

# Sex hormone receptors in varicose veins of women with premenstrual syndrome

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## Keywords

Sex hormones, varicose veins, premenstrual tension syndrome

## Schlüsselwörter

Geschlechtshormone, Varizen, prämenstruelles Spannungssyndrom

## Mots clés

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## Summary

The pathogenesis of premenstrual tension syndrome is not fully understood. It has been hypothesized that the interaction between sex hormones and target organs is the key event in its pathogenesis. **Aim:** The purpose of the study was to examine the differences in the prevalence of smooth muscle cells with sex hormones receptors in varicose veins of women with and without premenstrual tension syndrome (PS). **Patients, methods:** Samples of great saphenous vein were obtained from 50 women during varicose vein surgery. They were divided into group I (20 women with clinically diagnosed PS) and group II (30 women without PS). Estrogen and progesterone receptors were detected with an immunohistochemical method. Superficial densities of smooth muscle cells positive to estrogen and progesterone receptors were analyzed with densitometric program IM-AN and automatic image analyzer Magicall. The results were compared with t-Student test. **Results:** There were no differences in superficial density of smooth muscle cells positive to estrogen receptors between the groups. In contradiction to this, superficial density of smooth muscle cells positive to progesterone receptors was higher in group with PS than in patients without, 343 ( $\pm 171$ ) and 240 ( $\pm 123$ ), respectively. The difference was statistically significant ( $p < 0.05$ ). **Conclusion:** The amount of cells with sex hormone receptors, not the level of hormones, could play a role in PS pathology. Our results show that progesterone is more important than estrogen in effector organs.

## Zusammenfassung

Die Pathogenese des prämenstruellen Spannungssyndroms (PS) ist nicht geklärt. Es gibt eine Hypothese, nach der die Interaktion zwischen Geschlechtshormonen und betroffenen Organen eine Schlüsselrolle in der Pathogenese zukommt. **Ziel** dieser Untersuchung war die Prüfung der Unterschiede in der Prävalenz der glatten Muskelzellen mit Rezeptoren der Geschlechtshormone in Varizen der Frauen mit und ohne PS. **Patienten, Methode:** Proben aus der großen Rosavene bei 50 Frauen wurden während der Varizenoperation entnommen. Sie wurden unterteilt in Gruppe I (20 Frauen mit PS) Gruppe II (30 Frauen ohne PS). Die Östrogen- und Progesteronrezeptoren wurden immunhistochemisch nachgewiesen. Die oberflächliche Dichten glatter Muskelzellen, die auf Östrogen- und Progesteronrezeptoren positiv reagierten, wurden densitometrisch mit einem IM-AN-Programm und automatischer Bildanalyse Magicall analysiert. Die **Ergebnisse** wurden nach t-Student ausgewertet. Es wurden keine Unterschiede in oberflächlicher Dichte der glatten auf Östrogenrezeptoren positiv reagierenden Muskelzellen in den beiden Gruppen festgestellt. Dagegen war die oberflächliche Dichte der glatten auf Progesteronrezeptoren positiv reagierender Muskelzellen in der PS-Gruppe höher als bei Frauen ohne PS, entsprechend 343 ( $\pm 171$ ) und 240 ( $\pm 123$ ). Der Unterschied war statistisch signifikant ( $p < 0,05$ ). **Schlussfolgerung:** Progesteron scheint eine Rolle in der Pathogenese von PM zu spielen, die eher von der Zahl der auf Progesteronrezeptoren positiv reagierenden Muskelzellen in betroffenen Geweben als von dessen Konzentration abhängt.

## Résumé

La pathogenèse du syndrome prémenstruelle n'est pas complètement connue. Il y a une hypothèse selon laquelle l'interaction entre les hormones sexuelles et les organes de cible serait d'importance primordiale pour la pathogenèse de ce syndrome. Cet étude avait pour **but** d'examiner les différences de prevalence des cellules de muscles viscéraux avec récepteurs des hormones sexuelles dans les varices chez les femmes avec et sans syndrome de tension prémenstruelle. **Méthode:** Les échantillons ont été prélevés de la grande veine saphène chez 50 femmes pendant l'opération de varices. Les patientes ont été divisées en groupe 1 (20 femmes avec le syndrome de tension prémenstruelle cliniquement constaté) et groupe 2 (30 femmes qui n'étaient pas atteintes). Les récepteurs de l'estrogène et de la progesterone étaient détectés par une méthode immunohistochemique. Les densités superficielles des cellules de muscles viscéraux ayant la réaction positive aux récepteurs de l'estrogène et de la progesterone étaient analysées par programme densitométrique IM-AN et analyseur automatique d'images Magicall. Les **resultats** ont été analysés par test statistique de comparaison (t-Student). Il n'a pas été observé la différence de prevalence des cellules de muscles viscéraux ayant la réaction positive aux récepteurs de l'estrogène entre les deux groupes examinés. Par contre, la différence de prevalence des cellules de muscles viscéraux ayant la réaction positive aux récepteurs de la progesterone était plus élevée dans le groupe atteint du syndrome de tension prémenstruelle par rapport à l'autre, en se chiffrant respectivement à 343 ( $\pm 171$ ) et 240 ( $\pm 123$ ). La différence était statistiquement significative ( $p < 0,05$ ). **Conclusion:** La progesterone puisse jouer un rôle important dans la pathogenèse du syndrome prémenstruelle. Cela dépend plus du nombre des cellules réagissant positivement aux récepteurs de la progesterone dans les organes de cible que du niveau de cette hormone.

About 75% of women in the reproductive age suffer from symptoms related to the premenstrual tension syndrome (PS) (4). PS is defined as somatic and psychological symptoms severe enough to limit the daily activity of a woman regularly occurring in premenstrual phase (4, 16, 19). Primary varicose veins (PVV) are the most frequent symptoms of chronic venous insufficiency (CVI). Data shows that symptoms related to PVV are observed in up to 50–55% of adult women. So it may be assumed that in 20–55% of women both – PS and PVV – are present.

Research concerning PS or PVV is scarce. However, data demonstrate that 5–33% of women with PS suffer from symptoms related to varicose veins (i. e. pain, dilatation of varices) (4, 10). Clinical and epidemiological observations of PVV point to some influence of sex hormones on its incidence and prevalence. Increased venous stasis in the lower extremities was noticed during sex hormone therapy, the luteal phase of the menstrual cycle and the 1<sup>st</sup> trimester of pregnancy (15, 21). Symptoms of PS are observed after ovulation and intensify to fifth day before menstruation and are convergent to venous symptoms (4, 10, 16, 19). The symptoms of PS in the luteal phase of the menstrual cycle could be explained by the role of sex hormones in its pathogenesis. However, there is a lack of scientific data to prove this theory (17).

Recent research shows that women with PS after total hysterectomy with ovariectomy along with constant estrogen substitution therapy had no symptoms of PS. However, symptoms occurred when progesterone or progestagen therapy was administered and were positively correlated with hormone concentrations (16). Superficial vein dilatation was also observed during estrogen, progestagen therapy or hormonal contraception. It was proven that progesterone may influence the vascular endothelial complex (10, 13) by inducing

- an increase of vasodilatation and
- a decrease of vascular wall tone.

Based on our observations and data from the literature, it may be assumed that sex hormone receptors localized within venous walls play an important role in the develop-

ment of PVV (1, 9, 24). In vitro and in vivo research proves that sex hormones may have a direct influence on blood vessels (1, 5, 6, 12). Actual publications suggest that estrogen therapy just after menopause protects against atherosclerotic changes in coronary arteries not only indirectly through the metabolism of lipoprotein, but also directly through estrogen receptors localized in the intima and smooth muscle cells (9). It was observed that estrogen inhibits smooth muscle cell proliferation and stimulates cell migration and matrix protein secretion (5, 25). Similar observations of progesterone's influence on the vascular wall were published (18).

With regard to PS, it is believed that cyclic biochemical changes in the central nervous system or other target tissues result from physiological function of the ovaries and not from the hormonal balance disorder. Varicose veins are one of the target tissues which is easily available for histopathological verification. Our previous studies revealed the presence of sex hormone receptors both in healthy veins and varicose veins. They were localized mostly in smooth muscle cells (1, 16, 18). Thus, vein samples obtained from women with PS are an adequate material to study the pathogenesis of PS on the level of target organs. The aim of our study was to verify differences in the prevalence of smooth muscle cells with sex hormone receptors in varicose veins of women with and without PS.

## Patients, methods

A group of 50 women with PVV was analyzed. To be included they had to have regular menstrual cycles and did not receive any hormonal therapy. In 20 women PS was diagnosed, whereas the other 30 women were without this condition. For the diagnosis of PS a woman had to suffer from one or more of the following symptoms:

- psychological discomfort of average degree,
- bloating,
- increased body weight,
- breast tenderness,
- oedema of hands and feet,
- pain of various degree,

- concentration or sleep disorders,
- change of appetite,

and the symptoms had to

- start in luteal phase and
- disappear with the beginning of menstruation.

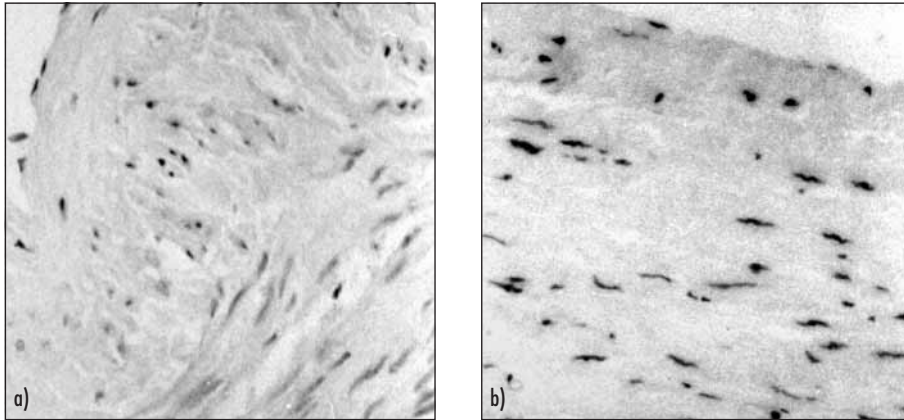
The average age of women with PS was 29 ( $\pm 6,5$ ) years and without PS 31 ( $\pm 7,3$ ) years. This difference was not statistically significant. With regard to the differences in symptoms between groups, the aching of varicose veins in premenstrual phase was more frequent in PS and PVV group. This difference did not reach statistical significance neither.

In all 50 women, samples of the great saphenous vein were obtained during varicose vein surgery. Estrogen and progesterone receptors were detected with an immunohistochemical method, that involved forming of avidine-biotin-peroxidase complex using Novocastra Laboratories reagents.

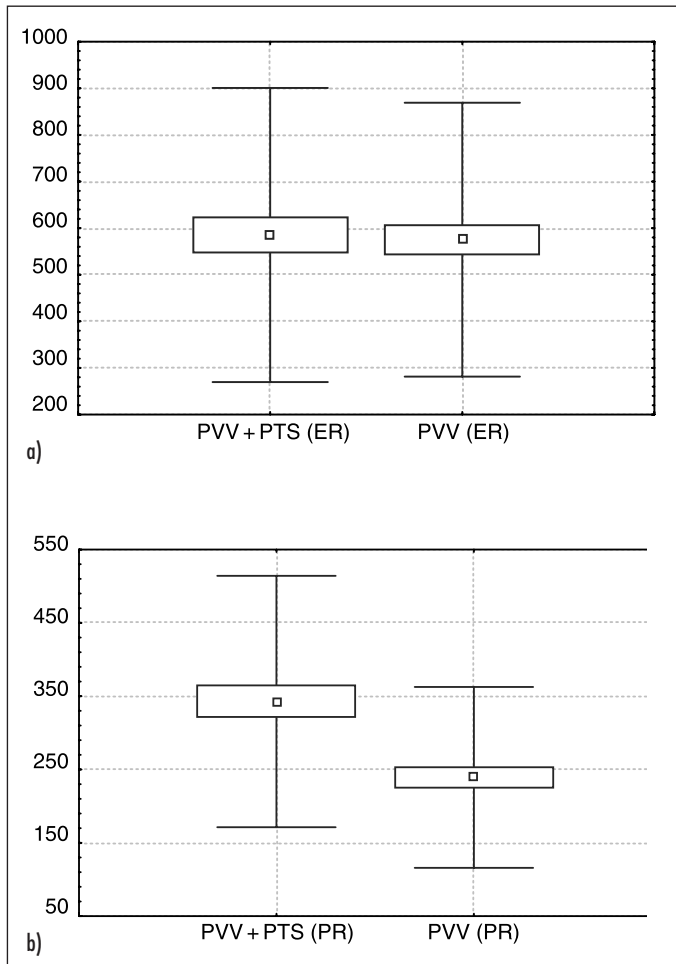
Collected samples were fixed in a 4% solution of formaldehyde in a pH of 7.4 for five days. Then they were rinsed with running water for 12 hours, dehydrated with alcohol series, fluoroscoped by benzene and embedded in paraffin. Ultrathin (3  $\mu$ m) paraffin sections were placed on slides covered by an adhesive substance (a 2% solution of 3-aminopropyltriethoxysilan in acetone).

## Estrogen and progesterone receptor detection

The samples were deparaffinated in xylene for one hour and rehydrated in an alcohol series (100%, 96%, 90%, 80%, 70%, 50%, distilled water). The endogenous peroxidase was blocked by 30 minutes bath in a 0.12% solution of hydrogen peroxide in methanol. Then the samples were rinsed for 10 minutes with running water and for another 15 minutes with TBS: 0.9% NaCl in buffer of tris-(hydroxymethyl)-aminemethane at pH 7.6. Thereafter, they were incubated in 3% goat's serum (Novocastra Laboratories) for one hour and then with primary rabbit polyclonal antibodies (Novocastra Laboratories) to estrogen/progesterone receptors diluted with TBS 1 : 200 for 24 hours at 4°C.



**Fig. 1** Transverse cross-section of venous wall with a positive reaction of smooth muscle cell nuclei to **a)** progesterone receptors; **b)** estrogen receptors



**Fig. 2** Superficial density of smooth muscle cells immunohistochemically positive to **a)** estrogen receptors (ER) in women with varicose veins and PTS (PVV + PTS) and with varicose veins and no PTS symptoms (PVV) **b)** progesterone receptors (PR) in women with varicose veins and PTS (PTS) and with varicose veins and no PTS symptoms (no PTS)

After incubation the samples were rinsed with TBS for 30 min. Then they were incubated with goat's polyclonal biotin labeled antibodies to rabbit antibodies (Novocastra Laboratories) for 45 minutes at room tem-

perature. After rinsing with TBS they were incubated with avidine-biotin-peroxidase complex (Novocastra Laboratories) for 45 minutes at room temperature, rinsed again with TBS and incubated in a solution of 1

mg of DAB (-3,3'diaminebenzidine) in 1 ml of TBS with 10  $\mu$ l 3% hydrogen peroxide for five minutes. Then the samples were rinsed with distilled water and dehydrated in alcohol series, fluoroscoped by benzene and embedded in canadian balm.

Immunohistochemical reactions to estrogen and progesterone receptors were evaluated by an image analysis computer system. Superficial density of smooth muscle cells immunohistochemically positive to estrogen and progesterone receptors were analyzed separately for estrogen and progesterone receptors and expressed as amount/ $\text{mm}^2$ . Analysis was performed with the densitometric program IM-AN and the automatic image analyzer Magicall (Applied Imaging, Great Britain).

## Statistics

Results were assessed as to whether the disposition was normal and were analyzed with t-Student's test (StatSoft, Inc. STATISTICA for Windows 5.1 (PL)).

## Results

Immunohistochemical analysis of samples of the great saphenous vein from both subgroups (with PS and control) showed positive reactions of smooth muscle cell nuclei to estrogen and progesterone receptors. Figure 1 shows a transverse cross-section of venous wall with a positive reaction of smooth muscle cell nuclei to progesterone and estrogen receptors.

The superficial density of smooth muscle cells immunohistochemically positive to estrogen and progesterone receptors in the group with PS as compared to that without PS is presented in Figure 2. There were no differences in superficial density of smooth muscle cells positive to estrogen receptors between the groups. In contradiction to this, the superficial density of smooth muscle cells positive to progesterone receptors was higher in the subgroup with PS than in patients without, 343 ( $\pm 171$ ) and 240 ( $\pm 123$ ), respectively. This difference was statistically significant ( $p < 0.05$ ).

## Discussion

Unfortunately, there is not much reliable information about the aetiology and treatment of PS (4, 15, 17, 20). This syndrome is frequently observed in women with mothers suffering from similar symptoms. Some researchers see PS as a result of stress, others as a result of affective or personality disorders (4, 19, 20). These hypotheses have never been confirmed. PS symptoms in the premenstrual phase have led to some interest in the insufficiency of the luteal body as possible reason for PS. Papers from the 1970's have pointed to progesterone secretion disorders, but the newer reports show a normal function of pituitary-ovarian axis hormones (4).

Hypotheses for the development of varicose veins, based on clinical, epidemiological and morphological studies, do not satisfactorily explain the pathogenesis of this condition. Clinical and epidemiological observations as well as some publications point to sex hormones as a cause of varicose veins and PS (1, 10, 13, 17, 19, 22). However, recently the higher prevalence of varicose veins in men than in women was reported (3, 7). There are also authors who do not agree with the role of sex hormone in development of PS (4, 16). There are no papers about PS as a risk-factor of varicose veins. Nevertheless, some association between PS and varicose veins may exist.

Because varicose veins are more common in women, it is possible that some hormonal factors (e. g. menstrual cycle hormone changes, pregnancy, hormonal contraception) determine this dominance (2). Mild physiological PS symptoms are observed in 75% of women in the reproductive age, but in 5% of women, PS symptoms are severe enough to significantly disturb their activity during the two weeks preceding menstruation (4). Conditions of the venous system change during the menstrual cycle, pregnancy, hormonal treatment and contraception (1, 5, 10). In 30% of cases, changes in superficial veins lead to pain of these veins, oedema, venostasis, telangiectases, development of new varicose veins or enlargement of existing ones. Sometimes, these symptoms precede the development of

varicose veins. About 30% of women with varicose veins have pain before menstruation (10).

As discussed, immunohistochemical analysis performed showed a positive reaction to estrogen and progesterone receptors in nuclei of smooth muscle cells (SMC) in varicose veins regardless of PS symptoms. These results are consistent with the literature, where the presence of sex hormone receptors was described irrespective of the technique that was used (20, 23). Analysis of sex hormone receptors was limited to SMC, because these cells and their products (i. e. collagen and elastin) are mostly responsible for tonus and distensibility of the venous wall. This is also warranted by data from the literature that show remodeling of the venous wall in the course of varicose vein development mainly affects the muscular layer (9, 11, 12). The presence of sex hormone receptors in SMC nuclei proves its hormonal dependence (5, 12, 23). It confirms the possibility of influential changes of blood hormonal concentration on the condition of the venous system during the menstrual cycle. Also, an exacerbation of symptoms in the 2<sup>nd</sup> phase of the cycle caused by an increase of venostasis might be explained by a high level of progesterone in this phase.

Analysis of the superficial density of sex hormone receptors in SMC showed a statistically significant higher number of cells with a positive immunohistochemical reaction to progesterone receptors in the group of patients with PS when compared to the group of patients without PS. This difference might be an argument to support the role of progesterone in the pathogenesis of PS.

A larger number of progesterone effector cells might explain PS symptoms in women with normal hormonal levels. This finding correlates with reports about symptom recurrence during progesterone treatment after total hysterectomy with bilateral ovariectomy and no symptoms with estrogen treatment. It is an indirect proof of familial presence of PS, caused by a probable inheritance of the amount of SMC with progesterone receptors. The difference in the amount of progesterone-sensitive cells in venous walls of women with varicose veins

with and without PS, on the assumption that there is no difference in blood progesterone level, may explain venous symptoms in the luteal phase of the menstrual cycle in women with an increased number of cells supplied with progesterone receptors.

It was proven that there are hormonal mechanisms which cause an increased blood flow in the reproductive system organs during the 2<sup>nd</sup> phase of the menstrual cycle (18). Assuming a similar hormonal influence on superficial veins explains inducing by progesterone morphological changes. It also suggests that an increased amount of PR positive cells could be a first marker of PS.

The study of hormonal influence on vein morphology needs further research. Current results show that hormonal concentration does not influence receptor expression. A correlation between receptor level and phase of the menstrual cycle was proven only in the female reproductive system (18). There was no difference between sex hormone receptor level in women before and after menopause (15, 18, 23). Also, pregnant women present with pathology in the venous system mostly in the 1<sup>st</sup> trimester (70%), although hormonal levels are 20 times lower than in the 40<sup>th</sup> week of pregnancy (2, 9). It may be postulated that the density of PR-positive SMC (not the amount of receptors) have a crucial role in the development of varicose veins during pregnancy.

## Conclusion

The amount of cells with sex hormone receptors and not their concentration, could play a role in the pathology of premenstrual syndrome. Our results show that progesterone is more important than estrogen in effector organs. Varicose veins seem to be an excellent tissue for investigation of sex hormone related disorders.

## Streszczenie

Patogeneza zespołu napięcia przedmiesiączkowego nie jest do końca wyjaśniona. Uważa się, że kluczowym elementem odpowiedzialnym za występowanie tego zespołu są interakcje pomiędzy hormonami płciowymi a narządami docelowymi. Cel. Celem badania było zbadanie różnic w występowaniu komórek mięśniowych gładkich z receptoami dla hormonów płciowych w ścianie żyłaków kobiet z i bez zespołu napięcia przedmiesiączkowego. Materiał i metody. Do badań wykorzystano fragmenty żył odpiszczelowych pobranych od 50 kobiet podczas operacji żyłaków kończyn dolnych. Chorzy zostali podzielni na dwie grupy. Grupę I stanowiło 20 kobiet z rozpoznaniem klinicznym zespołem przedmiesiączkowym. Grupę II stanowiło 30 kobiet bez tego rozpoznania. Obecność receptorów estrogenowych i progesteronowych badano przy użyciu metod immunohistochemicznych. Gęstość powierzchniową komórek mięśniowych gładkich z pozytywnym odczynem immunohistochemicznym dla receptorów estrogenowych i progesteronowych badano przy użyciu programu densytometrycznego IM-AN i automatycznego analizatora obrazu Magica. Do porównania statystycznego wyników użyto testu t-Studenta. Wyniki. Nie stwierdzono różnic w gęstości powierzchniowej komórek mięśniowych gładkich z pozytywnym odczynem dla receptorów estrogenowych pomiędzy grupami. Stwierdzono natomiast wyższą gęstość powierzchniową komórek mięśniowych gładkich z pozytywnym odczynem dla receptorów progesteronowych u chorych z zespołem przedmiesiączkowego niż u chorych bez tego zespołu, odpowiednio 343 ( $\pm$  171) i 240 ( $\pm$  123). Różnica ta była statystycznie istotna ( $p < 0.05$ ).

**Wnioski:** Wydaje się, że liczba komórek z receptorami dla hormonów płciowych, a nie ich poziom może odgrywać ważną rolę w patogenezie zespołu przedmiesiączkowego. Otrzymane wyniki wskazują, że progesteron może odgrywać większą rolę niż estrogen w narządach efektorowych.

**Receptory hormonów płciowych w żyłach kończyn dolnych u kobiet z zespołem przedmiesiączkowym**

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