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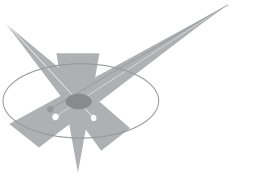

Original Paper

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Correlation between pre-therapeutic TPS and VEGF concentrations, in the serum of patients with cancer of the uterine cervix, and early effects of therapy

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Summary

Aim

To assess the relationship between pre-therapeutic serum TPS and VEGF levels and the results of treatment as measured immediately after completion of therapy.

Materials/Methods

The study included 146 women treated for cancer of the uterine cervix. Of these, 37 women were in stage I, 43 in stage II, 59 in stage III and 7 in stage IV of disease progression according to the FIGO classification. The ages of the patients ranged from 31 to 80 years. The dominant cancer observed was squamous cell carcinoma which accounted for more than 97% of cases. Samples were taken before commencement of treatment. Patients were treated by a combination of methods including radiochemotherapy, radical radiotherapy and palliative radiotherapy. The effects of therapy were graded after completion of irradiation according to generally accepted criteria. For tested criteria, ROC curves were drawn, determining cut-off points of 58 U/l for TPS and 500 pg/l for VEGF. For statistical calculations the U Mann-Whitney test was used.

Results

The level of VEGF expression varied between groups of patients in certain stages of disease progression (stages 1 & 2 and stages 2 & 3). Statistically significant differences were found between group PR in stage 2 and a control group of healthy women. Entry levels of TPS rose with tumor advancement (in stages 2, 3 & 4) and were higher than in a group of healthy women. Detected differences were statistically significant.

Conclusions

Only pre-therapeutic TPS levels show definite differences between degrees of clinical advancement and also with early therapeutic effects. Comparatively higher levels of serum TPS were found in patients with the worst prognosis prior to treatment (group P) than in the control group. This difference is statistically significant. Pre-therapeutic VEGF levels showed statistically significant differences between early (I, II) and advanced (III) clinical stages of the tumor as well as some effects of therapy assessed immediately after completion of treatment (PR vs S).

Key words VEGF • TPS • cancer of the uterine cervix

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Background

Cancer of the uterine cervix is characterized by long, localized, slow growth and occasionally, under conducive conditions, sudden accelerations in the tumor growth process. This process, as in other cancers, is closely related to increased angiogenesis and the ability to form metastases. Low immunogeneity of the cancer, and many levelled disturbances of connected processes of the immunological response, manifest themselves as the clinical aggressiveness of the cancer.

Characteristic pathomorphological features of a malignant tumor (structure, type, degree of differentiation) predetermine the course of the disease process. Many connected factors influence the final therapeutic effects on cancers of the uterine cervix and it is necessary to define their prognostic significance.

The growth of a malignant tumor is dependent on the distribution of necessary substances and equally on the removal of waste products from the environment of the neoplasm. Many clinical connections have been demonstrated between the vascularisation of a tumor and its aggression [1,2]. Under physiological conditions, angiogenesis is a complicated process, regulated by stimulatory and inhibitory mechanisms. Under pathological conditions these mechanisms are either damaged or do not function at all [3]. One of the factors regulating angiogenesis is Vascular Endothelial Growth Factor (VEGF) [4]. VEGF is a glycoprotein, which has the ability to bind heparin, and comprises at least 5 isoforms known as VEGF 121, VEGF 165, VEGF 189 and VEGF 205. The fifth isoform and its role in the process of angiogenesis has not been completely explored. The figures in each isoform name represent the number of amino acids each isoform contains [2]. Induction of VEGF dependent permeability is reliant on the penetration of certain protein isomers to the interstitium, from which endothelial cells later migrate. The VEGF receptors present on the above cells may be divided into three sub-types: FLT-1, FLK-1/KDR and VEGFR-3/FLT-4. Interactions between the VEGF family and its receptors initiate many processes: angiogenesis, lymph vessel and capillary formation, activation of enzymes in endothelial cells responsible for invasion of the stroma, endothelial cell mitosis and, as a consequence, the formation of new vessels in and around the tumor by several different methods [5]. Many tumors of the uterine cervix express high levels of VEGF, including some rare glandular cancers [6].

TPS (Tissue Polypeptide Specific Antigen) is a well known tumor proliferation marker [7]. It is created and secreted into the blood from late phase S to phase M. The best known is TPA which is made up of 35 epitopes, of which only 2 decide its specificity [8]. A fragment of cytokeratin 18 weighing 14kD, TPS may be identified by immunoenzymatic reactions through the use of the monoclonal antibody M3 [9,10]. Increased levels of TPS in the serum and effusion fluid may be observed in cases of ovarian cancer and other gynaecological tumors [11].

The proteins mentioned above directly or indirectly take part in, and therefore provide information about, the processes of tumor growth [1,12–19].

Table 1. Patient characteristics (Clinical advancement of the tumor).

Degree of clinical advancement	Number	%
I	37	25.34
II	43	29.45
III	59	40.41
IV	7	4.79

Aim

The purpose of the study was to assess the relationship between pre-treatment TPS and VEGF levels in the serum of patients, with invasive cancer of the uterine cervix, and the results of treatment – as assessed immediately after completion of therapy.

Materials and Methods

The study included 166 women as follows:
146 patients with invasive cancer of the uterine cervix aged between 31 and 80 years (average age 53.12 – standard deviation 11.04),
20 healthy women to form control group "S" (Sanus) aged between 30 and 76 years (average age 49.7 – standard deviation 14.10).

The ages of women in the control group was adequate for the study group and socio-economic conditions were similar.

Cancer of the uterine cervix was classified into 4 stages of clinical advancement, from IB to IVB, according to the criteria of the International Federation of Gynaecology and Obstetrics (FIGO), and in agreement with the TNM system.

Stage 1 was represented in the study by 37 cases (25.34%) while 43 patients (29.45%) were classified as being in stage II of clinical advancement. Stages III and IV were represented by 59 (40.41%) and 7 (4.79%) cases respectively. (Table 1).

The microscopic structure of the tumors was graded according to the criteria of the World Health Organization.

Among the investigated women, the most commonly observed tumor was non-keratinising squamous cell carcinoma, although other malignancies were observed (Table 2).

Prior to commencement of oncological therapy, 5 ml of blood was taken from each patient. The blood was centrifuged (1200 × g) and the resulting serum fraction was frozen and stored at –20°C. In order to identify proteins, immunoenzymatic methods were used – in the form of commercially prepared tests: TPS-Biotech, Broma, Sweden, ELISA-Kit, $VEGF_{165}$-R&D System, Quantikine, ELISA-Kit. The above tests were performed in the Department of Tumor Immunology of Wrocław Medical University.

Table 2. Patient characteristics (Histopathology).

Group	Histopathology	Number	%
A	Ca plano. Akeratodes	52	37.14
B	Ca plano. G-2 Keratodes	15	10.71
C	Ca plano. G-2 (Ak/Ke)	30	21.43
D	Ca plano. G-3 (indif/nondif/maledif)	15	10.71
E	Adenocarcinoma	4	2.86
F	Ca plano. (nonspecific structure)	24	17.14

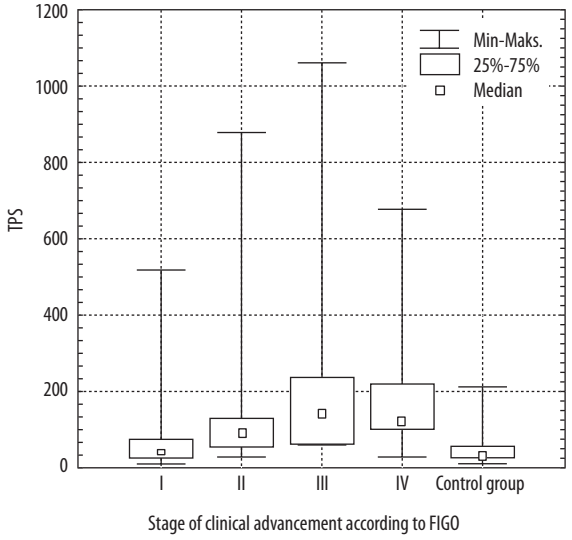

Figure 1. Ranges and median values for TPS concentration levels in each degree of clinical advancement and in control group 'S'.

Patients were treated in the years 2001 and 2002 in the Gynaecological Oncology Clinic of Wrocław Medical University if they satisfied the requirements for one of the following treatment schemes:

- surgical treatment (modified Wertheim-Meigs procedure) with follow-up complementary radiotherapy.
- a combination of 2 methods of radical radiotherapy – brachytherapy (Selektron Cs-137 MDR/LDR) with teletherapy (Megavolt 4,6,9,18 MeV).
- radical radiotherapy (as above) in combination with single drug chemotherapy – based on cisplatin at a dosage of 40 mg/m^2, once weekly – 6 courses.
- radiochemotherapy with subsequent surgical treatment.
- palliative radiotherapy.

The effects of therapy were graded immediately after completion and according to accepted criteria: CR, PR, SD, P.

For each investigated parameter, an ROC curve was drawn in order to establish cut-off points. These were 500 pg/l for VEGF and 58 U/l for TPS.

For statistical analysis, the non-parametric U Mann-Whitney test was applied.

Results

Comparison of TPS levels between individual stages of clinical advancement (Figure 1):

- I vs III – a difference in TPS levels was demonstrated (p=0.000069).
- I vs IV – a difference in TPS levels was demonstrated (p=0.027957).
- I vs S – a difference in TPS levels was demonstrated (p=0.014306).
- II vs III – a difference in TPS levels was demonstrated (p=0.012053).
- II vs S – a difference in TPS levels was demonstrated (p=0.000106).
- III vs S – a difference in TPS levels was demonstrated (p=0.000000).
- IV vs S – a difference in TPS levels was demonstrated (p=0.000604).

Similarly, for VEGF (Figure 2):

- I vs III – a difference in VEGF levels was demonstrated (p=0.036715).
- II vs III – a difference in VEGF levels was demonstrated (p=0.021804).

Comparison of TPS concentration levels in study groups and the control group 'S' (Figure 3):

- CR vs S – a difference in TPS levels was demonstrated (p=0.021088).
- PR vs S – a difference in TPS levels was demonstrated (p=0.000001).
- SD vs S – a difference in TPS levels was demonstrated (p=0.028467).
- P vs S – a difference in TPS levels was demonstrated (p=0.000220).
- CR vs PR – a difference in TPS levels was demonstrated (p=0.000168).
- CR vs P – a difference in TPS levels was demonstrated (p=0.006213).

Owing to early therapeutic effects at certain stages of clinical advancement, the following results were obtained for TPS (Figure 4):

- In stage I of clinical advancement – according to the FIGO classification:
  - CR vs P – a difference in TPS levels was demonstrated (p=0.038786).

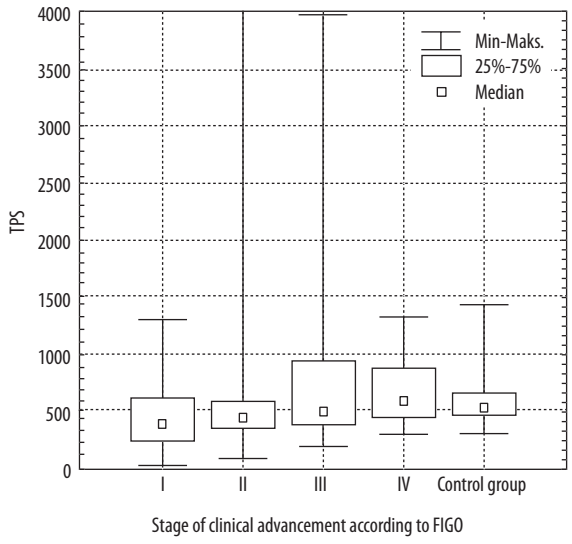

Figure 2. Ranges and median values for VEGF concentration levels in each degree of clinical advancement and in control group 'S'.

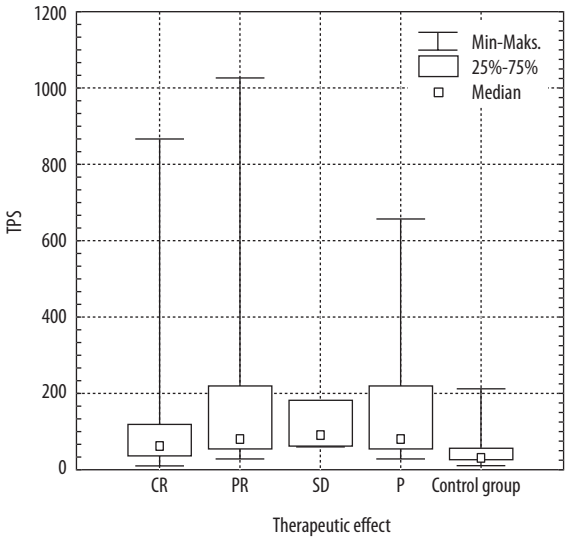

Figure 3. Ranges and median values for TPS concentration levels in groups (as defined by early results of treatment) and in the control group 'S'.

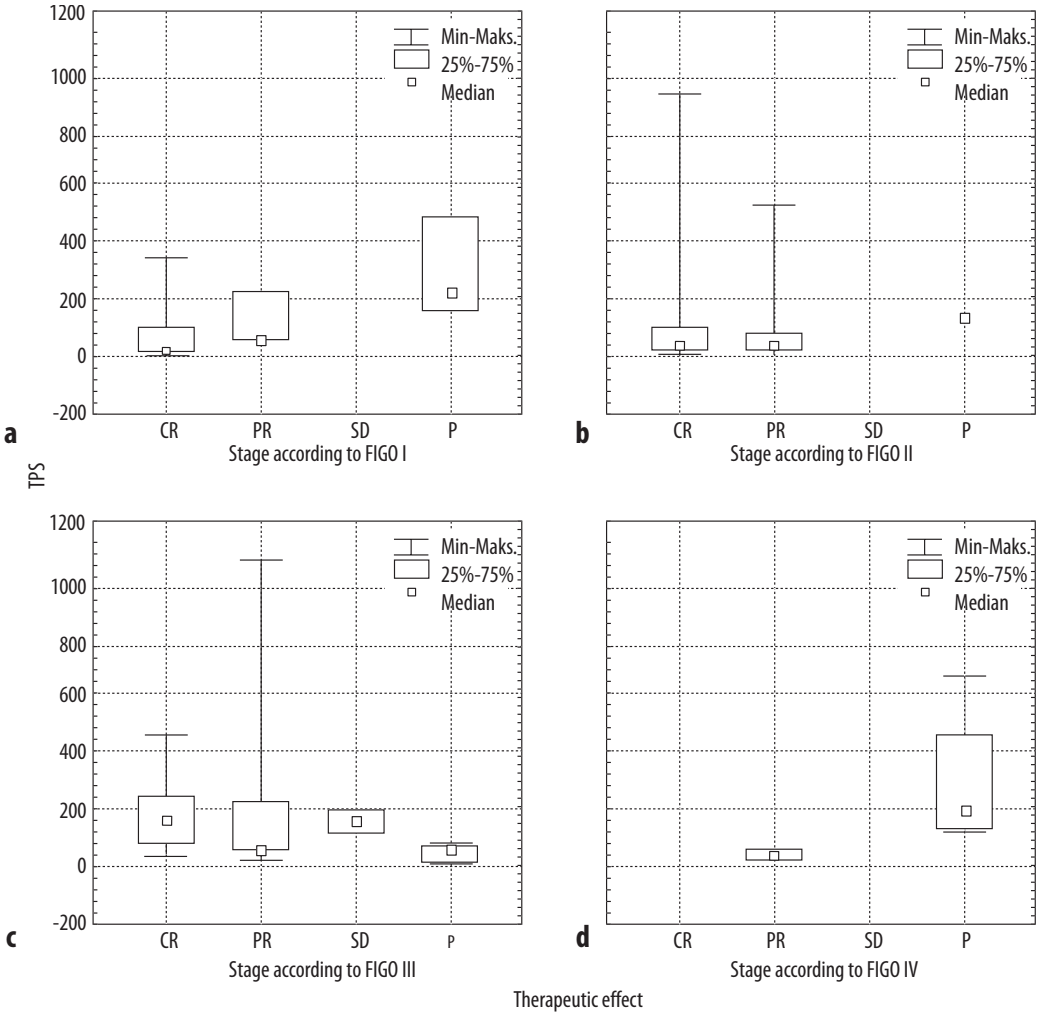

Figure 4 (a,b,c,d). Ranges and median values for TPS concentration levels in groups (as defined by early results of treatment and stage of clinical advancement).

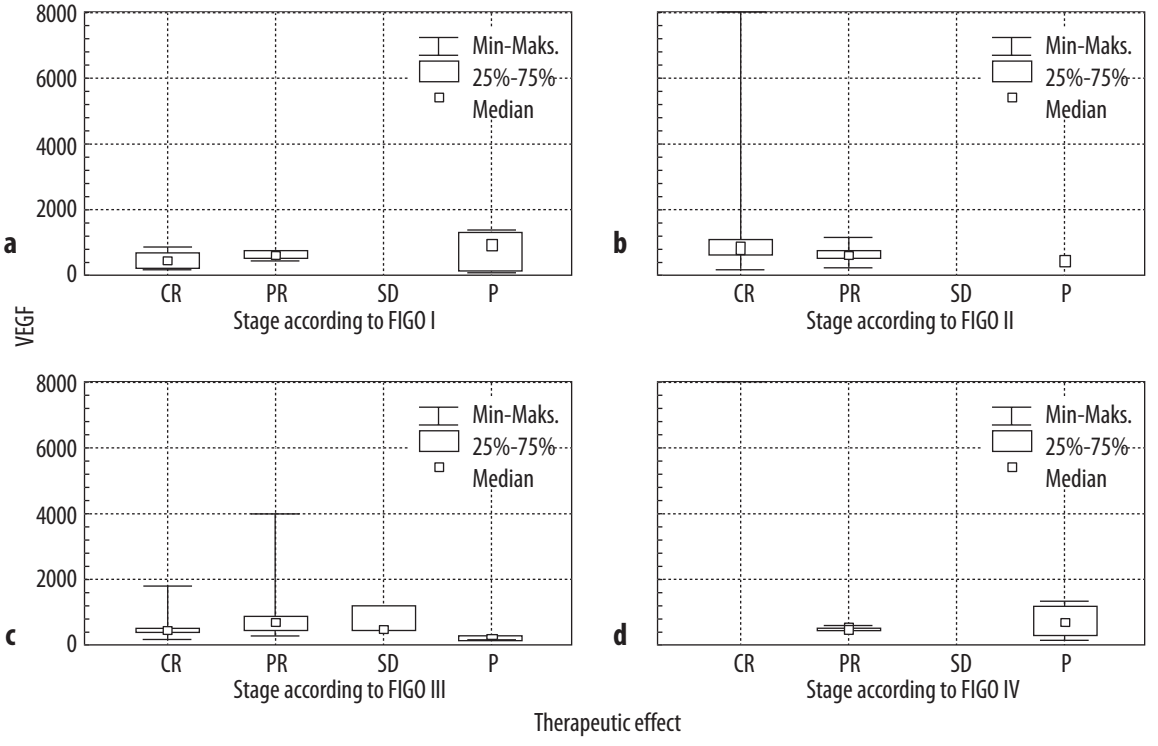

Figure 5 (a,b,c,d). Ranges and median values for VEGF concentration levels in groups (as defined by early results of treatment and stage of clinical advancement) present with several small gallstones (arrow).

  - P vs S – a difference in TPS levels was demonstrated (p=0.016383).
- In stage II of clinical advancement – according to the FIGO classification:
  - CR vs S – a difference in TPS levels was demonstrated (p=0.004749).
  - PR vs S – a difference in TPS levels was demonstrated (p=0.000195).
- In stage III of clinical advancement – according to the FIGO classification:
  - CR vs S – a difference in TPS levels was demonstrated (p=0.001721).
  - PR vs S – a difference in TPS levels was demonstrated (p=0.000000).
- In stage IV of clinical advancement – according to the FIGO classification:
  - PR vs S – a difference in TPS levels was demonstrated (p=0.022485).
  - P vs S – a difference in TPS levels was demonstrated (p=0.004160).

Similarly for VEGF (Figure 5):
- In stage II of clinical advancement – according to the FIGO classification:
  - PR vs S – a difference in VEGF levels was demonstrated (p=0.030236).

Discussion

Expression of VEGF mRNA in tumor tissues (cancers of the breast, lung, kidney, stomach, ovary, vulva and corpus and cervix of the uterus) has been the subject of many clinical studies [1,19–22]. In the case of cancer of the cervix of the uterus, both *in situ* and microinvasive tumors have been studied [18,23,24]. Attention is drawn to the fact that VEGF expression is proportional to tumor growth: in situ – microinvasion – macroinvasion, but only to a certain point. In clinical advancement stages III and IVa, according to the FIGO classification, VEGF levels suddenly drop [25]. An exception is stage IVB in which there is a general spread of processes, for example, metastasis via the blood vessels. The processes of angiogenesis, proliferation and spread play equally important roles, at this stage, in the breakdown of the majority of the body's defence systems [26]. The described phenomena are reflected in the concentration levels of VEGF in patients' serum, as observed in the above tests. Despite a lack of statistical significance, there exists a pattern of increasing VEGF concentration in the blood serum of patients with cancer of the uterine cervix. This phenomenon partly explains increases in the tumor stroma and also an increased rate in breakdown processes. The percentage of hypoxic cells and necrotic masses in tumors increase while the number of blood vessels running to the centre of the tumor decreases [3,5]. Advanced cancer of the uterine cervix is characterized by long local growth, with no blood-borne spread, in which intensive angiogenesis plays a secondary role; this situation is reversed in the cases of cancers of the ovarys and the corpus of the uterus. Both tumors, which are glandular in nature and are well perfused with blood (for reproductive function), have a tendency to spread within the peritoneal cavity, during the early clinical stages of the disease, generally via cell migration, lymph vessels and via the capillaries. In advanced cancer of the corpus

of the uterus, and especially in ovarian cancer with peritoneal spread, VEGF levels are always high [27,28]. A point worth noting is that the pre-therapeutic values for VEGF levels are reflected in the early therapeutic effects on cancers of the uterine cervix. Besides surgery, radio and chemotherapy are also used. Applied schemes must be maximally effective and appropriate to the clinical stage of the tumor and its microscopic structure. Both methods of threatment have effects on occurrence of apoptosis in tumor cells. VEGF plays the role of inhibiting apoptotic cell death in haemopoietic cells after radio and chemotherapy, under laboratory conditions [5,29]. High levels of VEGF should be inversely proportional to the measured effects [30,31].

This simple relationship is complicated by sudden tumor growth – rapid proliferation is dependent on vascularisation (reoxygenation in chemo and radiotherapy) [3,32]. Reoxygenation creates good conditions for therapy, but also for the repopulation of tumor cells. In clinical practice, rapid tumor proliferation responds well to applied therapies although, unfortunately, after completion of therapy, rapid recurrence also occurs [5,31]. There should be a measurable relationship between VEGF levels and the degree of differentiation of the tumor. In solid tumors, growth of the stroma results in decreases in angiogenetic factors. Additional interpretative complications arise from both early and late radiation reactions: patients in group PR (partial remission) are sometimes later included in group CR (complete remission) after postirradiation reactions recede. From the point of view of clinical oncology the results of treatment are easier to grade after 2,3 or 5 years, when the effects of radiation have passed and the frequency of complications has fallen. (After 5 years recurrence of cancer of the uterine cervix occurs very rarely.)

In the case of TPS levels, it is evident that secretion is related to DNA synthesis, protein synthesis and cell division. In this aspect, TPS may be correlated with both the number of tumor cells present and with stroma growth [7,9,12,33]. Logically, there should be a close relationship between the clinical stage of the tumor, its degree of differentiation, and its clinical aggression. In the above tests, TPS was used as a well known proliferation marker involved in many tumor processes. It is synthesized and released into the circulatory system from late in phase S to phase M of the cell cycle which, along with the characteristic growth and course of disease in cancers of the uterine cervix, is reflected in the results. These results are in agreement with those of other authors [7,12,14–17]. Because grading of early therapeutic effects is closely related to the observation (or lack thereof) of pre-existing infiltration, the results may confirm the final clinical diagnosis.

It should be underlined that the more complicated the observed process is, the harder it becomes to separate test parameters from the cascade of factors responsible for promotion or inhibition of the tumor. It is much easier to confirm the relationship between factors indirectly related to each other than between discrete factors controlling the phenomena of oncogenesis or destruction of the tumor. Many aspects are still not fully understood with regard to the course of malignant diseases through time and to changes in endogenous and exogenous factors. Further testing is required in order to define independent prognostic factors for new methods in prophylaxis and therapy.

CONCLUSIONS

1. TPS can be used to differentiate, to a statistically significant degree, between stages of clinical advancement in cancer of the uterine cervix. Furthermore, there exists a statistically significant difference between results from patients in each stage of clinical advancement, relative to a contol group.
2. Also observed was a statistical difference between pre-therapeutic TPS concentrations in the control group and each group of therapeutic effects.
3. Both the tested parameters can identify stage III of clinical advancement in cases of cancer of the uterine cervix.
4. The expression level of VEGF in the blood serum of patients with cancer of the uterine cervix increases in line with stage of clinical advancement. This increase is not statistically significant.
5. The level of VEGF identified pre-therapeutically in patients with cancer of the uterine cervix shows correlation with early therapeutic effects only in certain clinical stages.

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