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D-dimer, plasminogen activator inhibitor-1, prothrombin fragments and protein C – role in prothrombotic state of colorectal cancer.

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The relationship between malignant tumors and blood coagulation disorders is generally well known. The authors studied blood coagulation in patients with colorectal cancer and evaluated some prothrombotic markers.

The authors analyzed by latex-aglutination method, ProC Global test, Asserachrom PAI-1 test and Enzygnost F 1+2 test the group of 137 patients with malignant tumor of colon and rectum, drew attention to the relationship between level of D-dimer, PAI-1, F 1+2, Protein C and the progress of malignant tumor, its localization, clinical stage, histopathology type, method of surgery considering the stapling use.

Very aggressive and advanced tumors have significantly higher level of D-dimer, plasminogen activator inhibitor I (PAI-1). Prothrombotic fragments 1+2 were significantly higher by anastomosis dehiscence. Protein C level was lower in the age from sixty to seventy and in advanced clinical stage.

Pre-operative surveys of D-dimer, PAI-1, prothrombotic fragments and Protein C give informations about risk of thrombosis of malignant diseases, their clinical stage and histological type. D-dimer and PAI-1 have a real clinical value and can be reliable prothrombotic marker.

Key words: colorectal cancer, hypercoagulation,D-dimers, plasminogen activator inhibitor, protein C

Activation of mechanisms of blood coagulation and fibrinolysis in malignant disease is widely know fact, but the mechanisms of its origin hasn't been clearly illustrated yet. [1] Coagulation-fibrinolysis imbalance is in this case caused either directly – by interaction of tumor cells with specific element of chain producing the thrombin, or indirectly – due to increased level of procoagulation proteins like Tissue Factor (TF), Carcinoma Procoagulation Factor (CPF), Plasminogen activator inhibitor (PAI-I), antithrombin III, protein C and tissue plasminogen activator (t-PA). The role of TF is formation of common complex with Factor VII, which then activates both factors IX and X. TF is under normal circumstances produced in monocytes and endothelium. In cancer, the monocytes can be activated by immune complexes or by cytokines. [2] The substances with such an effect are for example Tumor Necrosis Factor (TNF) as well as immature malignant cells. [3]

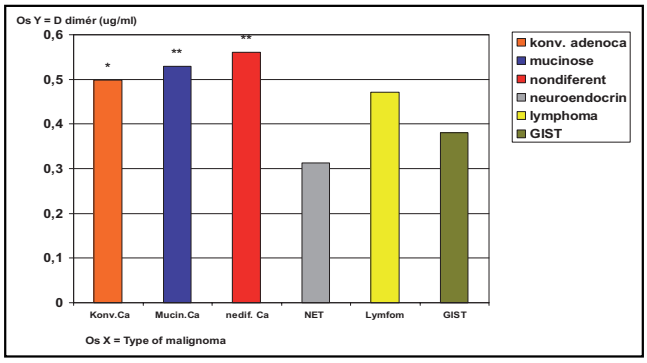
Carcinoma procoagulation factor is the direct activator of Factor X, even without necessity of Factor VII presence. CPF was found in malignant and foetal tissue, but not in normal tissue. [4] In the presence of F V, CPF can increase the production of thrombin more than threefold comparing to normal tissue. Actually, the changes are observed on all three levels of the Virchow's trias.

In our study we have established the objective to evaluate the coagulation components in compliance with the age and gender of patients, type, localization and progress of tumor process, type of tumor as well as the type of surgical technique use, approach and experience of surgeon.

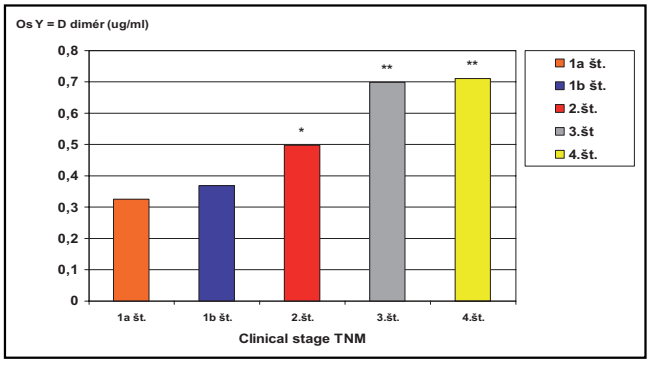
Materials and methods

We carried out the investigation of blood coagulation screening specifically including levels of D-dimer, PAI-1, prothrombin fragments 1+2 and procoagulation capacity of

Abbreviations: CEA: Carcinoembryonal antigen, CPF: Carcinoma procoagulation factor, CRC: Colorectal carcinoma, F 1+2: Fragments 1+2, IL-1: Interleukin 1, IL-6: Interleukin 6, PAI-1: Plasminogen activator inhibitor, PF: Prothrombin fragments, PC: Protein C, TF: Tissue factor, t-PA: Tissuae plasminogen activator, TNF: Tumour necrosis factor, VEGF: Vascular endothelial growth factor



Graph 1. The results of average D dimer in ug / ml of plasma in patients with colorectal malignoma by type of malignancy.



Graph 2. D dimer levels in plasma (ug / ml) in patients with colorectal malignoma by clinical stage (TNM classification).

Protein C in our group of 137 patients with colorectal malignant tumors undergoing surgery at our surgical department between 2007 – 2009. The blood was taken from peripheral cubital vein in amount of 10 ml in 3 samples: prior to surgery, 10 days and 3 months after/post surgery. The one part of blood samples was immediately tested for D-dimer level using latex-aglutination method and estimation of anti-coagulation capacity of Protein C was performed using the ProC Global test in the Department of Haematology FNsP Presov. On account of effective utilization of testing kits, the rest of blood samples were freezed to – 35 C and after accumulation of sufficient amount of blood samples were those altogether sent to National Centre for Treatment of thrombosis and Haemostasis in MFN Martin, where they were tested later in haematology lab for PAI-1 using Asserachrom PAI-1 test and prothrombin fragments was examined using Enzygnost F 1+2 micro test.

We performed the evaluation of obtained results following the comparison with control group of 40 healthy peoples – blood donors. Results of this study were evaluated by Student and Whitney test.

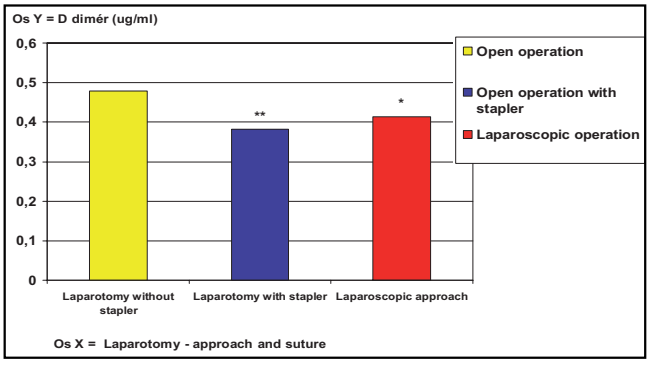
Results

D-dimer. The highest pre-operative results of D-dimer level were noticed in older patients in their 6^{th} – 7^{th} age decade, whilst we haven't noticed any statistically relevant aberration between male and female. The moderate elevation of levels is observed in location of tumor in sigmoid colon and rectum, but the results are not essentially discrepant.

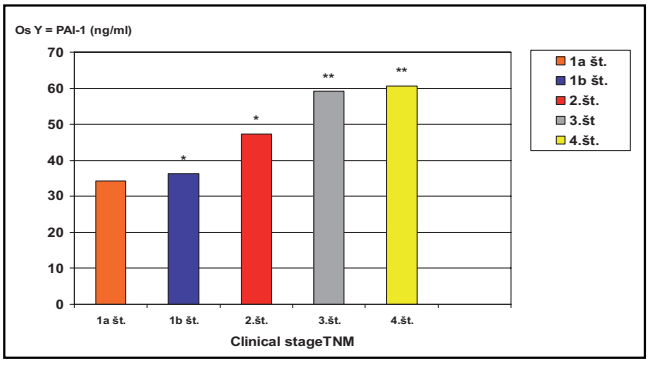
We have documented marked differences and elevation of D-dimer level with non-differentiated carcinoma (see the graph 1). Pre-operative plasma levels of D-dimer were increasing along with the advance of clinical stage and reflected the size of the tumor and lymph nodes affection (see the graph 2). The values measured out in clinical stage 3 were almost twofold higher than in stage 1a. Virtually, they directly and proportionally correlated with level of tumor-marker CEA (Carcinoembryonic Antigen). Due to our results, the application of staplers and mini-invasive therapeutic methods decreases the risk of thrombosis. (see the graph 3) In our study we have observed – with consideration of identical initial pre-operative levels of D-dimer – more rapid decline in post-operative D-dimer level, what was the most probably associated with more careful haemostasis and non-aggressive surgical technique. We've noticed that similar results were connected with more experienced surgeon (20 and more years of practice).

Plasminogen activator inhibitor (PAI-1). Whereas the measured plasma levels of PAI-1 in the 3^{rd} – 5^{th} age decade were rather balanced, with only slightly higher level in females, in the 6^{th} – 7^{th} decade – on the contrary – we've recorded marked elevation of levels equally in both genders. Regarding to localization of tumor we haven't noticed any significant changes.

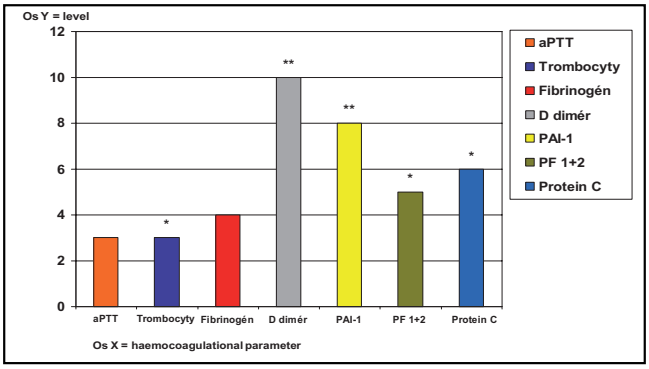
In evaluation regarding to the type of tumor we've found out the highest pre-operative levels of PAI-1 in non-differentiated and mucinous carcinoma, which remained elevated also in second sample on the 10^{th} day after surgery, with remarkably slowest decrease even after 3 months post-op (see the graph 4). According to the evaluation of clinical stage due to TNM classification, the plasma levels were increasing along with progress/advance of clinical stage and reflected the size of tumor and infiltration of lymph nodes (see the graph 5). The highest measured values were observed in the 3^{rd}-4^{th} clinical stage and, when comparing to CEA investigation, they very tightly correlated with level of this tumor marker! Even in the case of PAI-1, due to our results, the use of staplers and mini-invasive approaches minimize the risk of thrombosis, what is most likely associated again with careful haemostasis and delicate surgical technique. The similar results we've noticed in patients, whose surgery performed more experienced surgeon. When comparing the results in patients who underwent pre-operative neo-adjuvant therapy to those without neo-adjuvant therapy pre-operatively, to our astonishment, we have not observed any significant differences.



Graph 3. Postoperative plasma values of D dimer in ug / ml in patients with colorectal malignoma under approach and technique of anastomose suturing.



Graph 4. PAI-1 levels in plasma (ng / ml) in patients with colorectal malignoma byclinical stage to TNM classification.



Graph 5. Global analysis of various haemocoagulations parameters

Prothrombin fragments 1+2. By evaluation of the results of Prothrombin fragments 1+2 we've spotted significantly elevated levels even with the 1st sample pre-operatively: so high as threefold higher comparing to the control group, the levels in samples taken on 10th post-op day remained elevated in average twofold higher than in the control group. After 3 months post-op the levels in patients undergoing radical surgery gradually declined to almost normal levels. We have noticed significant elevation of F 1+2 levels in the 2nd sample in patients with anastomosis dehiscense. Contrariwise, with the application of the staplers was the restitution of normal values considerably marked. In further monitored criteria like regarding age, gender, localization of tumor, the sort and histopathology type of tumor, experience of surgeon and adjuvant therapy we haven't noticed any significant changes of F 1+2 in our group of the patients.

Protein C. In our group we have recorded average pre-operative plasma level of anti-coagulation capacity of Protein C = 0,62 (NR). After radical surgery we've observed the return to the normal level 0,82 (reference valuation > 0,8) in the 2nd blood sample taken on the 10th post-op day, and after 3 months was the average level = 1,22. The average level in control group was = 1,38. Pathological values in our group were recorded mostly in females and unlike to the literary data rather in middle age group of the patients, while patients in the 6th – 7th age decade had the levels more closer to normal. Regarding to the type of carcinoma, its location or experience of surgeon, stapling or even adjuvant therapy we haven't noticed any considerable changes. We've documented pathologically decreased levels of anti-coagulation capacity of Protein C mostly in advanced forms of malignant tumors.

Discussion

There were several studies published in the past showing elevation of pre-operative D-dimer level in the patients with colorectal malignant tumor. [5] The example is study referring to pre-operative high D-dimer level in patients with extensive tumors, deeply penetrating to the wall of colon, with signs of lymphogenous or haematogenous invasion and distant metastases, thus, in patients with progressive stages of cancer due to Dukes classification. [6,7] Deep venouse thrombosis can be the first symptom of unknown malignant tumor. [8]

Other studies suggest certain connection between CRC and blood coagulation disorders. [3,11] Following pathophysiology of its origin, in which the important role play TF, CP, cytokines, TNF, IL-1, IL-6, VEGF, protein C, PAI-1 with consequential effect on the abnormalities formation on endothelium [2,11], in the blood elements and in the blood stream, with direct impact on the process of the angiogenesis, they rather hypothetically analyze the possibility to make pre-operative diagnosis, including predictable survival length. [3,10] However, the studies investigating the pro-coagulation activity in colorectal carcinoma regarding to reference of monitoring several coagulation parameters at the same time are very rare.

We have analyzed the changes in mentioned 4 coagulation parameters in all patients with colorectal carcinoma and evaluated the results in relation to appearance of thromboembolic complication and to the relapse of malignant process, or alternatively to the dehiscence of anastomosis.

Based on our knowledge we'd like to present the following findings:

Coagulation disorders are manifest mostly in older patients in 6th – 7th age decade (D-dimer p<0,01, PAI-1 p<0,01) with prevalence of findings in female (PAI-1 p<0,05, PC p<0,05). Regarding the location of the tumor we have noticed higher D-dimer levels (p<0,05). The rest of the parameters didn't show any significant changes. The highest levels of D-dimer we have recorded with the location of tumor in caecum, less high in the area of sigmoid colon and rectum and the lowest levels were recorded when tumor was located in transverse colon.

Based on histopathology findings the results of clotting screen were significantly different when comparing non-differentiated carcinoma and mucinous adenocarcinoma, where the D-dimer and PAI-1 levels were significantly higher comparing to the rest of clotting parameters (p<0,01). The most parameters sensitively reflected the progress/advance of disease and clinical stage of tumor – practically all parameters more or less correlated with grade of TNM classification (D–dimer p<0,01, PAI–1 p < 0, 01, F 1+2 p < 0, 05, PC p < 0, 05)

Our results confirmed the hypothesis that non-aggressive surgical technique – application of stapling and laparoscopic method, experienced surgeon with the aim of meticulous haemostasis and avoidance of operative tissue devastation decrease the risk of postoperative complications. Hence, the measured levels of D-dimer (p< 0,01), PAI-1 (p< 0,01) and F 1+2 (p< 0,05) were significantly lower.

According to the two monitored parameters – D-dimer and PAI-1 – we have showed the relationship between their levels and level of CEA. Along with the above mentioned – as to the relations between D-dimer, PAI-1 level and clinical stage of disease – we can state that our findings confirm in literature published affirmation, that we can consider D-dimer (along with CEA and lymph nodes) for the 3rd prognostic marker of malignant disease progression.

Surprisingly, despite of our expectations, we haven't found any significant elevation of monitored parameters after neoadjuvant or adjuvant therapy in the group of our patients. But, to our interest, we have unexpectedly recorded significantly elevated levels of prothrombin fragments F 1+2 in all patients with dehiscence of anastomosis (p < 0, 01).

In conclusion, concerning seriousness of colorectal malignant disease with its high morbidity a mortality despite of already well known staging, thrombo-embolic prophylaxis and treatment, our used diagnostic parameters of coagulation could help to evaluate the advance/progress of cancer disease, quantification of risk of thrombosis and above all, to eliminate possible thrombo-embolic complications. Our findings shows higher protrombotic activity mainly in older patients and in aggressive forms of malignant tumors. They all approve the legacy and importance of delicate surgical techniques and methods. The application of delicate surgical techniques together with careful meticulous haemostasis provides lesser risk of microtrombotic formation in the area of anastomosis. This important fact we have clearly demonstrated with significantly lower levels of prothrombotic markers in our tests.

The results of our study as well as those published in several other studies proves that the coagulation tests, though not used so far for this purpose, can provide different possibilities of evaluation in future, as it is clearly demonstrated by example of D-dimer's related pre-operative estimation of disease's prognosis, its possible relapse, postoperative survival or risk of anastomosis dehiscence. Still, all our results calls for further investigations and research with prolonged monitoring of coagulation parameters in patients with colorectal carcinomas.

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