patch and vaginal ring. Conflicting results have been reported on the thrombotic safety of the contraceptive patch with estimates of the thrombotic risk varying between 0.9 (95%CI 0.5 to 1.6) (32) to 2.4 (95% CI 1.1 to 5.5) (33) compared to oral contraceptives containing norgestimate and EE (29;30;34).

Recently, the first study on the risk of venous thrombosis of the contraceptive ring has been published by the FDA (13). Use of the vaginal ring was associated with a 1.56-fold (95% CI 1.02 to 2.37) higher risk of thrombosis compared to a group of combined oral contraceptives with low estrogen. The study also observed a 1.55-fold (95% CI 1.02 to 2.37) higher thrombotic risk during use of the transdermal patch. In our study, users of the vaginal ring and the transdermal patch had the highest SHBG levels of all contraceptive users. These results are in agreement with earlier studies, reporting an increase in SHBG of ~260% for transdermal patch users and ~150% for vaginal ring users compared to pre-treatment levels (18;26). The increased SHBG levels in women using the patch and ring compared to women using combined oral contraceptives containing LNG suggest an increased thrombotic risk.

The increased risk of the vaginal ring might be explained by the fact that etonogestrel (ENG) is the active metabolite of DSG. According to recent literature, use of combined hormonal contraceptives containing DSG is associated with a 1.82-fold (95% CI 1.49 to 2.22) higher risk of venous thrombosis compared to use of combined oral contraceptives containing LNG/EE (6). However, in women using the contraceptive ring peak serum concentrations of EE and DSG are significantly lower than in women using a combined oral contraceptive containing DSG and EE (35).

The increased risk of the transdermal patch might be explained by the 60 percent higher exposure to EE as measured by the area under the curve and steady state concentration during use of the contraceptive patch compared to use of an oral contraceptive composed of norelgestromine (NGM) and EE. NGM exposure is similar during use of the contraceptive patch and pill (36;37). Since the increased SHBG levels in users of the patch and ring in our study are based on a small number of participants, further studies are indicated to confirm these results and to draw definite conclusions.

The difference in SHBG levels between the hormone preparations was not the result of differences between women but rather between contraceptive methods as evidenced by the women who switched from one contraceptive type to another in the original studies. For example, switching from a combined hormonal contraceptive containing CPA to a combined hormonal contraceptive containing LNG, resulted in a mean decrease of SHBG by 150 nmol/L (95% CI -206 to -94) (6;19;20).

Currently, a biological explanation for the association between the changes in SHBG and APC resistance induced by hormonal contraceptives is lacking. It is known that estrogen increases the risk of venous thrombosis and that a higher dose is associated with a higher risk. We propose that SHBG reflects 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. There are now different studies demonstrating an association between SHBG and the risk of venous thrombosis. However, the mechanism is still not known and further research is needed to unravel the association, changes in other proteins produced in the liver, changes of haemostatic parameters and the increased risk of venous thrombosis.

We acknowledge that caution is required when using surrogate markers since they can be severely misleading (38). Preferably, a surrogate marker should be validated in a prospective trial in which both the surrogate marker and the clinical endpoint are assessed. However, in case of very rare events, such as venous thrombosis during combined hormonal contraceptive use, a clinical study is almost unfeasible due to the required number of participants. In order to prospectively 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 must be followed for one year (27). Case-control studies only become possible post-marketing (27;39). Such a large sample size makes it almost impossible for a pharmaceutical company to evaluate the risk of venous thrombosis of a new preparation before market authorization.

There are now reasonably reliable data of the risk of venous thrombosis from several epidemiological studies showing that the combination of EE plus LNG carries the lowest risk of venous thrombosis of all combined hormonal contraceptives (1;3;5;6). Comparing the SHBG levels in users of a new preparation with that of EE plus LNG could give an estimation of the magnitude of the risk of venous thrombosis before a new preparation is launched and should be included in the general benefit-risk analysis of the new preparation. SHBG measurement is already recommended in guidelines during clinical development of a new combined hormonal contraceptive by the European Medicines Agency (EMA).

In conclusion, our data support that SHBG could be a useful marker for estimating the risk of venous thrombosis of a new hormonal contraceptive. Preferably, the effect of a new hormonal contraceptive on SHBG should be compared with the effect of the combined hormonal contraceptive with the lowest reported risk of venous thrombosis, i.e. an oral preparation containing EE plus LNG.

References

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

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

3 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.

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

5 Effect of different progestagens in low oestrogen oral contraceptives on venous thromboembolic disease. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Lancet 1995;346, 1582-1588.

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

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

8 Van Hylckama Vlieg A, Middeldorp S. Hormone therapies and venous thromboembolism: where are we now? J Thromb Haemost 2011;9:257-66.

9 Jick SS, Hernandez RK. Risk of non-fatal venous thromboembolism in women using oral contraceptives containing drospirenone compared with women using oral contraceptives containing levonorgestrel: case-control study using United States claims data. BMJ 2011;342:d2151.

10 Parkin L, Sharples K, Hernandez RK, Jick SS. Risk of venous thromboembolism in users of oral contraceptives containing drospirenone or levonorgestrel: nested case-control study based on UK General Practice Research Database. BMJ 2011;342:d2139.

11 Gronich N, Lavi I, Rennert G. Higher risk of venous thrombosis associated with drospirenone-containing oral contraceptives: a population-based cohort study. CMAJ 2011 ;183:E1319-E1325.

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

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

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

15 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.

16 Tchaikovski SN, Van Vliet HA, Thomassen MC, Bertina RM, Rosendaal FR, Sandset PM, Helmerhorst FM, Tans G, Rosing J. Effect of oral contraceptives on thrombin generation measured via calibrated automated thrombography. Thromb Haemost 2007;98:1350-6.

17 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.

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

19 Gardner DG. Greenspan's Basic & Clinical Endocrinology. 8th edn. New York: McGraw-Hill Companies, Inc., 2007.

20 Van der Vange N, Blankenstein MA, Kloosterboer HJ, Haspels AA, Thijssen JH. Effects of seven low-dose combined oral contraceptives on sex hormone binding globulin, corticosteroid binding globulin, total and free testosterone. Contraception 1990;41:345-52.

21 Knopp RH, Broyles FE, Cheung M, Moore K, Marcovina S, Chandler WL. Comparison of the lipoprotein, carbohydrate, and hemostatic effects of phasic oral contraceptives containing desogestrel or levonorgestrel. Contraception 2001;63:1-11.

22 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 V Leiden mutation: a randomized trial. Blood 2004;103:927-33.

23 Van Rooijen M, Silveira A, Hamsten A, Bremme K. Sex hormone-binding globulin --a surrogate marker for the prothrombotic effects of combined oral contraceptives. Am J Obstet Gynecol 2004;190:332-7.

24 Van Vliet HA, Tchaikovski SN, Rosendaal FR, Rosing J, Helmerhorst FM. The effect of the levonorgestrel-releasing intrauterine system on the resistance to activated protein C APC. Thromb Haemost 2009;101:691-5.

25 Van Vliet HA, Rodrigues SP, Snieders MN, van der Meer FJ, Frolich M, Rosendaal FR, Rosing J, Helmerhorst FM. Sensitivity to activated protein C during the menstrual cycle in women with and without factor VLeiden. Thromb Res 2008;121:757-61.

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

27 WHO. Medical eligibility criteria for contraceptive use. 4th ed. Geneva: WHO; 2009. Available at http://www.who.int/reproductivehealth/publications/family_planning/9789241563888/en/index.html 2011.

28 Tchaikovski S. Hormone-induced changes in the coagulation system. Thesis Maastricht University, 2009. Universitaire Pers Maastricht, Maastricht, the Netherlands.

29 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.

30 Jick SS, Hagberg KW, Hernandez RK, Kaye JA. Postmarketing study of ORTHO EVRA and levonorgestrel oral contraceptives containing hormonal contraceptives with 30 mcg of ethinyl estradiol in relation to nonfatal venous thromboembolism. Contraception 2010;81:16-21.

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

32 Jick SS, Kaye JA, Russmann S, Jick H. Risk of nonfatal venous thromboembolism in women using a contraceptive transdermal patch and oral contraceptives containing norgestimate and 35 microg of ethinyl estradiol. Contraception 2006;73:223-8.

33 Cole JA, Norman H, Doherty M, Walker AM. Venous thromboembolism, myocardial infarction, and stroke among transdermal contraceptive system users. Obstet Gynecol 2007;109:339-46.

34 Jick SS, Kaye JA, Li L, Jick H. Further results on the risk of nonfatal venous thromboembolism in users of the contraceptive transdermal patch compared to users of oral contraceptives containing norgestimate and 35ug of ethinyl estradiol. Contraception 2007;76: 4-7

35 Timmer CJ, Mulders TM. Pharmacokinetics of etonogestrel and ethinylestradiol released from a combined contraceptive vaginal ring. Clin Pharmacokinet 2000 Sep;393:233-42.

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

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

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

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