Bratisl Lek Listy 2009; 110 (5)
280 – 284

CLINICAL STUDY

Local changes in hemostasis in patients with gastric cancer

Kovacova E, Kinova S, Duris I, Remkova A

Ist Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia.
bll@fmed.uniba.sk

Abstract: Disorders of haemostasis and haemocoagulation are often seen in cancer patients as a part of the paraneoplastic syndrome. This study describes a novel compound that activates coagulation and also inhibits fibrinolytic system and fibrin degradation products in the gastric juice of 33 patients with gastric cancer. Similar, but less pronounced changes have been found in gastric juice of patients with gastric precancerosis. Procoagulant activity, induced by pathologically changed cells or monocytes, macrophages from tumor stroma, indicates the activation of local coagulation with the production of fibrin. It can be concluded that the local changes of coagulation and fibrinolysis may precede coagulopathies in cancer patients *(Tab. 2, Ref. 15)*. Full Text (Free, PDF) *www.bmj.sk.*
Key words: procoagulant activity of gastric juice, α1 acid glycoprotein, α1 antitrypsin, gastric cancer.

The etiopathogenesis of blood clotting in association with malignant tumour disease is complex and multi-factorial. Changes in haemostatic parameters can influence the course and prognosis of malignant diseases. The aim of this study was influenced by our struggle to contribute to the essence of this form of paraneoplastic syndrome and thus to judge the local changes in coagulation and fibrinolysis.

Assessment of local changes in coagulation and fibrinolysis

We have chosen the gastric cancer as our model. We were interested in the fact whether the gastric acid in patients suffering from gastric cancer or pre-cancer contains substances capable of activating or inhibiting the system of hemostasis. Further, we were interested in the fact whether local changes precede general changes in hemostasis. At the same time we tried to find out whether the assessment of these substances can be used as a marker of tumour diseases and contribute to the diagnosis, stratification as well as to the monitoring of patients suffering from cancer.

Assessment of general changes in hemostasis

Systemic changes in hemostasis can reflect the risk of thromboembolic complications. It was our endeavor to find out whether they involve the changes of primary hemostasis, hemocoagulation, inhibitors of coagulation or fibrinolysis. Based on this knowledge, it would be possible to choose optimal therapeutic or preventive procedures.

Ist Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Address for correspondence: E. Kovacova, MD, Ist Dept of Internal Medicine, LFUK, Mickiewiczova 13, SK-813 69 Bratislava, Slovakia.

Patients, material and methods

I. Local pro-coagulation and fibrinolytic activity of the gastric acid

Gastric acid was examined in 99 patients who were subject to gastrological examination. Based on the results of the gastroscopy and histological examinations, the group of patients was divided into the two subgroups.

A) The risk of pre-cancer was found in 31 patients (19 females and 12 males) with the age ranging from 34 to 83 years, (median 68 and 95 % confidential intervals (CI) ranging from 59 to 72). The group at risk was constituted by patients suffering from gastric ulcer and xanthoma, adenomatous polyps, post-resection states in patients with gastroscopy and histological findings of atrophic gastritis with intestinal metaplasia.
B) The group of gastric tumor was comprised of 33 patients (10 females and 23 males) with the age ranging from 45 to 92 years (median 72 and 95 % CI ranging from 60 to 76). The patients with gastric tumors suffered from variously differentiated gastric adenocarcinomas (21 patients) sigilocellular carcinomas (7 patients) or mucoid carcinoma (5 patients).

The control group consisted of 35 subjects (18 females and 17 males) with the age ranging from 17 to 89 years (median 54 and 95 % CI ranging from 45 to 62), in whom no pathologic finding was found on their gastric mucosa.

Pro-coagulant activity of the gastric juice (PCA)

PCA was examined by means of a modified version of the test assessing the time of plasmatic recalcification. The measurement was done by the FIBRINTIMER LABOR COA DAA 1000 (Germany).

Tab. 1. Studied parameters in the gastric juice and the results in individual groups.

Parameter	Control group	Pre-cancer group	Gastric cancer
Procoagulant activity (s)	0 (0–0) [0–0]	90 (36–125.7) [40–120.4]	145.6 (109.4–178.5) [117.8–176.7]
α1acidic glycoprotein (mg/l)	0.6 (0.29–0.98) [0.39–0.98]	1.96 (1.4–4.1) [1.4–3.1]	41.4 (25–89.6) [29.8–81.2]
α1antitrypsin (mg/l)	2 (1.19–2.55) [1.37–2.35]	5.9 (2.6–7.45) [2.9–5.9]	50.9 (35.3–60.8) [36–59]
Fibrinogen (mg/l)	0	0	0
pH	3.5 (3.4–4.5)	4.5 (3.5–5.5)	6.5 (6–7)

Presented are medians (quartiles) and [95 % confidential intervals]

α1 acid glycoprotein, α1 antitrypsin and fibrinogen

It was assessed by the immunoprecipitation using the commercial tests DIAGNOSTICA ORION; the measurement was done by the TURBOX, DIAGNOSTICA ORION (Espoo, Finland).When using these commercial tests, the serum was gradually replaced by the examined gastric juice following the recommended procedure.

Products of fibrin degradation – plasmin D dimer (D-di) – were examined by the semi-quantitative latex agglutination method using the diagnostic test D-di TEST, DIAGNOSTICA STAGO France.

Statistical methods

The statistical processing of quantitative indicators of coagulation and fibrinolysis in the gastric juice of the studied patients was done by the non-parametric analysis of dissemination according to Kruskal and Wallis; individual comparisons were done by Neményi's test. The investigated indicators were characterized by medians, their 95 % confidential intervals and we assessed the upper and lower quartiles. The hypotheses on qualitative indicators were tested by the chí^2 test. As we assessed four mutually coinciding indicators in the three groups, we used the Bonferroni's correction and the statistical significance was interpreted by the critical quantiles on the level α/12.

Results

The results of local pro-coagulation and fibrinolytic activity in gastric juice

The evaluation of physical properties of the gastric juice in patients with gastric cancer revealed a statistically significant decrease in the acidity of juice when compared to the control group ($p<0.001$) as well as to the group with stomach pre-cancer ($p<0.001$). When comparing the pH values in the group with stomach pre-cancer with that in the control group we also found a decrease in the acidity of gastric juice in pre-cancer, however after Bonferroni's correction, the difference was statistically insignificant.

When compared to the control group, the patients with malignant stomach tumors yielded marked pro-coagulation properties of the gastric juice ($p<0.001$). Similarly, when compared to the control group, the statistical significance of the gastric juice pro-coagulant activity in the group with pre-cancer was increased ($p<0.001$). When compared to the pre-cancer group, the significantly increased values of pro-coagulant activity were found also in patients with gastric carcinomas ($p<0.001$).

In the control group, the pro-coagulant activity of the gastric juice was not observed (median=0).

α1 acidous glycoprotein yielded significantly increased values in patients with gastric cancer not only when compared to the control group ($p<0.001$) but also when compared to the group with pre-cancer ($p<0.001$). However, it was increased also in patients with gastric pre-cancer when compared to the control group ($p<0.001$).

α1 antitrypsin was significantly increased in patients with gastric malignancies not only when compared to the control group ($p<0.001$), but also when compared to the group with pre-cancer ($p<0.001$). It was increased in the gastric juice of patients with pre-cancer also when compared to the control group ($p<0.001$). The Table 1 reviews the investigated indicators and their results in individual groups.

In our set, fibrinogen within the gastric juice was not detected in any group (median=0).

A significantly increased amount of the fibrin degradation product D-dimer was revealed in the gastric juice of patients with gastric tumors when compared to the control group ($p<0.001$). D-dimer was found to be increased also in the gastric juice of patients with gastric tumors not only when compared to the control group ($p<0.001$), but also when compared to the group of pre-cancer ($p<0.001$). The D-dimer results in individual groups are given in Table 2.

chí2 81.32; variance degree of 4; $p<0.0001$

Tab. 2. Results of D-dimer in individual groups.

	Group		
D-dimer	Control	Pre-cancer	Gastric cancer
Negative	27	2	0
+	8	22	10
++	0	7	23

Negative= <500 ng/l, positive (+)= >500 ng/l, Strongly Positive (++)= >1000 ng/l, chí² 81.32; variance degree of 4; p<0.0001

Discussion

Disorders of local regulatory mechanisms: In the recent years, the question of pro-coagulation and fibrinolytic properties of tumor cells has been arising in association with the growth and metastasis of tumors. In our study we have selected a group of patients with stomach tumors because in addition to the examination of a wide range of laboratory indicators of the overall hemostasis it provides the possibility to follow the local changes in coagulant and fibrinolytic systems straight from their gastric juice. The common gastroscopy can reveal not only samples of mucosa for the histological examination, but also the gastric juice. In our control group, we detected no pro-coagulant activity in the gastric juice, while in the group with gastric carcinoma we detected marked pro-coagulation properties of the gastric juice. However, the increased pro-coagulation activity was observed also in the gastric juice of patients with gastric pre-cancer. In patients with gastric cancer, the pro-coagulant activity in the gastric juice achieved significantly higher values than in the pre-cancer group. Based on our observations we can assume that intact cells of gastric mucosa have no ability to express the substances with pro-coagulant activity. In pre-cancer, gastric mucosa is attacked by chronic inflammation causing congestion and changes in microcirculation, which in turn lead to restructuring and transformation of glandular epithelial cells. Then the latter cells can gain pathognomonic properties. The pro-coagulant activity coming either from tumor, pathologically changed cells, or from monocytes and macrophages infiltrating the tumor stroma, can in the first instance initiate the activation of local coagulation actions. Microvasculature of tumor is increasingly permeable to fibrinogen. In response to pro-coagulant substances, fibrinogen quickly changes into fibrin. Tumor cells produce factor XIII, owing to which fibrin becomes stable. Being a part of stroma, fibrin further enables the tumor tissue to proliferate (1). There are several studies dealing with the issue of the expression of pro-coagulant activity of tumor cells (2). Okrucká already in 1991 revealed the presence of pro-coagulant activity in the gastric juice in patients with gastric cancer or pre-cancer. (3) Amirksawi et al found an increased expression of tissue factor by cancer cells in various types of tumors. A correlation has been described between the increased expression of pro-coagulant substances in cancer cells (breast, lung) and the unfavorable prognosis (4, 5).

In patients with pre-cancer however, especially in those with gastric cancer, we have found an increase in á1 acidic glycoprotein in gastric juice; its traces have been proved also in the gastric juice of the control group. A marked increase in patients suffering from cancer could have been related to pro-coagulant properties of glycoproteins produced and released by tumor. Mucoproteins within mucus, that is to say in vitro, are able to directly activate the factor X. In 19 patients suffering from gastric cancer (i.e. 57.6 %) we have proved the presence of H. pylori being one of the possible etiopathologic factors of the neoplastic diseases of stomach (6). Recently, some relation of H. pylori to some digestive tract lymphomas has been taken into consideration, especially in association with stomach lymphomas. In our group of patients however, no patient had such a type of gastric tumor. Malignant cells and infectious organisms can either directly or indirectly activate the coagulant and fibrinolytic enzymatic systems. Also the originating thrombin per se can support local inflammatory changes by its chemotactic effect on polymorphonuclear leukocytes. Activated polymorphonuclear leukocytes release a significant amount of proteolytic enzymes (elastase, cathepsin G, collagenase), which also contribute to local destructive processes. The current consumption and proteolytic degradation of regulatory proteins contributes firstly to hypercoagulation, then to fibrinolysis, and later to hypocoagulation, which can lead to local thrombosis and hemorrhage. Iversen et al (7) also observed an increase of local coagulation activity with the formation of microthrombi in perianastomotic area in patients after surgery due to colorectal carcinoma.

The cellular proliferation and inflammation in the site of solid tumor expansion lead to the release of various mediators (TNF, IL1, IL6) regulating the synthesis of the so-called reactive proteins as e.g. α1 acidic glycoprotein, á1 antitrypsin or fibrinogen. That is why some of the authors recommend observing their levels in order to monitor not only the inflammatory processes, but also the malignant disease and the effect of anti-tumor therapy (8, 9). In addition to their ability to regulate the immune response, interleukins also modulate pro-coagulation, anti-coagulation and fibrinolytic properties of cells. In this way they form an important joining link between the hemostasis and immunity (10). In our study, fibrinogen was detected in the gastric acid of no patients. The negative finding of fibrinogen in the gastric acid of both control group and patients thus does not prove any direct

production by cells of gastric mucosa. However, many immunohistochemical analyses have proved the presence of fibrin in the site of tumor proliferation (5, 11). The source of fibrinogen needed for the production of fibrin bed surrounding the tumor is in blood plasma. From plasma, fibrinogen can get into the extravascular space owing to the disturbed vascular permeability.

In our study, we did not examine the specific components of fibrinolytic system within the gastric juice, however based on the increased amount of fibrin degradation products by means of plasmin (D-dimer) in the gastric juice of patients with gastric pre-cancer and especially in the juice of patients suffering from gastric cancer we can deduce that the formation of fibrin is local, the fact of which subsequently increases the fibrinolytic activity. There are many studies, in which authors either directly or indirectly indicate that the fibrinolytic activity in gastric cancer is local (2, 12). Despite the fact that in both groups of patients we observed an increase in á1 antitrypsin, the presence of D-dimers indicates that the local activity of fibrinolysis prevails. The increase in α1 antitrypsin can be a reaction to locally increased fibrinolytic potential or inflammation (13). The components of fibrinolytic system together with leukocytic proteases can contribute to the regulation of tumor angiogenesis by damaging the surrounding (14). The growth of tumors depends on the vascular bed providing the tumor cells with nutrition. The stage of tumor vascularization coincides with the clinical prognosis and increases the probability of metastases. This is why the course of tumor angiogenesis can be an important clinical target for therapeutic intervention. Many tumors increase the expression of fibrinolytic proteins when compared to normal tissue surrounding them. According to immunohistochemical properties, the major part of malignant fibrinolytic activities is represented by the urokinase activator of plasminogen. An increased number of its receptors were found directly in the tumor tissue of stomach (12). The increased expression of fibrinolytic proteins in association with cancer can contribute to hemorrhagic diathesis. But the expression of pro-coagulant substances in tumor cells, provable also within the gastric juice, contributes to the explanation of the differences between massive hemorrhage from ulcer and frequent occult bleeding in gastric cancer (15). Despite the fact that not all mechanisms of activation of local coagulation and fibrinolytic reactions have been clarified, the results of some of our examinations give a hint that the examination of PCA, α1 KGP, α1 AT and D-dimer directly within the gastric juice could possibly support the diagnosis to a significant extent. They can be useful as non-specific tumor markers distinguishing the processes of tumor proliferation from benign lesions. The observation of the dynamics of changes in patients with pre-cancer can stimulate the intensity or change in therapy (surgical solution). Based on a number of our observations we can state, that local changes in coagulation and fibrinolysis can precede the systemic changes of hemocoagulation. The factors co-operating in the activation of systemic coagulation include the continual neovascularization, the release of cytokines and reactants of the acute phase as well as the entrance of tumor cells and their products into circulation.

Conclusion

The gastric juice of patients with gastric cancer was found to contain substance activating the coagulation system, components of fibrin degradation products, however also the compounds inhibiting the fibrinolytic system. The gastric juice of patients with gastric pre-cancer also contained substances activating the coagulation system, components of fibrin degradation, compounds inhibiting the fibrinolytic system, however in a lower amount when compared to the group with cancer. The intact cells of gastric mucosa probably do not have the ability to express the substances with pro-coagulant activity.

1) The presence of marked pro-coagulant activity within the gastric juice of patients with cancer implies that local coagulation processes have been activated with a subsequent formation of fibrin.

2) The high level of α1 acidic glycoprotein might probably coincide with pro-coagulation properties of the tumor produced and released by glycoproteins.

3) The presence of plasmin-derived products of fibrin degradation (D-dimer) in the gastric juice of patients with gastric cancer indicates that the fibrinolytic activity is local.

4) The presence of α1 antitrypsin indicates that fibrinolysis is inhibited.

5) Despite the fact that not all mechanisms of the activation of local coagulation and fibrinolytic reaction are clarified, based on our results we can state that the examination of PCA, α1 KGP, α1 AT and D-dimer straight from the gastric juice can be of supportive diagnostic significance.

6) Based on our observations we can further state that local changes in coagulation and fibrinolysis precede the systemic changes in hemocoagulation

Clinical conclusions

1) Examinations of PCA, α1 KGP, α1 AT and D-dimers being non-specific tumor markers in gastric juice can be useful in distinguishing the tumor proliferation from benign lesions. These examinations are available and economical.

2) The observation of levels of the above markers in patients with pre-cancer can stimulate an intensive monitoring as well as therapy in order to detect the possibility of cancer transformation.

3) The laboratory diagnosis of hemocoagulation disorders and hemostasis in patients with malignant diseases requires multiple laboratory indicators and their complex judgment.

References

1. Dvorak HF. Thrombosis and cancer. Hum-Pathol 1987; 18 (3): 275—284.

2. Okrucká A. Zmeny hemostázy pri malígnych ochoreniach. Folia Facultatis Medicae 1991; 29 (2): 9—67.

3. Amirkhosravi A, Warnes G, Biggerstaff JP, Francis JL. Tumor procoagulant activity is predominantly due to tissue factor expression.

Thrombosis and Haemostasis, XVIth Congress of the International Society on Thrombosis and Haemostasis. Florence, Italy, 1997, Abst. 27.

4. Inufusa H, Nakatiny Y, Adachi T, Wakano T, Nakajima A, Nakamura M, Suzuki M, Ando O, Kurimoto M, Miyake M, Shindo K, Yasutomi M. Correlation of prognosis of brest cancer patients and expresion of Ley which acts as a cofactor of tumor procoagulant. Inj J Oncol 1998; 13 (3): 481—487.

5. Shoji M, Hancock WW, Abe K, Micko C, Casper KA, Baine RM et al. Activation of coagulation and angiogenesis in cancer: imunohistochemical localization in situ of clotting proteins and vascular endothelial growth factor in human cancer. Amer J Pathol 1998; 152 (2): 399—411.

6. Sipponen P, Hyvärinen K, Seppälä M, Blaser MJ. Review article: pathogenesis of the transformation from gastritis to malignancy. Aliment Pharmacol Ther 1998; 12 (Suppl 1): 61—71.

7. Iversen LH, Thomsen GH, Thorlacius-Ussing O. Systematic coagulation activation and anastomotic leakage after colorectal cancer surgery. Dis Colon Rectum 1999; 42 (1): 56—65.

8. Piver MS, Moyer M, Diakun K, Lele SB, Chu TM. Serum Alpha1-acid glycoprotein in epithelial ovarial Cancer. Gynecol Oncol 1988; 29 (3): 305—308.

9. Lee K, Kye M, Jang JS, Lee OJ, Kim T, Lim D. Proteomic analysis revealed a strong association of a high level of alpha1-antitrypsin in gastric juice with gastric cancer. Proteomics 2004; 4 (11): 3343—3352.

10. Vitkovsky Y. Interleukins modulate procoagulant, anticoagulant and fibrinolytic properties of lymphocytes. XVIth Congress of the International Society on Thrombosis and Haemostasis. Florence, Italy, J. Thrombosis and Haemostasis 1997, 111.

11. Bini A, Mesa-Tejada, Fenoglio R. Immunohistochemical characterization of fibrin(ogen)-related antigens in human tissues using monoclonal antibodies. Lab Invest 1989; 60 (6): 814—821.

12. Ho CH, Chao Y, Lee SD, Chau WK, Wu CW, Liu SM. Diagnostic and prognostic values of plasma levels of fibrinolytic markers in gastric cancer. Tromb Res 1998; 91 (1): 23—27.

13. Aliustaoglu M, Yumuk PF, Gumus M, Ekenel M, Bolukbas F, Bolukbas C, Mutlu N, Basaran G, Avsar E, Turhal NS. D-dimer-can it be a marker for malignant gastric lesions? Acta Oncol 2004; 43 (8): 770—771.

14. Brooks PC. Cell adhesion molecules in angiogenesis. Cancer Metast Rev 1996; 15 (2): 187—194.

15. Hromec A, Okrucká A, Ďuriš I. Zmeny hemostázy a hemokoagulácie pri vredovej chorobe gastroduodéna a pri malignómoch tráviaceho traktu komplikovaných krvácaním. Bratisl Lek Listy 1986; 85 (2): 209—219.

Received October 31, 2007.
Accepted January 22? 2009.