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# Soluble Urokinase-Type Plasminogen Activator Receptor and Ferritin Concentration in Patients with Advanced Alimentary Tract Carcinoma. Relationship to Localization, Surgical Treatment and the Stage of the Disease – Preliminary Report

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**A** – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

## Abstract

**Background**. The urokinase plasminogen activation system is associated with metastatic potential of cancer in several tumors. Its specific membrane receptor (uPAR) is released from cancer cells and can be detected as the soluble fraction of the plasminogen activator receptor (suPAR). Ferritin (FRT) is a poor prognostic factor in various neoplasms.
**Objectives.** We analyzed the serum concentrations of suPAR and FRT in patients with gastrointestinal cancer. Tumor localization, stage of the disease, possibility of surgery and histopathological diagnosis were considered.
**Material and Methods.** The analysis involved 48 patients (8 females/40 males) treated in the Department of Gastrointestinal and General Surgery, Wroclaw Medical University. Thirty two patients had esophageal, 7–gastric, 9-colorectal cancer. Fifteen patients underwent resection surgery, 33 palliative therapy. The control comprised 10 healthy donors. The serum concentration of suPAR was determined by enzyme-linked immunosorbent assay (ELISA), expressed in pg/mL. Concentration of the serum FRT was detected using immunonephelometry method, expressed in μg/L.
**Results.** Serum concentration of suPAR ranged from 1789 –7320, x = 3676.2, SD = 1042 and was significantly higher ($p = 0.0002$) than in the control group. In patients who underwent palliative therapy, the concentration of suPAR was significantly higher ($p = 0.05$) than in those after resection, also in patients with esophageal cancer compared to those with colorectal one ($p = 0.02$). Serum concentration of FRT in patients with gastrointestinal cancer was significantly higher than in control group. Serum FRT concentration was higher in patients with esophageal cancer compared to patients with gastric cancer ($p = 0.05$), in persons with IV compared to patients with I–III stage of the disease, patients who underwent palliative compared to surgical therapy.
**Conclusions.** In patients with gastrointestinal cancer the level of suPAR is high, with highest values in advances disease with remote metastases. The FRT concentration is sensitive indicator of the disease process: its level is highest in pts with IV stage who underwent palliative therapy (**Adv Clin Exp Med 2014, 23, 6, 959–967**).

**Key words:** colorectal cancer, esophageal cancer, gastric cancer, suPAR concentration, FRT concentration.

The stage of the tumor is one of the most important prognostic factors in alimentary tract cancers, and the evaluation of the clinical stage of the disease is based on the assessment of infiltration of the wall, lymph nodes involvement and presence or lack of remote metastases.

The invasion of cancer cells, both, *in situ*, as well as leading to metastasis, is affected by the urokinase-type plasminogen activator (uPA) system which consists of the urokinase-type plasminogen activator (uPA), tissue-type plasminogen activator (tPA), the uPA receptor (the urokinase-type plasmonogen activator receptor: uPAR or CD87), its form deprived of the glycolipid anchor, the soluble form of uPAR: suPAR, and 2 major inhibitors: the plasmionogen activator inhibitor-1 (PAI-1) and plasminogen activator inhibitor-2 (PAI-2). The tPA is mainly involved in intravascular thrombolysis, while uPA is involved in pericellular proteolysis, and plays the main role in the process of plasmin formation from plasminogen. Plasmin hydrolyses fibrin bonds, fibronectin and lamin and activates metalloproteinases, which digest collagen and other matrix proteins [1, 2]. This tissue remodeling together with matrix desorganization stimulate the development of endothelium and the formation of new blood vessels which supply the tumor tissue and enable the tumor cells to pass the anatomical barrier separating the tumor from the host's healthy tissues [3–5]. Recent studies have demonstrated that neovascularisation is an essential metastasis risk factor in patients suffering from colorectal cancer [6–8]. It was shown that blocking the activity of uPA and/or uPAR, e.g. by means of endostatin, may inhibit angiogenesis and the invasion of the tumor [9]. Przybyłowska demonstrated a correlation between the level of uPAR and the density of blood vessels in cancer tissue of patients with colorectal cancer [10]. Thus uPAR is functionally engaged in cancer invasion and its high levels affect the survival time in patients with gastric, endometrial and breast cancers [11–13]. As a result of enzymatic exfoliation of uPAR from the surface of tumor cells and/or matrix cells, the plasma level of suPAR in patients with cancer of lung, breast, ovarian and colorectal was significantly higher than in healthy subjects [14–17]. Stephens, who examined 591 patients before surgical treatment, was first to prove that high level of suPAR is a poor prognostic factor in cancers in the lower part of alimentary tract [18]. Similar observations were reported by Fernebro and Riisbro [17, 19].

Association between the level of stored iron and cancers risk has been investigated by researchers for many years. As the data shows, a high level of iron may be observed in cancer of the pancreas, liver, esophagus, stomach, colon, breast, as well as multiple myeloma and other hematological malignancies [20–25].

FRT is the major Fe storage protein in the cells and at the same time an acute phase protein. One FRT molecule is able to store about 4500 $Fe^{3+}$. FRT is present in the liver, spleen, bone marrow cells and serum. An estimation of serum concentrations of FRT is a good indicator of resources of Fe and is the most common clinical method of assessing the concentration of Fe in patients without conditions that cause acute phase responses such as inflammation, infection. The main reason for the increased concentration of FRT are states of Fe overload, or block of the availability of Fe.

In Fe overload conditions, unbound Fe generates oxygen free radicals which are mutagens and cause damage to cellular and sub-cellular membranes, cellular organelles membranes, leading to failure of cells, tissues and organs [26]. Stevens first reported that there is a link between the saturation of the transferin (iron binding transporter), and the risk of colorectal cancer [27]. In 33 studies analyzed by Nelson in 2001 it has been shown that there is a positive association between iron in diet and colon cancer risk [21]. Nelson stated that the development of precancerous lesions in colon, colonic adenomas and polyps is correlated with body iron storage [20]. In 2003 Elmberg described an increased risk of extrahepatic cancers and hepatocellular cancer in patients suffering from haemochromatosis [28]. In addition, a risk of cancers among blood donors is lower, and it correlates with the frequency of donation and in the same time iron loss [29]. Data may indicate the participation of Fe in the process of carcinogenesis.

The study was undertaken to evaluate the serum concentration of suPAR and ferritin in patients with alimentary tract cancer in relation to tumor localization, stage of the disease, possibility of surgery and histopathological diagnosis.

## Material and Methods

We analyzed 48 patients (pts) treated in the Department of Gastrointestinal and General Surgery, Wroclaw Medical University. The group consisted of 8 females (F) aged 50–71 years (mean = 60.6 years) and 40 males (M) aged 39–82 years (mean = 58.7 years). Thirty-two patients (3/29 F/M) were diagnosed with esophageal, 7 (2/5 F/M) – with gastric and 9 (1/8 F/M) – with colorectal cancer. All patients were histologically verified. All the patients underwent standard staging examinations, including endoscopic evaluation together with biopsy studies, ultrasound examination of the neck and abdomen, and CT scans of the thorax and abdomen. Stage of the disease was based on TNM classification. Fifteen patients underwent resection surgery, 5 of them for esophageal, 4 – for gastric and 6 – for colorectal cancer. Table 1 lists the demographic data of the analyzed group.

**Table. 1.** Basic clinical data of patients with advanced alimentary tract carcinoma at diagnosis

| | | |
|---|---|---|
| Number of patients (F/M) | 48 (8/40) | |
| Age (years), range, mean | 39–82 (mean: 61.2) | |
| Localization (F/M) | esophagus | 32 (3/29) |
| | stomach | 7 (2/5) |
| | colon | 9 (1/8) |
| Clinical stage | esophagus II//III/IV | 1/3/28 |
| | stomach I/III/IV | 1/3/3 |
| | colon III/IV | 4/5 |
| Treatment | palliative | 26 |
| | resection (esophagus/stomach/colon) | 15 (5/4/6) |

Blood samples (5 mL) for analysis were taken at diagnosis; the serum concentration of suPAR was determined by enzyme-linked immunosorbent assay (ELISA) and expressed in pg/mL. In all patients serum FRT concentration was detected using immunonefelometry method and expressed in μg/L. Patients with iron deficiency anemia were excluded, all were free of infectious complication at the time of study.

The control group comprised 10 (5/5 F/M) healthy donors. Written informed consent was obtained from all patients and donors.

Statistical evaluation was performed using the Student's $t$, $\chi^2$ and Fisher's exact tests. All the results were considered statistically significant for $p < 0.05$. All statistical analyses were performed in Statistica software (StatSoft, Inc. (2011). STATISTICA (data analysis software system), version 10. www.statsoft.com.).

# Results

The serum concentration of suPAR in patients with gastrointestinal cancer ranged from 1789 to 7320, x = 3676.2, SD = 1042 and was significantly higher (p = 0.0002) than in the control group: 1791–2646, x = 2211, SD = 329 (Fig. 1). The serum concentration of suPAR in patients with esophageal and gastric cancer as well as colorectal cancer was significantly higher than in the control group p = 0.00002, p = 0.0001, p = 0.01 respectively. In patients with esophageal cancer, suPAR concentration was significantly higher than in patients with colorectal cancer (p = 0.02) and ranged respectively 2799–7320, x = 3693, SD = 1752 and 1789––3722, x = 2955, SD = 1090. The suPAR concentration did not differ between patients with gastric and colorectal cancer; however, patients with gastric cancer revealed a tendency to have a higher suPAR concentration (p = 0.06) (Table 2).

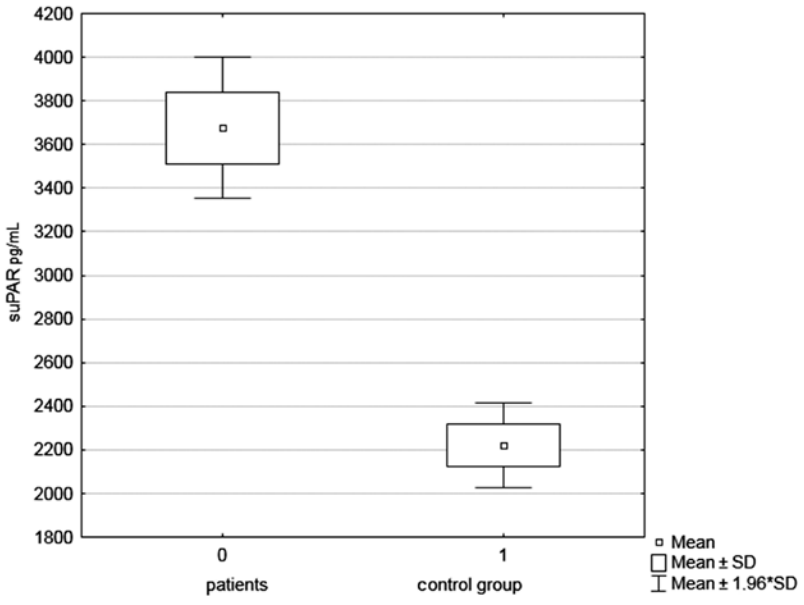


**Fig. 1.** suPAR concentration in patiens with alimentary tract carcinoma and in control group

**Table 2.** The serum concentration of suPAR and FRT in patients with alimentary tract carcinoma according to localization

| Data | Localization | | | | |
|---|---|---|---|---|---|
| | **esophagus I (n = 32)** | **stomach II (n = 7)** | **colorectal III (n = 9)** | **control IV (n = 10)** | **p value** |
| | **range, mean, ± SD** | **range, mean, ± SD** | **range, mean, ± SD** | **range, mean, ± SD** | |
| suPAR* (pg/mL) | 2799–7320<br>3693 ± 1752 | 2866–4887<br>3584 ± 1203 | 1789–3722<br>2955 ± 1090 | 1791–2646<br>2211 ± 329 | p I/III = 0.02<br>p II/III = 0.06<br>p I/IV = 0.00002<br>p II/IV = 0.0001<br>p III/IV = 0.01 |
| FRT** (µg/L) | 84–873<br>290 ± 211 | 32–300<br>189 ± 114 | 92–258<br>171 ± 59 | 20–280<br>128.5 ± 113 | p I/III = 0.05<br>p I/IV = 0.001<br>p II/IV = 0.05<br>p III/IV = 0.01 |

*suPAR – soluble urokinase-type plasminogen activator receptor.
** FRT – ferritin.

The serum concentration of FRT in patients with esophageal and gastric cancer as well as colorectal cancer was significantly higher than in the control group p = 0.001, p = 0.05, p = 0.01 respectively. The serum FRT concentration was the highest in patients with esophageal cancer compared with patients with colorectal cancer (p = 0.05) and ranged respectively 84–873 (mean = 290, SD = 211) and 92–258 (mean = 171, SD = 59).

Patients in the clinical stages I, II and III (subgroup A) had a lower expression of suPAR than patients in stage IV (subgroup B), but the difference was not statistically significant (Table 3). The serum FRT concentration displayed a similar trend with range of 32–271 (mean = 126, SD = 156) and 65–873 (mean = 293, SD = 210) respectively, and the difference was statistically significance (p = 0.01).

Fifteen patients who underwent total resection of the neoplastic lesion were markedly younger (p = 0.02) than those with advanced stage who were subjected for palliative therapy; it ranged 58 ± 6.2 years and 65 ± 8.4 years respectively. The suPAR concentration in patients who underwent resection was significantly lower (p = 0.05) than in patients who underwent palliative therapy, it being 1789–3745, mean = 3078, SD = 922 and 2573–7320, mean = 3898, SD = 1155 respectively (Table 4). A similar trend was observed for FRT concentration, which was lower in the surgical group compared with the palliative one (p = 0.01).

Patients with squamous cell esophageal carcinoma had the same concentration of suPAR as the patients with gastric or colorectal adenocarcinoma (Table 5). However, the serum concentration of FRT was higher in patients with squamous cell

**Table 3.** The serum concentration of suPAR and FRT in patients with alimentary tract carcinoma according to stage of the disease

| | Stage of the disease | | |
|---|---|---|---|
| | **I/II/III** | **IV** | **p value** |
| **Data** | **A (n = 12)** | **B (n = 36)** | |
| | **range, mean, ± SD** | **range, mean, ± SD** | |
| suPAR* (pg/mL) | 1789–4203<br>3178 ± 972 | 2751–7320<br>3842 ± 1159 | p A/B = ns.<br>p A/C = 0.005<br>p B/C = 0.00004 |
| FRT** (µg/L) | 32–271<br>126 ± 156 | 65–873<br>293 ± 210 | p A/B = 0.01 |

* suPAR – soluble urokinase-type plasminogen activator receptor.
** FRT – ferritin.

**Table 4.** The serum concentration of suPAR and FRT in patients with alimentary tract carcinoma qualified for surgery or palliative therapy

| Data | Treatment | | |
|---|---|---|---|
| | surgical (n = 15) | palliative (n = 33) | p value |
| | range, mean, ±SD | range, mean, ± SD | |
| suPAR* (pg/mL) | 1789–3745<br>3078, ± 922 | 2573–7320<br>3898, ± 1155 | p = 0.05 |
| FRT** (µg/L) | 32–408<br>84–873 | 96.8 ± 56<br>295.7 ± 214 | p = 0.01 |

* suPAR – soluble urokinase-type plasminogen activator receptor.
** FRT – ferritin.

**Table 5.** The serum concentration of suPAR and FRT in patients with alimentary tract carcinoma according to histopathological diagnosis

| Data | Histopathological diagnosis | | |
|---|---|---|---|
| | squamous cell carcinoma (n = 32) | adenocarcinoma (n = 16) | p value |
| | range, mean, ± SD | range, mean, ± SD | |
| suPAR* (pg/mL) | 2799–7320<br>3693 ± 1752 | 1789–4887<br>3269 ± 1213 | p = ns. |
| FRT** (µg/L) | 84–873<br>290 ± 211 | 96.8 ± 56<br>295.7 ± 214 | p = 0.01 |

* suPAR – soluble urokinase-type plasminogen activator receptor.
** FRT – ferritin.

carcinoma compared to adenocarcinoma (p = 0.04) and ranged 84–873 (mean = 292, SD = 211) and 32–300, (mean = 148, SD = 118) respectively.

Hemoglobin levels ranged from 7.6 to 15.8 g/dL. (mean = 12.8, SD = 1.8 g/dL). Fifteen patients (10/32 with esophageal, 2/7 with gastric and 3/9 with colorectal cancer) were diagnosed with anaemia (Hb 7.6–11.0 g/dL). All the patients had normocytic anaemia; they did not demonstrate bleeding from the digestive tract (negative benzidine test, normal iron level) or haemolysis (normal or decreased reticulocyte number, normal serum bilirubin level). In patients with anaemia (subgroup A) suPAR concentration was significantly higher (p = 0.04) than in the patients with normal Hb levels (subgroup B), it being x = 3823.3, SD = 1609 and x = 3437, SD = 1010.9 respectively. A similar trend was observed for FRT concentration (Table 6). The serum concentration of FRT in subgroups A and B was significantly higher than in the control group, p = 0.05. The sTfR (serum transferrin receptor)/log ferritin ratio (sTfR/FRT index) in both group was below one, 0.248–0.938, mean = 0.742 and 0.615–1.25 mean = 0.97.

# Discussion

According to the world data of 2003 on the incidence of malignant tumors, every third patient from 10 million new cancer cases in the world was diagnosed with digestive cancer [30]. Second place in this group is occupied by colorectal cancer, with the same incidence rate for males and females. On the other hand, as far as mortality rate is concerned, stomach cancer occupies 2$^{nd}$ place as the reason of death in men and 4$^{th}$ in women, while oesophageal carcinoma, which has the poorest prognosis among digestive cancers, with high prevalence among men, occupies 8$^{th}$ place among malignant tumors [30–33].

Surgical treatment remains the central therapeutic modality. The selection of an adequate therapeutic modality is strictly correlated with the severity of the tumor [34–36]. In patients with esophageal carcinoma, despite extensive surgical intervention, 5-year survival after surgery alone is observed in only about 20% of patients. Almost half of the patients with locally advanced tumors will die following cancer recurrence within the first 2 years after surgical resection [32, 36]. This means that at the time of diagnosis and surgical

**Table 6.** The serum concentration of suPAR, and FRT, TRF and sTfR in patients with alimentary tract carcinoma in relation to the level of hemoglobin

| Data | Hb level (g/dL) | | |
|---|---|---|---|
| | < 11 g/dL (n = 15) | >11 g/dL (n = 33) | p value |
| | range, mean, ± SD | range, mean, ± SD | |
| suPAR* (pg/mL) | 2760–7320<br>x = 3823 ± 1609 | 1789–4800<br>x = 3437 ± 1010.9 | p = 0.04 |
| FRT** (µg/L) | 84.7–873<br>x = 266 ± 239 | 48.8–463<br>x = 213 ± 139 | p = 0.06 |
| TRF*** (g/L) | 0.87–2.18<br>x = 1.7 ± 0.9 | 1.17–2.55<br>x = 2.0 ± 0.77 | |
| sTfR**** (mg/L) | 0.48–2.73<br>x = 1.8 ± 2.0 | 1.04–2.81<br>x = 1.34 ± 1.26 | |

* suPAR – soluble urokinase-type plasminogen activator receptor.
** FRT – ferritin.
*** TRF – transferrin.
**** sTfR – soluble transferrin receptor.

intervention the cancer has already spread far beyond its primary focus in the gastrointestinal tract. Thus, the search for parameters which would predict systemic changes in the course of oesophageal carcinoma has become an urgent need.

Numerous changes in the activity of uPA, uPAR and PAI-1 in matrix cells, among others, uPA expression on fibroblast-like cells, uPAR on macrophages and cancer cells as well as PAI-1 expression on myofibroblasts were demonstrated in digestive cancers. The changes evidence a direct participation of fibrinolysis in remodeling and dissemination of the tumor [37–39]. Seetoo demonstrated that the overexpression of uPA, uPAR and PAI 1 was significantly associated with metastasis to the liver in patients suffering from colorectal cancer [40]. Shiomi examined 56 patients after esophagectomy and did not observe the presence of uPA, uPAR and PAI-2 in healthy oesophageal tissue, while uPA was found on cancer cells on the tumor margins. Patients with uPA-positive cells more often displayed tumor infiltration beyond muscularis propria and metastases to the lymph nodes [41].

Moreover, other authors reported a negative correlation between uPAR, uPA and/or PAI-1 expression in cancer cells and the survival time and stage of tumor in gastric and colorectal cancer [42, 43].

In our material the serum concentration of suPAR was significantly higher than in the control group. The highest levels were observed in patients with oesophageal carcinoma and they were significantly higher than in colorectal cancer. Oesophageal carcinoma develops insidiously and for a long time does not produce characteristic symptoms. Difficulties with swallowing, as the first and primary symptom of cancer, as a rule occur in advanced stages of the disease, when the neoplastic infiltration has already involved all layers of the oesophageal wall and extended beyond them. The detection of a potentially early form of carcinoma in the asymptomatic stage is associated with implementation of a screening programme, which unfortunately has not been considered justified yet. In the studied group of 32 patients with oesophageal carcinoma, only 4 patients (12.5%) were in a stage lower than IV, and resection surgery was possible only in 5 persons (15.6%). Among patients with stomach cancer and colorectal cancer, 50% were staged lower than IV and 62.5% were qualified for resection surgery. The level of suPAR in the subgroup of patients in stage I–III of the disease was lower than in patients in stage IV, this finding was similar to data reported by Stephens and Riisbro; moreover, in patients undergoing resection it was significantly lower in comparison to patients who did not qualify for resection surgery [17, 18]. In the study by Stephens, high preoperative level of suPAR increased significantly the risk of death [18]. Our studies may also correspond to the findings by other authors who analyzed the levels of uPA and uPAR as well as uPA and uPAR mRNA in tumour tissue. Chen investigated 67 patients with stomach cancer and demonstrated that the level of uPA and uPAR was significantly increased in stomach cancer; moreover, patients with uPA and uPAR expression had significantly lower survival time in comparison to patients who were free from such an expression [11].

In the studies by Zhanga performed on 105 gastric tumor tissue specimens, cells with uPA and

uPAR expression were found to infiltrate the muscular coat, the peritoneum and the greater omentum [44]. Hogdall analyzed 567 patients suffering from colorectal cancer and demonstrated that increased levels of suPAR in association with low levels of PAI-1 were a poor prognostic factor and indicated the presence of remote metastases [45]. As soon as in 1995, Heiss proved that the expression of uPAR in solid tumors may be an indicator of tumor cells dissemination in the bone marrow [46]. Prior examinations revealed the presence of microdeposits of epithelial cells in bone marrow of patients with oesophageal carcinoma [46].

Already in 1983, Ludwig and Linkesch showed that the concentration of FRT in myeloma patients correlated with tumor mass and the concentration of beta-2-microglobulin [48]. Ludwig research published in 2014 confirmed the importance of ferritin as an independent prognostic factor for PFS and OS in myeloma patients undergoing autologous transplantation [22]. Italian research group showed that ferritin subunit L increases angiogenesis and cells proliferation rate in an iron-independent manner [49]. In cases of inhibition of ferritin expression, the growth of cells arrested in G1/S phase was resumed. An antiapoptotic effect of FRT not related with its iron-biding capacity was shown [50]. FRT is inducted by proinflamatory cytokines, on the other hand, FRT inhibits apoptosis inducted by TNF-alpha by suppressing reactive oxygen species. [51]. The correlation between NF--kappa B and ferritin has been shown for inflammatory cells and fibroblasts, which play a great role in the microenvironment of tumor in cancer.

The serum concentration of FRT in patients with esophageal and gastric cancer as well as colorectal cancer was significantly higher than in the control group, in particular it was high in patients with esophageal cancer compared to patients with colorectal cancer ($p = 0.05$), in persons with IV stage compared to patients with I, II and III stage of the disease, patients who underwent palliative therapy, compared to patients who underwent to surgical therapy. Consistent with our observations are Alkhateeb et al. studies in breast cancer, which indicated that the concentration of FRT correlates with tumor stage and histological grade. Moreover, the authors demonstrated that ferritin may be produced by tumor infiltrating macrophages.

The level of FRT in squamous cell carcinoma was significantly higher than in adenocarcinomas. Squamous cell carcinomas involved patients with oesophageal carcinoma in most advanced stages. However we cannot exclude that the difference may have resulted from different metastatic potentials of both histological types of the tumor. Significantly elevated level of FRT in comparison to the control group was also observed among patients with anaemia. In this group iron deficiency anemia was excluded by calculation of sTfR/log ferritin ratio (sTfR/FRT index), which was 0.742. Interestingly, both the level of suPAR as well as FRT concentration were highest in oesophageal carcinoma. Just as in myeloma patients, FRT can be produced by stromal cells of the tumor and stimulate tumor cell proliferation. This requires further research and observation on a larger group of patients.

Concluding, suPAR as well as the serum level of FRT in patients suffering from oesophageal, stomach and colorectal cancers, who did not develop bleeding from the digestive tract, is a sensitive marker of the activity of the pathological process and may be a significant prognostic marker while making therapeutic decisions.

According to Dass, the investigation of the uPA system may be a useful tool in the treatment of cancer patients, the evaluation of the efficacy of prophylaxis and sensitivity of neoplasms to new chemotherapeutic agents [52]. Already in 1994, Fazioli presented a concept of therapy for patients suffering, among others, from colorectal cancer, which consisted in the administration of recombined uPA receptor or receptor-blocking antibodies in order to inhibit the activity of uPA system [53]. Studies on gene therapy used to inhibit uPAR expression are currently undergoing [54].

## References


[1] **Dano K, Andreasen PA, Grondahl-Hansen J, Kristensen P, Nielsen LS, Skriver L:** Plasminogen activators, tissue degradation and cancer. Adv Cancer Res 1985, 44, 139–266.

[2] **Egeblad M, Werb Z:** New function for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002, 2, 163–176.

[3] **Yu HR, Schultz RM:** Relationship between secreted urokinase plasminogen activator activity and metastatic potential in murine B16 cells transfected with human urokinase sense and antisense genes. Cancer Res 1990, 50, 7623–7633.

[4] **Shapiro RL, Duquette JG, Roses DF, Nunes I, Harris MN, Kamino H, Wilson EL, Rifkin DB:** Induction of primary cutaneous melanocytic neoplasms in urokinase-type plasminogen activator (uPA)-deficient type mice: cellular blue *nevi* invade but do not progress to malignant melanoma in uPA-deficient animals. Cancer Res 1996, 56, 3597–3604.

[5] **Fisher JL, Mackie PS, Howard ML, Zhou H, Choong PF:** The expression of urokinase plasminogen activator system in metastatic murine osteosarcoma: an *in vivo* mouse model. Clin Cancer Res 2001, 7, 1654–1660.

[6] **Shpitz B, Gochberg S, Neufeld D, Grankin M, Buklan G, Klein E, Bernheim J:** Angiogenic switch in earliest stages of human colonic tumorigenesis. Anticancer Res 2003, 23, 5153–5157.
[7] **Pang RW, Poon RT:** Clinical implications of angiogenesis in cancers. Vasc Health Risk Manag 2006, 2, 97–108.
[8] **Rajaganeshan R, Prasad R, Guillou PJ, Chlmers CR, Scott N, Sarkar R, Poston G, Jayne DG:** The influence of invasive growth pattern and microvessel density on prognosis in colorectal cancer and colorectal liver metastases. Br J Cancer 2007, 96, 1112–1117.
[9] **Dkhissi F, Lu H, Soria C, Opolon P, Griscell F, Liu H, Khattar P, Mishal Z, Pericaudet M, Li H:** Endostatin exhibits a direct antitumor effect in addition to its antiagiogenic activity in colon cancer cells. Hum Gene Ther 2003, 14, 997–1008.
[10] **Przybyłowska K, Szemraj J. Kulig A, Dziki A, Ulanska J, Blasiak J:** Antigen levels of urokinaze type plasminogen activator receptor and its gen polymorphism relate to microvassel density in colorectal cancer. Acta Bioch Pol 2008, 2, 357–363.
[11] **Ji F, Chen YL, Jin RY, Wang WL, Yang ZL, Li YM:** Relationship between matrix metalloproteinase-2 mRNA expression and clinicopathological and urokinase-type plasminogen activator system parameters and prognosis in human gastric cancer. World J Gastroenterol 2005, 11, 3222–3226.
[12] **Memarzadeh S, Kozak KR, Chang L, Natorajan S, Shintaku P, Reddy ST, Farias-Eisner R:** Urokinase plasminogen activator receptor: Prognostic biomarker for endometrial cancer. Proc Natl Acad Sci USA 2002, 99, 10647––10652.
[13] **Stillfried GE, Saunders DN, Ranson M:** Plasminogen binding and activation at the breast cancer cell surface: the integral role of urokinase activity. Breast Cancer Res 2007, 9.
[14] **Pappot H, Høyer-Hansen G, Rønne E, Hansen HH, Brünner N, Danø K, Grøndahl-Hansen J:** Elevated plasma levels of urokinase plasminogen activator receptor in non-small lung cancer patients. Eur J Cancer 1997, 33, 867–872.
[15] **Riisbro R, Christensen IJ, Piiroren T, Greenall M, Larsen B, Stephens RW, Han C, Heyer-Hansen G, Smith K, Brünner N, Harris AL:** Prognostic significance of soluble urokinase plasminogen activator receptor in serum and cytosol of tumor tissue from patients with primary breast cancer. Clin Cancer Res 2002, 8, 1132–1141.
[16] **Sier CF, Stephens R, Bizik J, Mariani A, Bassan M, Pedersen N, Frigerio L, Ferrari A, Dano K, Brünner N, Blasi F:** The level of urokinase – type plasminogen activator receptor is increased in serum of ovarian cancer patients. Cancer Res 1998, 58, 1843–1849.
[17] **Riisbro R, Christensen IJ, Nielsen HJ, Brünner N, Nilbert M, Fernebro E:** Preoperative plasma soluble urokinase plasminogen activator receptor as prognostic marker in rectal cancer patients. An EORTC-Receptor and Biomarker Group collaboration. Int J Biol Markers 2005, 20, 93–102.
[18] **Stephens RW, Nielsen HJ, Christensen IJ, Thorlacius-Ussing O, Sorensen S, Dano K, Brünner N:** Plasma urokinase receptor levels in patients with colorectal cancer: Relationship to prognosis. J Nat Cancer 1999, 10, 869–874.
[19] **Fernebro E, Madsen RR, Fernö M, Brünner N, Bendahl P, Christensen IJ, Johnson A, Nilbert M:** Prognostic importance of the soluble plasminogen activator receptor, suPAR, in plasma from rectal cancer patients. Eur J Cancer 2001, 37, 486–491.
[20] **Nelson RL, Davis FG, Sutter E, Sobin LH, Kikkendal JW, Bowen P:** Body iron stores and risk of colonic neoplasia. J Natl Cancer Inst 1994, 86, 455–460.
[21] **Nelson RL:** Iron and colorectal cancer risk: Human studies: Nutr Rev 2001, 59, 140–148.
[22] **Strasser-Weippl K, Ludwig H:** Ferritin as prognostic marker in multiple myeloma in patient undergoing autologous transplantation. Leukemia Lymphoma 2014, in press.
[23] **Kalousová M, Krechler T, Jáchymová M, Kuběna AA, Zák A, Zima T:** Ferritin as an independent mortality predictor in patients with pancreas cancer. Results of a pilot study. Tumor Biol 2012, 33, 1685–1700, 1695–177.
[24] **Alkhateeb AA, Han B, Connor JR:** Ferritin stimulates breast cancer cells through an iron independent mechanism and is localized within tumor-associated macrophages. Breast Cancer Res Treat 2013, 137, 733–744.
[25] **Kikuchi S, Kobune M, Iyama S, Sato T, Murase K, Kawano Y, Takada K, Ono K, Hayashi T, Miyanishi K, Sato Y, Takimoto R, Kato J:** Prognostic significance of serum ferritin level at diagnosis in myelodysplastic syndrome. Int J Hematol 2012, 95, 527–534.
[26] **Dizdaroglu M, Jaruga P:** Mechanisms of free radical-induced demage to DNA. Free Radic Res 2012, 26, 382–419.
[27] **Stevens RG, Jones DY, Miccozzi MS, Taylor PR:** Body iron stores and the risk of cancer. N Engl J Med 1988, 319, 1047–1052.
[28] **Elmberg M, Hultcrantz R, Ekbom A, Brandt L, Olsson S, Olsson R, Lindgren S, Lööf L, Stål P, Wallerstedt S, Almer S, Sandberg-Gertzén H, Askling J:** Cancer risk in patients with hereditary hemochromatosis and in their first-degree relatives. Gastroenterology 2003, 125, 1733–1741.
[29] **Edgren G, Reilly M, Hjalgrim H, Tran TN, Rostgaard K, Adami J, Titlestad K, Shanwell A, Melbye M, Nyrén O:** Donation frequency, iron loss, and risk of cancer among blood donors. J Natl Cancer Inst 2008, 100, 572–579.
[30] **Lambert R:** Overview of the epidemiology and prevention of digestive cancer. World Gastroenterology News 2003, 82, 21–25.
[31] **Brenner H, Rothenbacher D, Arndt V:** Epidemiology of stomach cancer. Methods Mol Biol 2009, 472, 467–477.
[32] **Crew KD, Neugut AI:** Epidemiology of gastric cancer. World J Gastroenterol 2006, 12, 354–362.
[33] **Eslick GD:** Epidemiology of esophageal cancer. Gastroenterol Clin North Am 2009, 38, 17–25.
[34] **Lerut T, Coosemans W, Decketr G, De Leyn P, Moons J, Nafteux P, Van Raemdonck D:** Extended surgery for cancer of the esophagus and gastroesophageal junction. J Surg Res 2004, 117, 58–63.

[35] **Khosravi Shahi P, Diaz Munoz de la Espada VM, Garcia Alfonso P, Encina Garcia S, Izarzugaza Peron Y, Arranz Cozar JL, Hernandez Maria B, Perez Manga G:** Management of gastric adenocarcinoma. Clin Transl Oncol 2007, 9, 438–442.
[36] **Wilkinson N, Scott-Conner CE:** Surgical therapy for colorectal adenocarcinoma. Gastroenterol Clin North Am 2008, 37, 253–267.
[37] **Romer J, Nielsen BS, Ploug M:** The urokinase receptor as a potential target in cancer therapy. Curr Pharm Des 2004, 10, 2359–2376.
[38] **Illemann M, Hansen U, Nielsen HJ, Andreasen PA, Høyer-Hansen G, Lund LR, Danø K, Nielsen BS:** Leading-edge myelofibroblast in human colon cancer express plasminogen activator inhibitor 1. Am J Clin Pathol 2004, 122, 256–265.
[39] **Beyer BC, Heiss MM, Simon EH, Gruetzner KU, Babic R, Jauch KW, Schildberg FW, Allgayer M:** Urokinase System expression in gastric carcinoma. Prognostic impact in an independent patient series and first evidence of predictive value in preoperative biopsy and intestinal metaplasia specimes. Cancer 2006, 106, 1026–1035.
[40] **Seetoo DQ, Crowe PJ, Russell PJ, Yang JL:** Quantitative expression of protein markers of plasminogen activation system in prognosis in colorectal cancer. J Surg Oncol 2003, 82, 184–193.
[41] **Shiomi H, Eguchi Y, Tani T, Kodama M, Hattori T:** Cellular distribution and clinical value of urokinase-type plasminogen activator, its receptor and plasminogen activator inhibitor-2 in esophageal squamous cell carcinoma. Am J Pathol 2000, 156, 567–575.
[42] **Heiss MM, Allgayer H, Gruetzner KU, Babic R, Jauch KW, Schildberg FW:** Clinical value of an extended biological staging by bone marrow micrometastases and tumor associated proteases in gastric cancer. Ann Surg 1997, 226, 726–745, 736–744, discussion 744–745.
[43] **Mulcahy HE, Duffy MJ, Gibbons D, McCarthy P, Parfrey NA, O'Donoghue DP, Sheahan K:** Urokinase type plasminogen activator and outcome in Dukes'B colorectal cancer. Lancet 1994, 344, 583–684, 583–584.
[44] **Zhang L, Zhao ZS, Ru GQ, Ma J:** Correlative studies on uPA mRNA, uPAR mRNA expression with vascular endothelial growth factor, microvessel density, progression and survival time of patients with gastric cancer. World J Gastroenterol 2006, 12, 3970–3976.
[45] **Hogdall CK, Christensen IJ, Stephens RW, Sorensen S, Norgaard-Petersen B, Nielsen HJ:** Serum tetranectin is an independent prognostic marker in colorectal cancer and weakly correlated with plasma suPAR, plasma PAI-1 and serum CEA. APMIS 2002, 110, 630–638.
[46] **Heiss MM, Allgayer H, Gruetzner KU, Funke I, Babic R, Jauch KW, Schildberg FW:** Individual development and uPA receptor expression of disseminated tumour cells in bone marrow: a reference to early systemic disease in solid cancer. Nat Med 1995, 1, 1035–1039.
[47] **Usnarska-Zubkiewicz L, Strutyńska-Karpińska M, Podolak-Dawidziak M, Nienartowicz M, Grabowski K, Prajs I, Kuliczkowski K:** Epithelial bone marrow cells in patients with advanced esophageal squamous cell carcinoma. Neoplasma 2009, 56, 245–251.
[48] **Linkesch W, Ludwig H:** Serum ferritin and beta-2-microglobulin in patients with multiple myeloma. Cancer Detect Prev 1983, 6, 297–301.
[49] **Cozzi A, Corsi B, Levi S, Santambrogio P, Biasiotto G, Arosio P:** Analysis of the biologic functions of H and L ferritin in HeLa cells by transfection with siRNAs and cDNAs: evidence for a proliferative role of L-ferritin. Blood 2004, 103, 2377–2383.
[50] **Cozzi A, Levi S, Corsi B, Santambrogio P, Campanella A, Gerardi G, Arosio P:** Role of iron and ferritin in TNF alpha induced apoptosis in HeLa cells. FEBS lett 2003, 537, 187–192.
[51] **Pham CG, Bubici C, Zazzeroni F, Papa S, Jones J, Alvarez K, Jayawardena S, De Smaele E, Cong R, Beaumont C, Torti FM, Torti SV, Franzoso G:** Ferritin heavy chain upregulation by NF-kappa B inhibits TNF alpha-induced apoptosis by suppressing reactive oxygen species. Cell 2004, 119, 529–542.
[52] **Dass K, Ahmed A, Azmi AS, Sarkar SH, Sarkar FH:** Evolving role of uPA/uPAR system in human cancers. Cancer Treat Rev 2008, 34, 122–136.
[53] **Fazioli F, Blasi F:** Urokinase-type plasminogen activator and its receptor: new targets for anti-metastatic therapy? Trends Pharmac Sci 1994, 15, 25–29.
[54] **Pillay V, Dass CR, Choong PF:** The urokinase plasminogen activator receptor as a gene therapy target for cancer. Trends Biotechnol 2007, 25, 33–39.

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