Apoptosis-related biomarkers in patients with gastrointestinal cancer

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Introduction

More than 128,000 new cases of gastrointestinal cancer were diagnosed in 2008 in Germany. Of these, 65,390 were colorectal cancer, 15,870 involved cancer of the stomach, 14,960 patients had pancreatic cancer, 13,010 patients oral cancer, 7,610 patients presented with liver cancer, 6,180 with cancer of the esophagus, and 5,160 cases with cancer of the gall bladder. Approximately 67,000 patients died from gastrointestinal cancer in 2008, mainly patients with colorectal and pancreatic and cancer of the stomach [1]. Screening programs using the fecal (immunological) occult blood test (FOBT, FIT) and colonoscopy have been established for colorectal cancer with the aim of detecting the cancer in an early and still curable stage. Although these markers have limitations regarding tumor specificity and sensitivity, these programs have led to a reduction in colorectal cancer-related mortality [2]. In other types of gastrointestinal cancer, tumor markers are available supporting the differential diagnosis, monitoring of the therapy and follow-up, e.g., AFP in the case of liver cancer, CA 19-9 in pancreatic and gall bladder cancer and CEA in colorectal cancer [3]. Nevertheless, additional biomarkers and combinations of these are needed to improve the diagnostic accuracy for to be used in screening programs and also for providing a differential diagnosis when patients are admitted to the hospital with specific symptoms. One promising approach is the use of blood-based biomarkers that are associated with tumor development and the reaction of the tumor environment and immune system to neoplastic growth.

Of the many dysregulated pathways in the development of cancer, the loss in the ability of cells to undergo apoptosis is one of the most important. However, during the long period of cancerogenesis, immune-mediated tumor cell death to some extent is able to counterbalance the high proliferation rate. When the rate of cell death is impaired and/or the rate of tumor progression is accelerating, tumor manifestation and progression occur. In the later stages of cancer, the rate of cell death increases again since many dysplastic tumor cells undergo apoptosis or necrosis. In addition, tumor counterattack leads to massive cell death of bystander and immune cells. The resulting products of apoptosis are released and can be detected in the blood [4, 5]. The extrinsic apoptosis pathway is initiated by the interaction of FAS ligand (FASL) and the cell-surface receptor FAS leading to the transmission of the cell death signal into the cytoplasm after trimerization of the FAS receptor. The FAS/FASL system is involved in the escape of cancer cells from the immune system since apoptosis in immune cells expressing FAS is induced by cancer cells expressing FASL on their surface or producing soluble FASL [5, 6]. In addition, cleaved soluble FAS (sFAS) derived from tumor cells, peripheral blood cells or the surrounding stromal tissue can inhibit FAS-mediated apoptosis by neutralizing FASL. As a consequence, elevated levels of sFAS have been found in various types cancer both before and during anticancer treatment [5, 6, 7]. Furthermore, tumor necrosis factor-related apoptosis-inducing ligand (TRAII)}
Biomarkers in gastrointestinal cancer can induce apoptosis by extrinsic stimulation of its cell surface receptor.

The macrophage migration inhibitory factor (MIF), originally described as T-cell-derived cytokine, exhibits a broad range of immune-stimulatory and pro-inflammatory activities, e.g., by promoting mitogen-activated-protein-kinase (MAPK) signaling and the secretion of tumor necrosis factor-α (TNF-α) [8]. MIF is not only released from T-cells, but also from parenchymal and tumor cells and plays an essential role in the regulation of cell homeostasis, carcinogenesis, inhibition of p53-dependent cell death, and tumor angiogenesis [8]. In the investigation described here we examined the usefulness of the apoptosis-related biomarkers, FAS, FASL, TRAIL and MIF in the differential diagnosis of gastrointestinal cancer.

**Patients and methods**

Serum samples from a total of 231 subjects were collected at the University Hospital Bonn between 2010 and 2012. The subjects included 107 patients with gastrointestinal cancer (20 cases with gastroesophageal cancer, 35 cases with colorectal cancer, 19 cases with cancer of the liver, 14 cases with pancreatic cancer, and 19 cases with cholangiocellular cancer). There were 73 cases with benign gastrointestinal diseases, and 51 healthy controls. Blood samples were obtained from all patients during the active stage of the disease i.e., prior to surgery and other therapeutic interventions. Blood samples were centrifuged within two hours after venous puncture and aliquots cryopreserved at –80 °C until analysis was carried out using the Biofluid Biobank of the University Hospital Bonn.

The four biomarkers FAS, FASL, TRAIL, and MIF were quantified using the MILLIPLEX® MAP Human Circulating Cancer Biomarker Magnetic Bead Panel 1 assay (Merck Millipore, Billerica, MA, USA) that was run on the Bio-Plex® 200 System (Bio-Rad, Hercules, CA, USA). This kit is an immunoassay on the surface of fluorescent-coded magnetic beads where the determination of varying proportions of two fluorescent dyes allows the parallel and simultaneous

![Figure 1. Robust means and 95% confidence intervals of serum MIF, sFAS, and TRAIL in groups of healthy controls (HC), benign, malignant colorectal (A; CR ben, CRC), liver (LIV), pancreatic (PAN), and gall bladder (GAL) cancer disease (B). If confidence interval bars do not overlap, the difference between groups is significant.](image-url)
measurement of multiple biomarkers. Antibodies in prediluted serum samples bind to the beads which are coated with specific antibodies. After incubation with biotinylated detection antibody and streptavidin-phycoerythrin (PE), the bead-bound complexes are detected and quantified by flow cytometry on the Bio-Plex® 200 system.

Measurement procedures were performed according the instructions of the manufacturer as described earlier [9]. Standard material for calculation of the concentrations was provided in the kit. Controls and serum pools were included in all runs to assure inter-assay comparability. Serum samples were measured as single determinations. Prior to the clinical evaluation, intra- and interassay precision and preanalytical robustness of the method were assessed [9]. Robust means and 95% confidence intervals were calculated for each patient group. Comparisons between groups were done using the t-test, the Welch-test, and the Wilcoxon-test depending on the distribution of the values.

**Results**

sFAS and MIF serum levels in colorectal cancer patients were significantly elevated compared with healthy controls but not when compared with values from patients with benign colorectal disease. It is noteworthy that sFAS values were also increased in benign disease, but an increase was not seen in benign disease as compared to healthy controls in the case of MIF. The concentration of FASL and TRAIL were similar in all groups investigated. In liver cancer, only sFAS showed significantly higher values compared with healthy controls. Once again, sFAS levels were elevated in benign hepatic diseases limiting its differential diagnostic potential. Interestingly, TRAIL values showed a tendency to be lower in liver cancer patients and the same was true in the case of patients with pancreatic cancer and cancer of the gallbladder: in patients with cancer of the gallbladder sFAS was markedly increased whereas TRAIL values were lower than in healthy controls. FASL was unchanged in both types of cancer and unexpectedly, MIF in diseases of the gallbladder, was decreased. No clear results were obtained for subjects with esophageal and gastric cancer where MIF and TRAIL values were lower and sFAS and FASL values unchanged.

**Discussion**

The present findings showing elevated sFAS levels in colorectal, liver, and pancreatic cancer and also elevated MIF levels in colorectal cancer are in accord with earlier reports describing an increase in apoptotic markers in the blood of cancer patients [5, 7]. However, due to an increased rate in cell death in benign diseases and the marked overlap in serum concentrations, these markers cannot be used for differential diagnosis of malignant and benign disease. The finding of a lower level of TRAIL in some cancer entities is of interest. This could point to the existence of different regulation and induction mechanisms of apoptosis in different types of cancer patients. Further studies will show whether the combination of apoptosis-related markers with more specific tumor-associated markers is able to improve the diagnostic accuracy in gastrointestinal cancer and provide information on the prognostic relevance of the markers in general.

**References**


