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COLORECTAL CARCINOMA AND MARKERS OF BIOLOGICAL ACTIVITY

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Postgradual Dissertation
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CONTENTS

ABBREVIATIONS

CRC	colorectal carcinoma
CEA	carcinoembryonic antigen
CA 19-9	carbohydrate (cancer) antigen 19-9
WHO IARC	World health organisation, International agency for research on cancer
HHMI	Howard Hughes Medical Institute
DNA	deoxyribonucleotic acid
ACF	aberrant crypt foci
HGD	high-grade dysplasia
TGF	transforming growth factor
AI	incidence of apoptosis
DCC	deleted in colorectal cancer, encoding transmembrane glykoprotein of imunoglobulins
FAP	Familial adenomatous polyposis
CDK	cyklin dependent kinase
Ki-ras	Kirsten murine sarcoma viruses onkogens
β-CTN	β catenin
erB2	onkogene tyroxin kinase (EGFR group)
THF-β	transforming growth factor β
MMP	metalloproteinases
TF	tissue factor
Tiam-1	activator of invasivity in the lymphoma cells and inhibitor of invasivity in the epithelial cells
mts1	calcium binding proteins encoding gene
LR67	laminin receptor
MUC-1	mucin stimulating adhesion of carcinoma cells to the endothelial cells
APC	adenomatose polyposis coli protein
IGF-IIR	insulin-like growth factor II receptor
p53	nuclear protein inhibiting replication of involved DNA
Rb	retinoblastoma protein encoding phosphoprotein participating regulation of cell cycle
TGF-βR-II	TGF-β receptor type II
E-CAD/CTN	E-cadherin / catenin complex
Smad4	tumour suppressor
β-catenin	oncogene
Smad2	tumour suppressor
N-Ras	oncogene
HER-2/NEU	oncogene
VEGF	vascular endothelial growth factor
ECM	extracellular matrix
NO	nitric oxide
PCR	Polymerase Chain Reaction
MSI	microsatellite instability
HNPCC	Hereditary Non-Polyposis Colorectal Cancer
MSH2	gene
MLH1	gene
PMS1	gene
PMS2	gene
IBD	inflammatory bowel disease
UC	ulcerative colitis
IGF	insulin-like growth factors
HCG	human chorionic gonadotropin
GTD	gestational trophoblastic disease
CA 15-3	carbohydrate (cancer) antigen
CA 125	carbohydrate (cancer) antigen
CA 50	carbohydrate (cancer) antigen
CA 72-4	carbohydrate (cancer) antigen
CA 242	carbohydrate (cancer) antigen
PSA	prostate-specific antigen
EGTM	European Group on Tumour Markers

CA 72-4	carbohydrate (cancer) antigen
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NIH	National Institutes of Health
ASCO	American Society of Clinical Oncology
TPA	tissue polypeptide antigen - cytokeratinin tumor markers
TPS	tissue polypeptide specific antigen - cytokeratinin tumor marker
CYFRA 21-1	cytokeratinin tumor markers
SCC	cytokeratinin tumor markers
ICAM	intercellular adhesion molecules
VCAM	vascular cell adhesion molecules
CD44	adhesive molecules
TK	thymidine kinase
TMP	thymidine mono phosphate
ACRP-30	adipocyte compliment related protein-30
APM-1	adipose tissue most abundant gene transcript-1
AJCC/UICC	American Joint Committee on Cancer/ International Union Against Cancer
TNM	staging system
VLA	very late antigens
LFA	leukocyte function-associated antigens
gp	platelet glycoproteins
MAdCAM	mucosal addressin cell adhesion molecule
PECAM	platelet endothelial cell adhesion molecule
EGF-R	epidermal growth factor receptor
TGF-R	transforming growth factor receptor
VEGF-R	vascular endothelial growth factor receptor
IGF-R	insulin-like growth factor receptor
PD-ECGF	platelet-derived endothelial cell growth factor
c-Met	hepatocyte growth factor/scatter factor receptor
bFGF	basic fibroblast growth factor
MT-MMP	membrane-bound matrix metalloproteases
uPA-R	urokinase-type plasminogen activator receptor
TNF-R	tumor necrosis factor receptor
IMA	inferior mesenteric artery
PET	positron emission tomography
CT	computed tomography

INTRODUCTION

Colorectal cancer (CRC) remains a major public health problem throughout the world.

The goal of all cancer research and treatment is to prevent people dying from the disease. Knowledge has been accruing rapidly about actions and interventions that could lead to a reduction in death from colorectal cancer by **reducing the risk** of developing the disease, **identifying the disease at a stage when it is more curable**, or **improving the outcome of treatment.**

Surgical part of this task is mainly the last but not least part of this statement - it means improving the outcome of treatment. Improving the outcome of „surgical" treatment does not mean only improving surgical procedure results. As in most malignant diseases it is a **complex** of precise preoperative staging, adequate radicality to achieve curative operation with integral adjuvant or neoadjuvant therapy and the very important long term follow up with treating of asymptomatic relapse.

The precise preoperative staging is fundamental for surgical strategy, incomplete staging means incomplete treatment and poor outcome. Postoperative active follow up of patients helps to improve resecability of relapse.

Both staging and follow up with restaging derive benefit from using **tumor markers.**

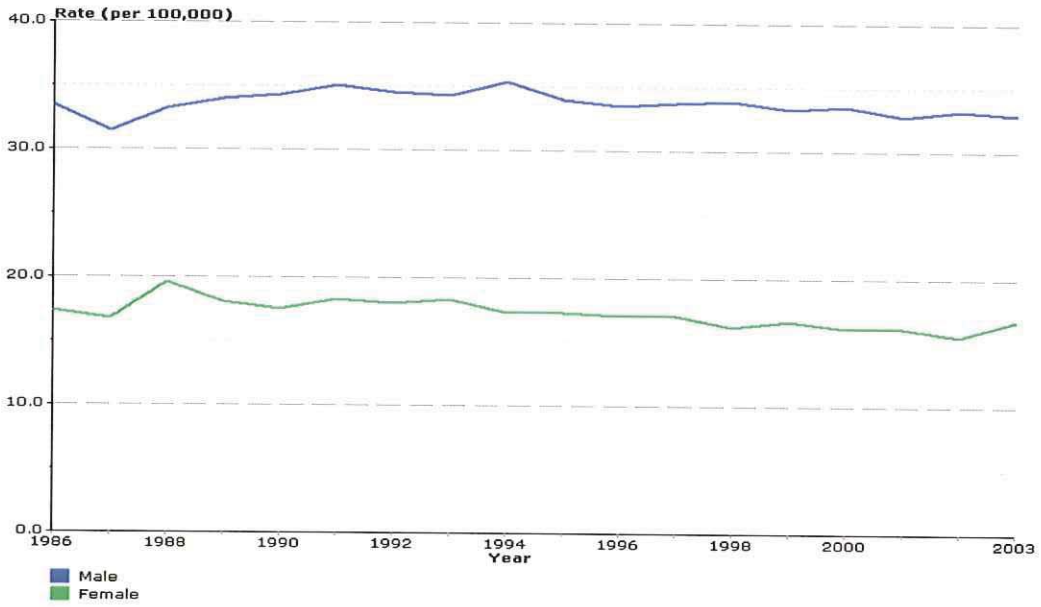
Large-scale clinical evaluations of predictive markers are currently in progress, including determination of their ability to predict response of patients to therapy for advanced disease and for adjuvant treatment.

Our Surgical Department has a long time experience using CEA and CA19-9 in staging and follow up of patients with colorectal cancer. In this thesis, results of study examining other markers are presented.

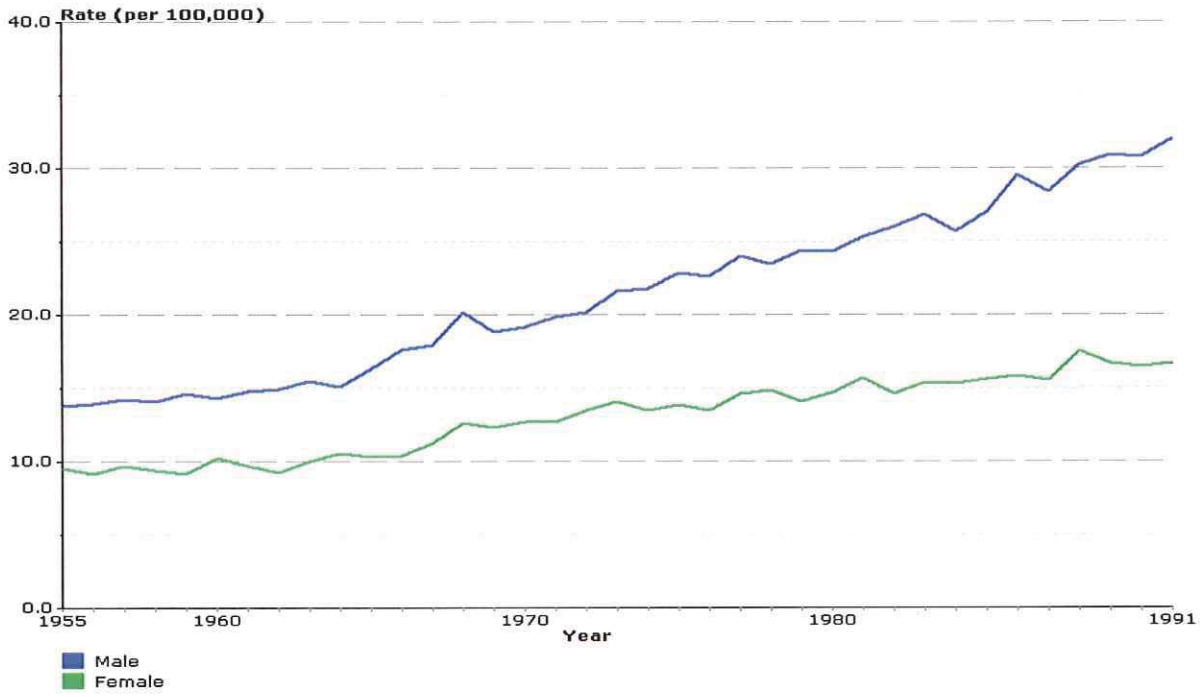
REVIEW OF THE LITERATURE

EPIDEMIOLOGY OF COLORECTAL CANCER

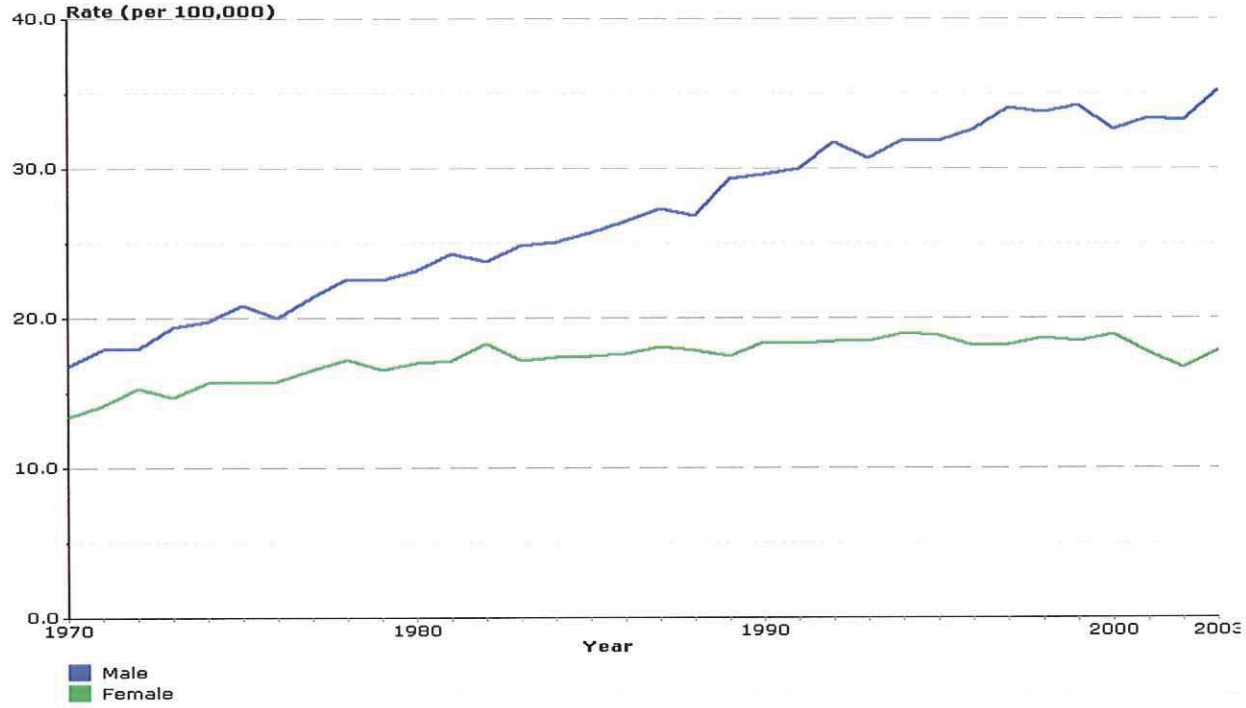
Worldwide, the mortality from colorectal cancer is estimated to be 500,000 a year. In the United States, CRC is the third most frequently diagnosed cancer in both men and women and the second most common fatal cancer (behind lung cancer). During the year 2004, there were an estimated 106,000 cases of colon cancer and 41,000 cases of rectal cancer in the United States, resulting in 57,000 total deaths[1]. The cost of treating colorectal cancer in the United States is believed to be between 5.5 and 6.5 billion dollars a year. Worldwide, the risk of death from CRC is highest in developed countries and especially low in Asia and Africa[2]. Until the year 2000 Czech Republic was the first in the world in the incidence of CRC. The latest data show that Hungary has replaced Czech Republic since the year 2000.



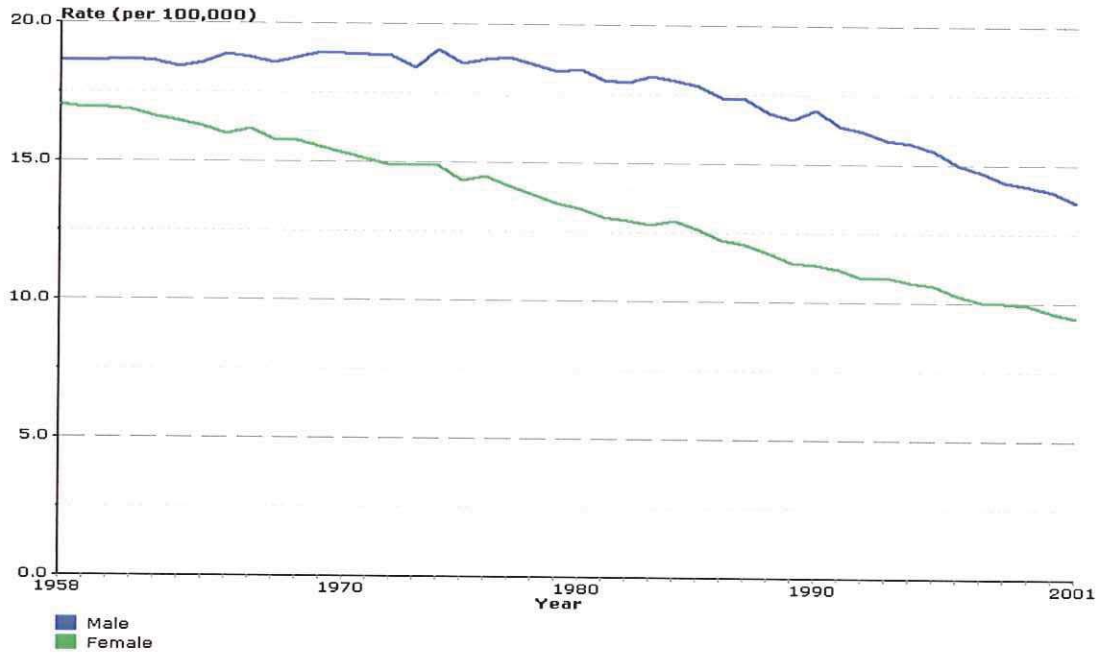
Graph 1: Age-Standardised Rate per 100,000, colorectal cancer, Czech Republic- source WHO IARC



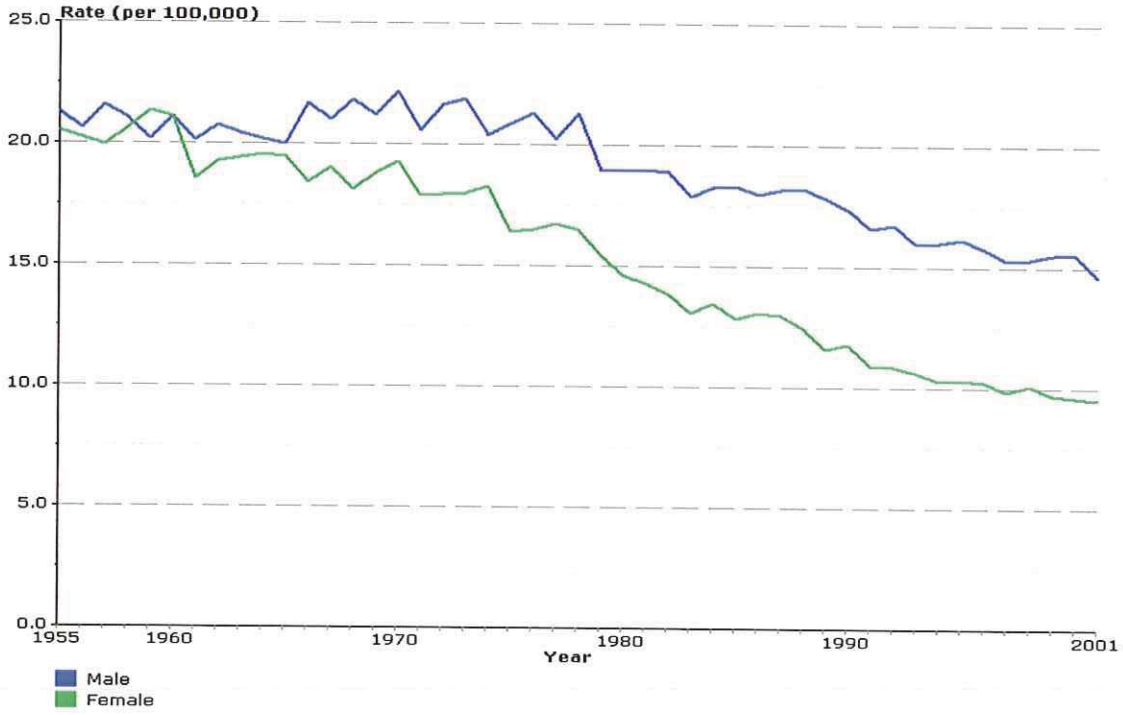
Graph 2: Age-Standardised Rate per 100,000, colorectal cancer, Former Czechoslovakia - source WHO IARC



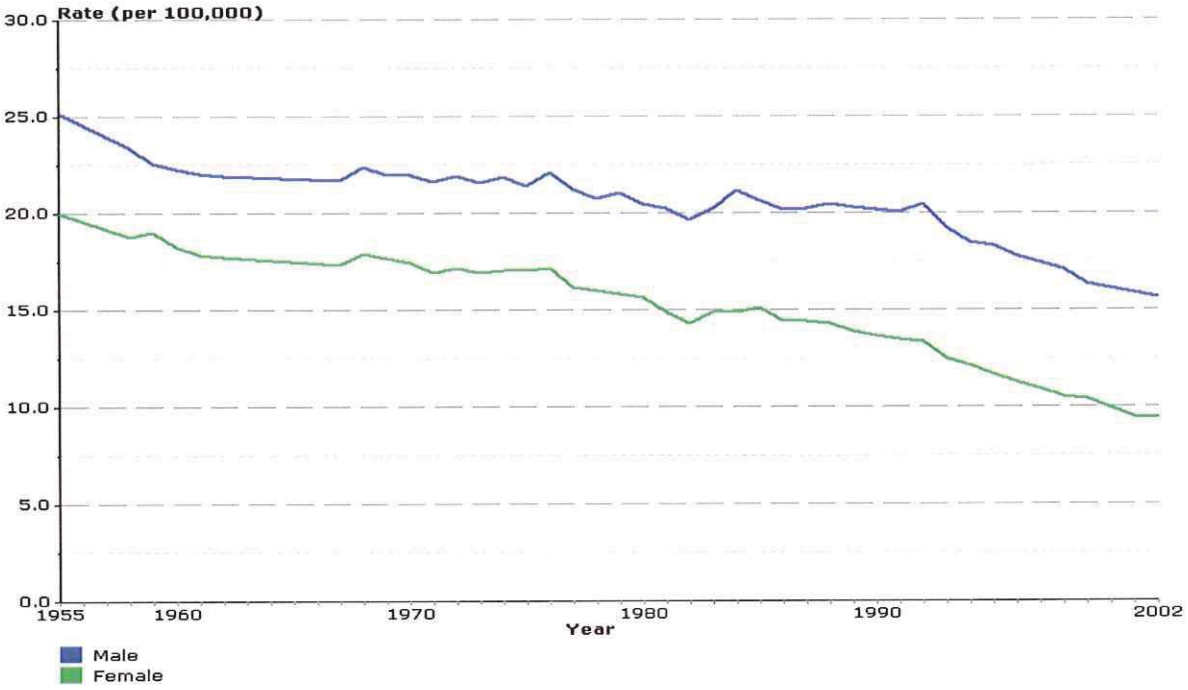
Graph 3: Age-Standardised Rate per 100,000, colorectal cancer, Hungary - source WHO IARC



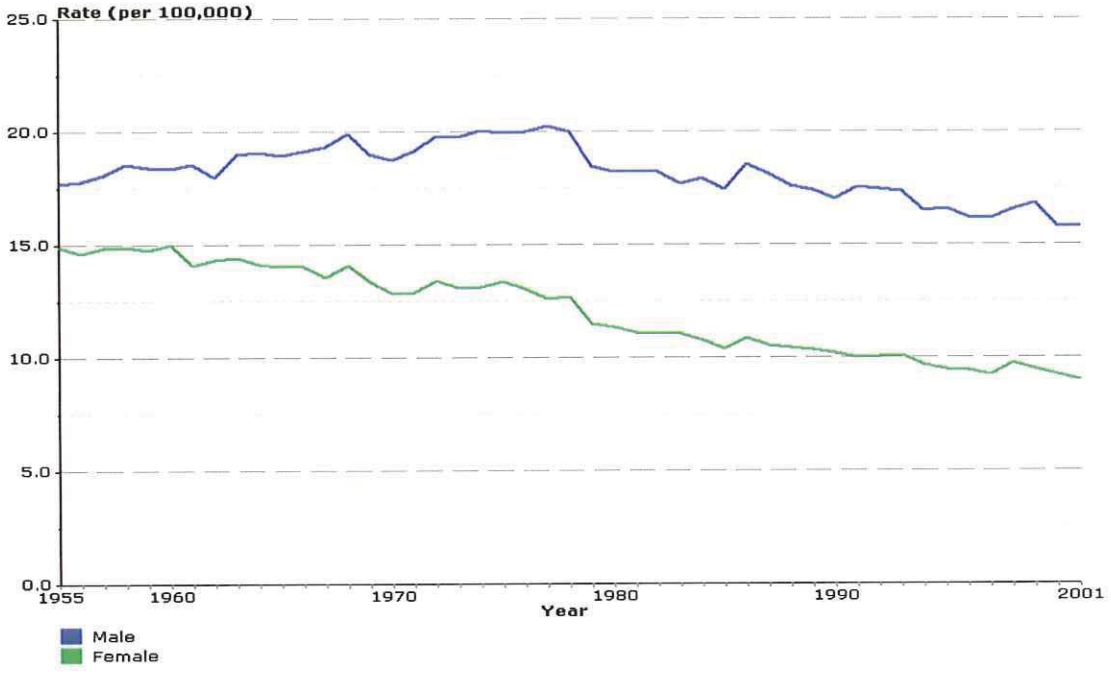
Graph 4: Age-Standardised Rate per 100,000, colorectal cancer, USA - source WHO IARC



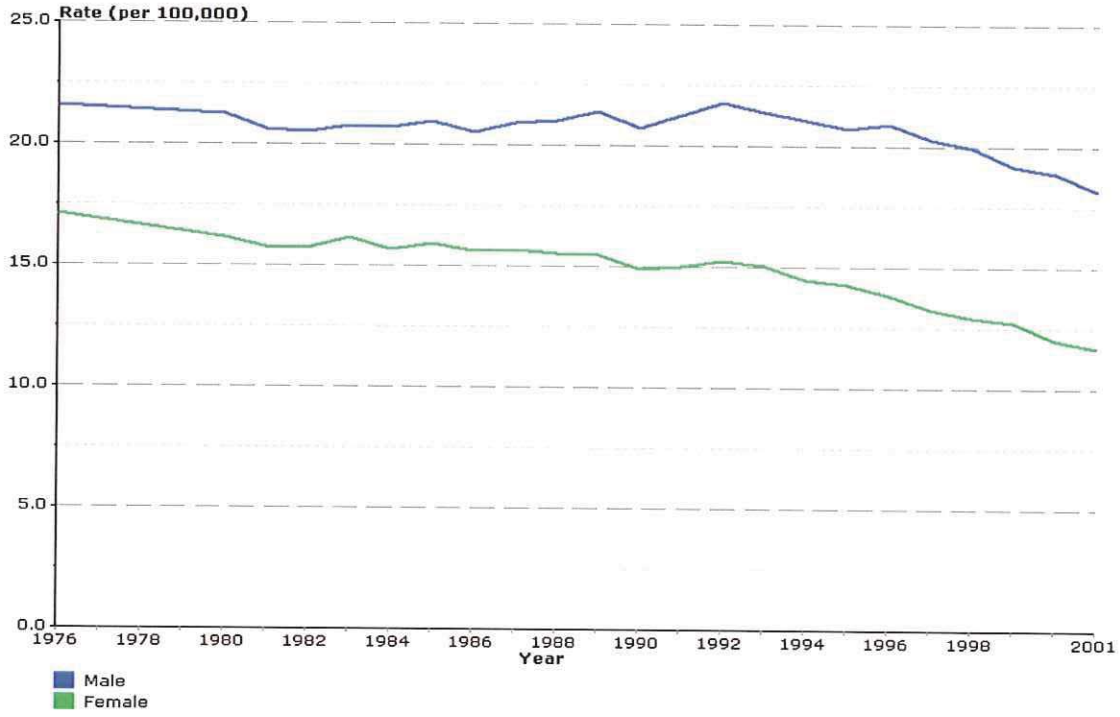
Graph 5: Age-Standardised Rate per 100,000, colorectal cancer, Canada - source WHO IARC



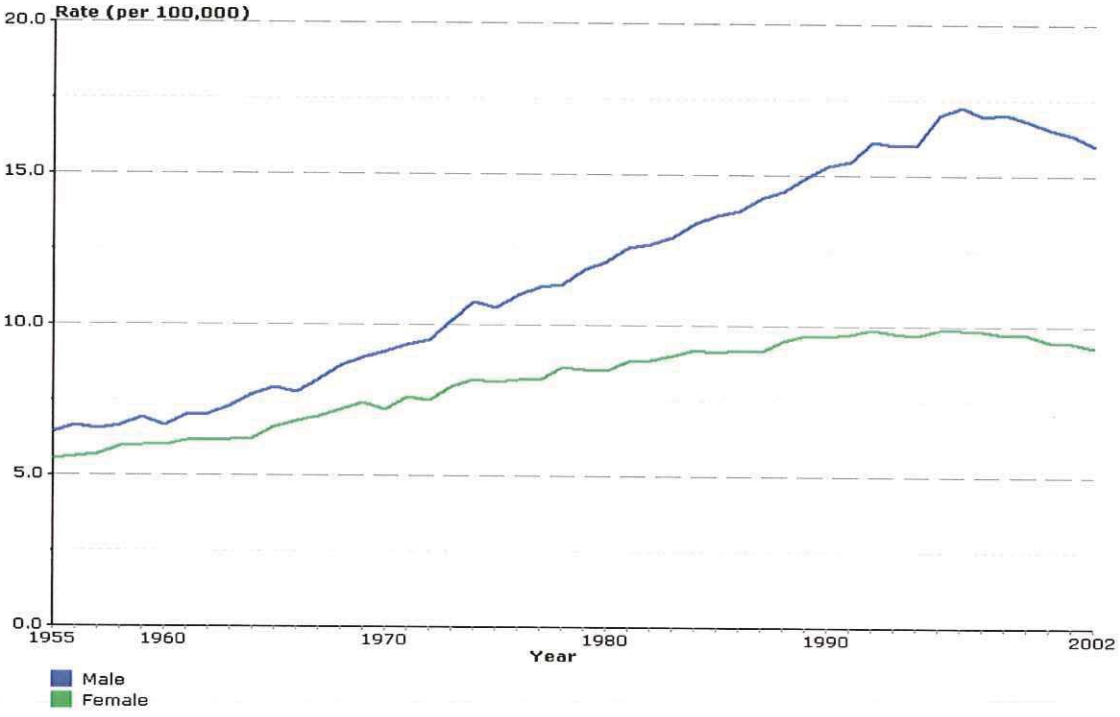
Graph 6: Age-Standardised Rate per 100,000, colorectal cancer, United Kingdom - source WHO IARC



Graph 7: Age-Standardised Rate per 100,000, colorectal cancer, France - source WHO IARC



Graph 8: Age-Standardised Rate per 100,000, colorectal cancer, Germany - source WHO IARC



Graph 9: Age-Standardised Rate per 100,000, colorectal cancer, Japan - source WHO IARC

Different populations worldwide experience different levels of colorectal cancer, and these levels change with time. Populations living in one community whose lifestyles differ from those of others in the same community also experience different levels of colorectal cancer. Groups of migrants quickly lose the risk associated with their original home community and acquire the patterns of the new community, often starting within one generation of arrival.

Ethnic and racial differences in colorectal cancer, as well as studies on migrants, suggest that environmental factors play a major part in the etiology of the disease. In Israel male Jews born in Europe or the United States are at higher risk of colon cancer than those born in Africa or Asia. Risk in the offspring of Japanese populations who have migrated to the United States has changed - incidence now approaches or surpasses that in white people in the same population and is three or four times higher than among the Japanese in Japan.

Country	Deaths	Crude Rate	ASR(W)	Cumulative Rate
Albania	39	2.4	2.9	-
Armenia	132	7.1	6.6	-
Australia	2569	27.0	18.0	-
Austria	1240	31.5	18.2	-
Belarus	1125	24.0	18.8	-
Bulgaria	1169	29.4	16.7	-
Canada	3549	23.3	15.6	-
Croatia	841	39.9	30.4	-
Czech Republic	2517	50.3	33.6	-
Denmark	983	37.3	20.3	-
Estonia	159	25.2	17.3	-
Finland	462	18.3	11.2	-
France	8345	29.2	15.8	-
Georgia	176	8.3	6.0	-
Germany	13658	34.0	18.9	-
Greece	952	17.6	8.8	-
Hong Kong	743	22.7	16.3	-
Hungary	2514	51.7	32.6	-
Iceland	24	17.1	11.8	-
Ireland	493	26.2	19.3	-
Israel	610	19.7	16.2	-
Italy	8807	31.4	15.3	-
Japan	20000	32.5	16.6	-
Kazakhstan	690	9.6	13.1	-
Kuwait	17	1.3	3.9	-
Kyrgyzstan	108	4.5	7.2	-
Latvia	263	24.1	16.9	-

Lithuania	423	25.8	18.9	-
Luxembourg	60	27.8	17.2	-
Macedonia	166	16.4	13.2	-
Malta	39	20.4	15.4	-
Mauritius	20	3.5	4.6	-
Netherlands	2140	27.2	17.2	-
New Zealand	571	30.2	20.7	-
Norway	777	35.1	18.8	-
Poland	4373	23.3	18.0	-
Portugal	1598	32.4	17.5	-
Republic of Korea	2253	9.4	11.4	-
Republic of Moldova	291	16.7	16.2	-
Romania	2028	18.5	12.7	-
Russian Federation	15369	22.7	18.8	-
Singapore	287	17.6	18.1	-
Slovakia	1009	38.4	31.1	-
Slovenia	323	33.7	22.9	-
Spain	6464	32.9	17.3	-
Sweden	1231	28.1	13.2	-
Switzerland	929	26.3	14.3	-
Tajikistan	39	1.3	2.6	-
UK, England and Wales	7497	28.7	15.7	-
UK, Northern Ireland	198	23.8	16.2	-
UK, Scotland	844	34.7	19.2	-
Ukraine	5771	25.2	18.1	-
United States of America	28462	20.6	14.1	-
Uzbekistan	232	1.9	3.3	-

Table No 1: Colorectal cancer, year 2000, males, Age-Standardised Rate[3]

For reasons such as these, colorectal cancer is widely believed to be an environmental disease, with „environmental" defined broadly to include a wide range of ill defined cultural, social, and lifestyle practices. As much as 70-80% of colorectal cancers may owe their appearance to such factors; this clearly identifies colorectal cancer as one of the major neoplasms in which causes may be rapidly identified, and a large portion of the disease is theoretically avoidable.

ETIOLOGY AND PATHOGENESIS OF COLORECTAL CANCER

Gene–environment interactions play an important role in the underlying cause of many cancers, including colorectal cancer.

Cancer is a genetic disease," says Bert Vogelstein, an HHMI investigator at The Johns Hopkins Oncology Center in Baltimore. "But it differs from most other genetic diseases in two ways." Traditional genetic diseases such as hemophilia or cystic fibrosis develop because of errors in the DNA of a fertilized egg and, therefore, of every subsequent cell in the person who develops from that egg. By contrast, though people may inherit predispositions to certain types of cancer, cancer itself generally results from mutations in the DNA of a single cell in the body that then begins to multiply uncontrollably. As that cell gains a selective advantage over the less-frequently dividing normal cells, it eventually produces a tumor made up of its descendants.

A second difference between cancer and other genetic diseases is that cancer arises "not from a single mutation, but from the accumulation of several mutations," Vogelstein says. Different kinds of cancers require a different number of mutations or "hits" to a cell's DNA.

Cancerogenesis

The stepwise nature of tumorigenesis was first observed and has been best worked out in colon cancer. It is well understood that certain polyps are precursors to most colon cancers.

Colon cancer evolves through epithelial cell deregulation and inappropriate proliferation. These histopathological characteristics are exemplified in the biochemical, immunohistochemical, genetic and epigenetic elements detected within colonic mucosa. Early detection is paramount for the prevention of colon cancer deaths. **Aberrant crypt foci (ACF)** are thought to be the earliest identifiable neoplastic lesions in the colon carcinogenetic model. The progression of ACF to polyp and, subsequently, to cancer parallels the accumulation of several biochemical alterations and mutations whereby a small fraction of ACF evolve to colon cancer. The transition from adenoma to high-grade dysplasia (HGD) appears to involve the TP53 gene, considered a guardian of the genome . Once HGD occurs, it has been suggested that „genetic chaos" ensues, setting the stage for malignant transformation. The study of ACF and their relationships to growth factors, such as TGF-α, TGF-β, EGFR, TGF- β RII, phosphorylated cellular tyrosine (P-tyr) revealed a strong correlation between altered expression of TGFs in all ACF that have been examined and the degree of dysplasia and crypt multiplicity. TGF-β was undetectable in ACF, which had a high incidence of apoptosis (AI). The result was similar to that both in adenomas and in carcinomas. Apoptosis provides a protective mechanism against neoplasia by moving genetically damaged stem cells from the epithelium before they can undergo clonal expansion. Manifestations are indicative of a high level of apoptosis in human ACF and carcinogen-treated animal ACF, in which apoptosis was said to eliminate cells damaged by carcinogen administration[4].

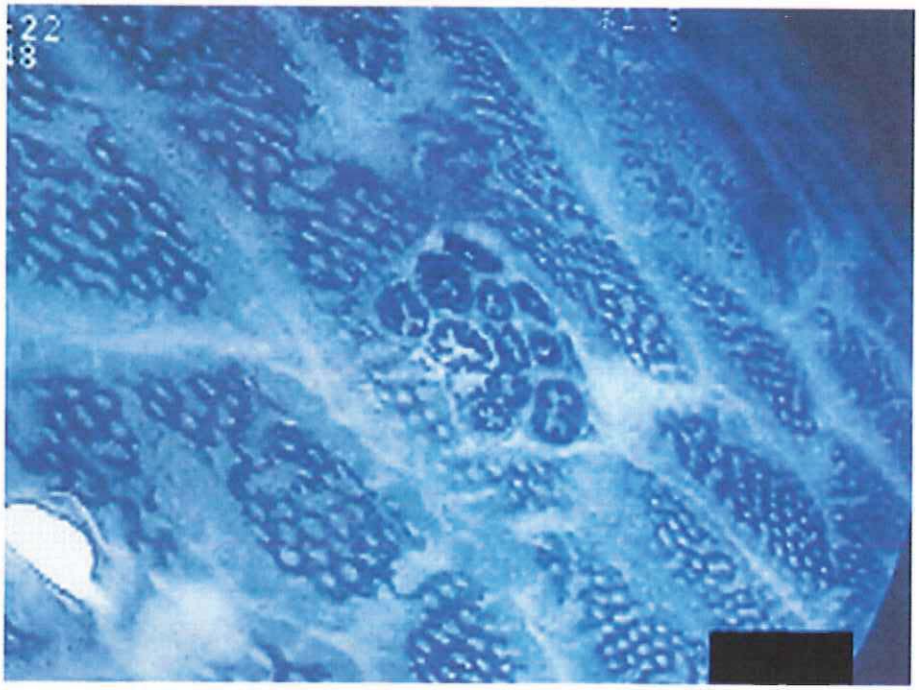


PHOTO No 1: Endoscopy with methylene blue staining reveals a small focus consisting of crypts with semicircular or oval lumens. The aberrant crypts stained more darkly, were larger, and had a thicker epithelial lining and a larger pericryptal zone than normal crypts[5]. (Department of Public Health (H.M.), Sapporo Medical University)

In the above cited study[5] the prevalence of aberrant crypt foci in normal subjects under the age of 40 was 10.0 percent, from 40 to 49 years of age it was 53.6 percent, and from 60 to 69 years of age it was 65.7 percent. Three of the four patients with adenoma who were under the age of 40 had aberrant crypt foci, and the prevalence increased gradually with age, reaching 90.2 percent by the age of 60. In patients with cancer, the prevalence was 100 percent in all age groups examined.

		Normal epithelium
APC gene mutation (5q, 9q chromosome)	⇒	⇓
		Aberant crypt focus
DNA hypomethylation	⇒	⇓
		Adenoma – low-grade dysplasia
K ras mutation (12p chromosome)	⇒	⇓
		Adenoma – medial-grade dysplasia
DCC deletion (18q chromosome)	⇒	⇓
		Adenoma – high-grade dysplasia
p53 deletion (17p chromosome)	⇒	⇓
		Carcinoma
Other alterations (1p deletion)	⇒	⇓
		Metastases

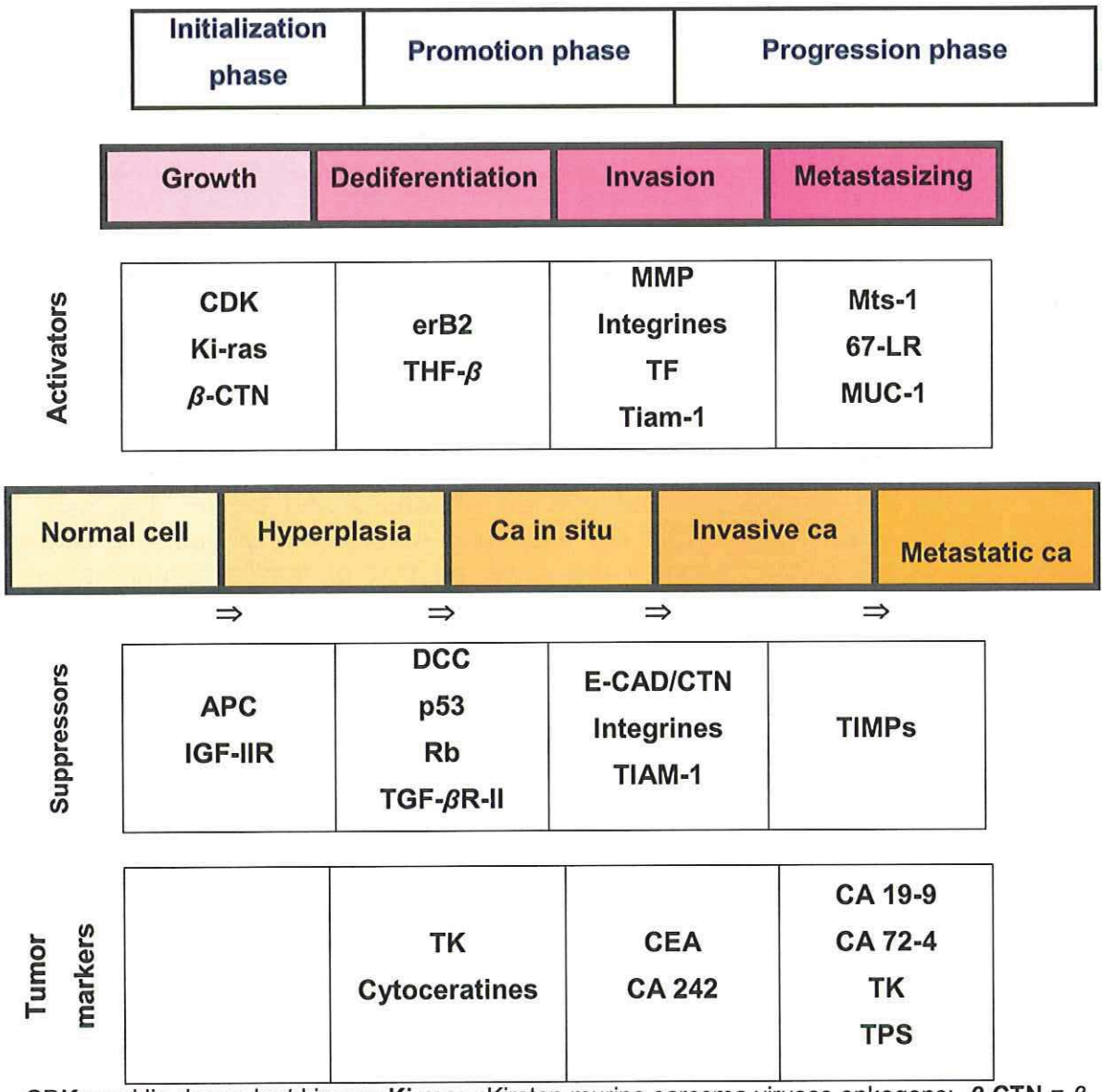
Table No 2: Vogelstein´s model of carcinogenesis.

Most FAP ACF are histopathologically, phenotypically and genetically different from sporadic ACF. Apart from the differences in ACF density between FAP ACF and sporadic ACF, there are significant differences in regard to dysplasia. Most FAP ACF were dysplastic, whereas sporadic ACF had the histopathological features of hyperplastic polyps with little or no dysplasia. The degree of dysplasia in FAP ACF was severer than that of sporadic ACF. Most ACF from FAP patients have phenotypic characteristics of adenomas, which are vital to carcinogenesis, and lack ras gene mutations, while sporadic ACF and a subset of FAP ACF closely resemble hyperplastic polyps, which are benign, but usually have ras gene mutations.

It was found that CpG island methylation was present in more ACF from sporadic cancer than in FAP ACF, implying that FAP ACF usually lacked methylation or K-ras mutations and were frequently dysplastic, while sporadic ACF usually had methylation and/or K-ras mutations and lacked dysplasia[6]. These results may suggest that the molecular mechanism of sporadic colon carcinogenesis is not necessarily the same as that of familial adenomatous polyposis. It was shown that ACF acquired resistance to apoptosis induced by bile salts, whereas normal colonic epithelial cells are turning over consistently by apoptosis. This apoptosis resistance was closely associated with glutathione S-transferase P1-1 expression. One of the most important clinical applications of ACF observation with magnifying endoscopy is its use as a target lesion for chemoprevention. Because ACF are tiny lesions, they should be eradicated during a short time by administration of chemopreventive agents[7].

It is thought that most colorectal cancers arise from preexisting **adenomas**. Such potentially premalignant lesions should be distinguished from juvenile polyps, hamartomas, and inflammatory polyps, which are not thought to progress to colorectal cancer. Recent evidence suggests that serrated adenomas, hyperplastic polyps, and admixed polyps may arise through a pathway different from that of conventional adenomatous polyps—that is, through abnormalities in mismatch repair.

Adenomatous polyps are common: autopsy studies have demonstrated that such lesions are present in more than 30% of persons older than 50 years and that their prevalence increases with age. However, fewer than 1% of adenomatous polyps ever become malignant. This cancer develops as a result of the pathologic transformation of normal colonic epithelium to an adenomatous polyp and ultimately an invasive cancer. The multistep progression requires years and possibly decades and is accompanied by a number of recently characterized genetic alterations. Mutations in two classes of genes, tumor-suppressor genes and proto-oncogenes, are thought to impart a proliferative advantage to cells and contribute to development of the malignant phenotype.



CDK = cyklin dependent kinase; **Ki-ras** = Kirsten murine sarcoma viruses onkogens; **β-CTN** = β catenin, **erB2** = onkogene tyroxin kinase (EGFR group); **THF-β** = transforming growth factor β; **MMP** = matrixmetalloproteinases; **TF** = tissue factor; **Tiam-1** = aktivator of invasivity in the lymphoma cells and inhibitor of invasivity in the epithelial cells; **mts1** = calcium binding proteins encoding gene; **LR67** = laminin receptor; **MUC-1** = mucin stimulating adhesion of carcinoma cells to the endothelial cells; **APC** = adenomatose polyposis coli protein, **IGF-IIR** = insulin-like growth factor II receptor; **DCC** = deleted in colorectal cancer, encoding transmembrane glykoprotein of imunoglobulins; **p53** = nuclear protein inhibiting replication of involved DNA; **Rb** = retinoblastoma protein encoding phosphoprotein, participating regulation of cell cycle; **TGF-βR-II** = TGF-β receptor type II; **E-CAD/CTN** = E-cadherin / catenin complex

Table No 3: Several parts of cancerogenesis and production of tumor markers during the growth of colorectal carcinoma

Part of metastatic process:	Factors:
a. **Local invasion**	• Matrix metalloproteinases • Angiogenetic factors • Adhesiv molecules
b. **Invasion from extracellular matrix through the vessel wall to lumen (intravasal invasion)**	• Matrix metalloproteinases • Cystein a serin proteases • Angiogenetic factors • Adhesive molecules • Hemocoagulative factors
c. **Transportation of tumorous cells**	• Pasiv transport • Hemocoagulative factors
d. **Invasion from lumen to extracellular matrix (extravasal invasion)**	• Matrix metalloproteinases • Cystein a serin proteases • Angiogenetic factors • Adhesive molecules • Hemocoagulative factors
e. **Nidation of tumorous cells and growth of metastases in the new tissue**	• Hemocoagulative factors • Adhesive molecules • Proliferative factors • Angiogenetic factors • Apoptotic factors

Table No 4: Parts of metastatic process and participating factors

The gradual transformation of benign adenomatous polyps into dysplasitic lesions and ultimately to invasive cancer is known as the adenoma-carcinoma sequence. Colorectal cancers are believed to develop through two molecular pathogenetic pathways, chromosomal instability and microsatellite instability. About 80% of all colorectal cancers are aneuploid, they have extra or missing chromosomes. The remaining tumours are diploid but have DNA microsatellite instability, the result of acquired or inherited DNA mismatch repair deficiency. The visible changes that appear when a benign polyp becomes malignant accompany a sequence of underlying genetic mutations that results ultimately in unbridled cell proliferation and metastatic behaviour. The genetic changes in colorectal cancer are not random. Instead, in most cases only a small number of genes are mutated. These are shown in the accompanying table[8].

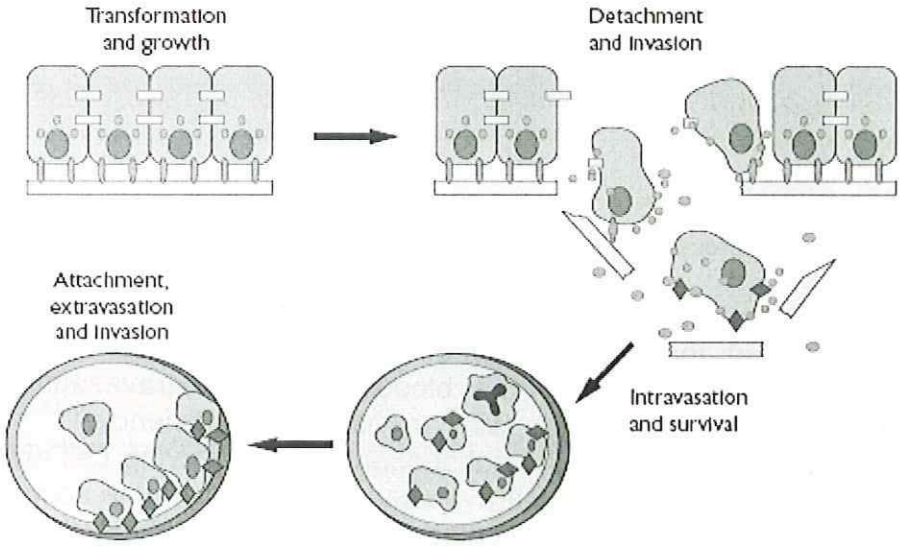
Gene	Class	Frequency in colorectal cancer
APC	Tumour suppressor	75 - 80%
p53	Tumour suppressor	60 - 70%
K-Ras	Oncogene	40 - 50%
TGFβ type II receptor	Tumour suppressor	25 - 30%
MLH1 & other DNA mismatch repair genes	Tumour suppressor	15% (often silenced by methylation)
Smad4	Tumour suppressor	10 - 30%
DCC	Tumour suppressor	> 10%
β-catenin	Oncogene	2 - 10%
Smad2	Tumour suppressor	< 5%
N-Ras	Oncogene	< 5%
HER-2/NEU	Oncogene	< 5%

Table 5: Some of gene mutations in colorectal cancer

Colorectal cancer results from an accumulation of mutations in tumor suppressor genes and oncogenes. An additional defining characteristic of colorectal cancer is its genetic instability. Two main types of genetic instability have been identified. Microsatellite instability leads to an increased point mutation rate, whereas chromosomal instability refers to an enhanced rate of accumulating gross chromosomal aberrations. All colon cancer cell lines are genetically unstable.

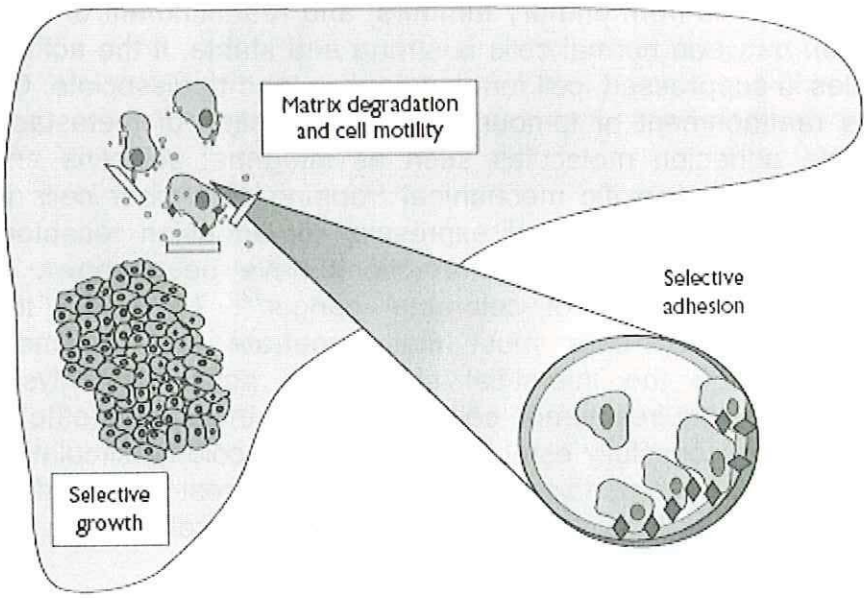
Angiogenesis. Invasive tumor growth and metastasis cannot occur without the growth of new capillary blood vessels. In fact, invasiveness always occurs in conjunction with an "angiogenic switch" -- a change in the balance of proangiogenic factors, such as vascular endothelial growth factor (VEGF), and antiangiogenic factors, such as angiostatin and endostatin. In tumors, this switch leads to a balance in favor of proangiogenic factors, and thus to vascularization of the tumor and metastasis[9]. Blood vessel formation begins with activation of an endothelial cell that forms the wall of an existing small blood vessel. The endothelial cell makes matrix metalloproteinases, which break down the extracellular matrix (ECM); the activated cell then invades the matrix and begins to proliferate[10]. These new endothelial cells eventually organize into hollow tubes that comprise new networks of vasculature, thus enabling the growth and repair of tumors. In addition, the degree of angiogenesis, as indicated by microvessel density, has been correlated with cancer invasion and metastasis in human colorectal cancers and numerous other tumor types[11].

Tumour metastasis involves two independent processes relevant to cell adhesion: detachment of cells from primary tumours, and reattachment of cells to new sites. Adhesion between normal cells is strong and stable. If the activity of adhesion molecules is suppressed, cell forming tissues tend to dissociate. On the other hand, the reattachment of tumour cells to new sites of metastasis could rely on multiple adhesion molecules such as integrins, selectins and cadherins, in addition to non-specific mechanical trapping of tumour cells in capillaries. Alterations in the function and expression of adhesion receptors which mediate cellcell and cell-substrate interactions have been shown to determine the malignant behaviour of colorectal cancer[12]. To initiate the metastatic cascade, neoplastic cells must first penetrate the basement membrane and then invade the interstitial stroma by active proteolysis. Subsequently,intravasation requires tumor cell invasion of the subendothelial basement membrane. To successfully establish a metastatic colony, circulating neoplastic cells must survive immunological surveillance, arrest at a distant vascular step, and extravasate. Finally, cells must invade and proliferate in the secondary organ.



Picture No 1: The metastatic process[13]

Molecular basis of site-specific tumor metastasis involves three major mechanisms. First, selective growth maintains that tumor cells extravasate ubiquitously but selectively grow only in the organs with the appropriate growth factors or extracellular matrix environment. Second, selective adhesion to the endothelial surface only at the site of organ homing. Third, matrix degradation and cell motility



Picture No 2: The metastatic process[14]

More recent studies using mouse and rat models and *in vivo* video microscopy have demonstrated that the initial steps of the haematogenous metastatic process, from cancer cells entering the bloodstream to extravasating into secondary organs, are completed with remarkable efficiency[14],[15]. The inefficiency is more associated with the subsequent steps involving cell division and formation of micrometastases by extravasated cancer cells in the secondary site[16]. In contrast, other studies have indicated that the majority of disseminating tumor cells die rapidly in the blood circulation and can not pass the first capillary bed they encounter. Recent *in vivo* and *in vitro* experimental evidence from various laboratories strongly suggests that, during the interactions between an organ microvascular bed and intravascular tumor cells, nitric oxide (NO) plays a significant role as a cytotoxic natural defensive effector, produced by the vascular endothelial cells, to exert toxic effects on invading tumor cells, interact with endothelial adhesion molecules and regulate the subsequent metastatic tumor formation in the secondary organ[17].

Genetics

Colorectal cancer is a heterogeneous disease arising from a complex series of molecular changes. The successive evolution of normal colonic mucosa to a benign adenoma, then to an adenomatous polyp containing cancer, and then to a potentially life-threatening invasive cancer is associated with a series of genetic events occurring over a long period.

The majority of colon cancer cases fall into category called **sporadic cancer**. Sporadic cancer refers to cancer that does not have a hereditary component and appears to have developed by random or chance occurrence.

Genomic Instability: Using inter-(simple sequence repeat) PCR, Stoler (1999) found a mean of 11,000 genomic alterations per colorectal carcinoma cell. A similar number of genetic events were detected in colonic polyps. Since colonic polyps are early in the tumor progression pathway this suggests that genomic destabilization is an early step in sporadic tumor development. Stoler and colleauges state that this supports a model in which genomic instability is a *cause* rather than an *effect* of colorectal carcinogenesis

The accumulation of genetic errors caused by mutations in any of the mismatch repair genes can often be seen in small genetic segments called microsatellites that are present throughout the genome. Although their function is unknown, microsatellites are repeating sequences of nucleotide bases within the genome. Microsatellites do not cause a malignancy to develop, but fluctuations in the length of microsatellites (termed instability) can mean that mismatch repair genes are not functioning correctly. Testing can be performed to determine if a tumor exhibits microsatellite instability (MSI) by comparing the microsatellites in the tumor specimen to normal tissue from that individual. If the tumor specimen exhibits alterations within the microsatellite regions, it is indicative of a probable defect in the mismatch repair genes. MSI testing demonstrating instability in the tumor specimen is suggestive of HNPCC, although not diagnostic since 10-15% of sporadic colon cancers will also exhibit MSI.

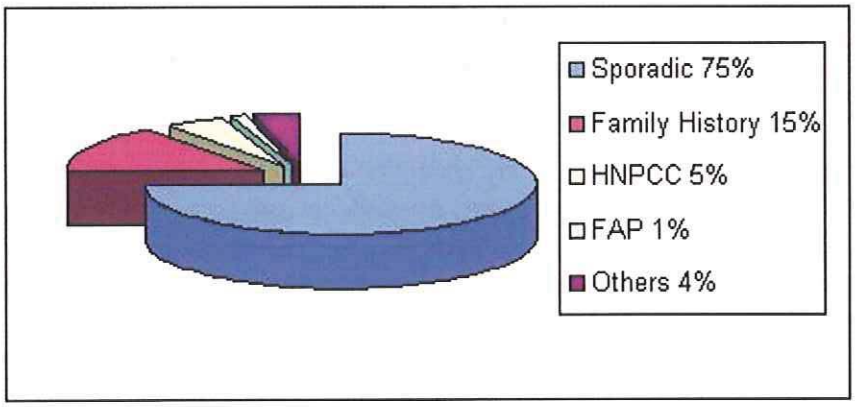
Between 15-20% of all colorectal cancers are thought to be familial. Some colon cancers and pre-disposing conditions are known to have an inherited element:

Hereditary Non-Polyposis Colorectal Cancer (HNPCC), or Lynch syndrome, is an autosomal dominant disease characterised by early onset of colorectal cancer and increased risk of other malignancies including endometrial and renal cell carcinomas. HNPCC is associated with germline mutations of DNA mismatch repair genes, individuals who inherit HNPCC have an 80% lifetime risk of developing colorectal cancer.
In a study of 48 HNPCC kindreds (Liu et al, 1996) identified mutations in known mismatch repair genes;

31%: had mutations in the MSH2 gene
33%, had mutations in the MLH1 gene
2%, had mutations in the PMS1 gene
4% had mutations in the PMS2 gene

These highly penetrant mutations result in microsatellite instability in the tumour (referred to as the replication error phenotype, RER+). These tumours are predominantly diploid and occur more frequently in the right colon, and often have characteristic mutations including TGFBR2 and/or BAX [18].

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder which typically presents with colorectal cancer in early adult life secondary to extensive adenomatous polyps of the colon. FAP accounts for about 1% of all colorectal cancers.Polyps also develop in the upper gastrointestinal tract and malignancies may occur in other sites including the brain and the thyroid. Helpful diagnostic features include pigmented retinal lesions known as congenital hypertrophy of the retinal pigment, jaw cysts, sebaceous cysts, and osteomata. The APC gene at 5q21 is mutant in FAP[19].



Graph No 10: Types of colorectal cancer

IBD

Human patients afflicted with IBD and chronic ulcerative colitis (UC) are at increased risk of developing colorectal cancer. The relative risk for colorectal cancer in UC patients is 10-fold greater compared to the general population[20].

The relationship between chronic intestinal inflammation and the development of dysplasia and neoplasia is complex. In human patients with IBD, the degree of inflammation has been reported to correlate with dysplasia and cancer, yet not all patients with chronic inflammation develop dysplasia or neoplasia suggesting the involvement of other factors. Helicobacter spp. may be a good prototype bacterial organism to study the mechanisms of bacterial-induced intestinal hyperplasia, dysplasia, and neoplasia and studies using both human and animal isolates have explored the pathogenesis[21].

Enviromental, diet and life style factors

Numerous lifestyle and dietary factors have been put forward as potential causes of increased CRC risk. Lower levels of physical activity and increased body mass are associated with an increased risk of CRC in both men and women. The Western-style diet, which is high in calories and fat and low in fiber, is associated with high rates of CRC. There is evidence that increased dietary intake of calcium may confer some protection against the development of CRC and adenomatous polyps. The Calcium Polyp Prevention Study, a large randomized trial done in the United States, reported a small but statistically significant reduction in the incidence of recurrent colorectal adenomas with dietary calcium supplementation. To date, the evidence from randomized trials has not shown dietary fiber supplementation to have a similar effect. In Japan, where the incidence of CRC has traditionally been low, CRC has become considerably more common in the past few decades. This increased incidence is believed to be the result of post—World War II lifestyle changes (e.g., increased consumption of animal fat and decreased expenditure of energy) that mirror Western habits[2].

The strong relationship between Westernization and colon cancer incidence has spawned a number of explanatory hypotheses, many focused on the influence of dietary fat and fiber on the colonic lumenal contents. Dietary fat induces secretion of bile acids, which are converted to secondary and tertiary bile acids by colonic bacteria. These bile acid products may promote tumors by increasing colonic cell proliferation or by mutagenesis. Fiber presumably dilutes fecal carcinogens and bile acids and reduces colonic transit time, further limiting exposure of the colonic mucosa to carcinogens. However, recent case-control, cohort and some randomized studies have cast doubt on the hypotheses that fat and fiber play the central role in colon carcinogenesis. In contrast, an increasing and diverse body of evidence indicates that variations in the levels of insulin and insulin-like growth factors (IGF) could account for many of the nutritional and other risk factors of colon cancer and for its high incidence in Western countries[22].

TUMOR MARKERS IN COLORECTAL CANCER

History of Tumor Markers

The first modern tumor marker used to detect cancer was human chorionic gonadotropin (HCG), the substance doctors look for in pregnancy tests. Women whose pregnancy has ended but whose uterus continues to be enlarged are tested for the presence of HCG. A high level of HCG in the blood may indicate the presence of a cancer of the placenta called gestational trophoblastic disease (GTD). This cancer continues to produce HCG. Some testicular and ovarian cancers resemble GTD because they arise from reproductive cells called germ cells. These cancers also make HCG, so this marker is used to help in their diagnosis and to monitor their response to therapy.

The hope in the search for tumor markers was that all cancers could someday be detected by a single blood test. Both GTD and germ cell tumors of the ovaries and testicles are too rare to look for these cancers by testing everyone. But cancers such as colon, breast, and lung are more common. A simple blood test that would be able to detect these cancers in their earliest stages could prevent the deaths of millions of people. Many scientists began working toward this goal.

The first success in developing a blood test for a common cancer was in 1965, when carcinoembryonic antigen (CEA) was found in the blood of some patients with colon cancer. By the end of the 1970s several other blood tests had been developed for different cancers. The new markers were often given numeric labels. There was CA 19-9 for colorectal and pancreatic cancer, CA15-3 for breast cancer, and CA 125 for ovarian cancer. Many others were also discovered, but because they did not show an advantage over the already discovered markers, they were not studied further.

Unfortunately, none of these markers, including CEA, met the original goal of discovering cancer at an early stage. Almost everyone has a small amount of these markers in their blood, and it is very hard to spot early cancers by using these tests. Only when there is a significant amount of cancer present are the levels of these markers substantially higher. Some people with cancer simply never have elevated levels of these markers. Even when levels of these markers are high, they are not specific enough. For example, patients with lung cancer or breast cancer can often have an elevated CEA, even though this marker was first discovered in people with colon cancer. CA 125 can be high in women with gynecologic conditions other than ovarian cancer. Because of this, these markers are used mainly in patients who have already been diagnosed with cancer to monitor their response to treatment or detect the return of cancer after treatment.

The only tumor marker that currently allows doctors to detect early disease and is used in screening is the prostate-specific antigen (PSA) test. It was discovered around the same time as the others, but it's been in widespread use for screening since the early 1990s because it has some advantages over them. First, it is made only by prostate cells, so a rise in PSA is fairly specific to a

prostate problem. And the PSA level usually rises even in early cancers, so most prostate cancers can be detected at an early stage, when they are most likely to be curable. The test is not perfect, however. Some men may have an elevated PSA because of other prostate conditions (or prostate cancer that would never need treatment), and some men with prostate cancer may not have an elevated PSA. Because of this, doctors and medical organizations do not agree about whether all men should be tested.
Many other tumor markers have been found in recent years and are currently under study. Some of these are different from traditional markers, which were proteins found in the blood.

Tumor markers are substances, usually proteins, that are produced by the body in response to cancer growth or by the cancer tissue itself. Some tumor markers are specific, while others are seen in several cancer types. Many of the well-known markers are also seen in non-cancerous conditions. Consequently, these tumor markers are not diagnostic for cancer. Tumor markers can be classified in two groups: Cancer-specific markers and tissue-specific markers, can be detected from body fluids- blood, urine ascites etc, or tissues- mostly tumor cells.

What are the intrinsic characteristics that would define an **ideal tumor marker**? The level of such a marker would rise in the presence of the smallest neoplastic lesions, and would increase only with the existence of tumors. The marker would be produced by all neoplastic cells, thus making it possible to correlate between marker levels and tumor extent. All patients would generate such a marker. For the public, the examination must have an accessible cost, be minimally invasive and can be performed in any location. The marker should precisely indicate the diagnosis, staging, prognosis and occurrence of neoplastic relapsee. There is a consensus on the fact that the ideal tumor marker does not exist.

The use of tumor markers:

Screening
Diagnostics
Staging
Determining prognosis
Treatment guiding
Treatment monitoring
Determining recurrence
Monitoring cancer recurrence

Prognostic markers - associated with clinical outcome

Predictive markers - associated with response to therapy

Tumour markers in gastrointestinal cancers - EGTM recommendations[23]

Diagnosis and screening

The most useful and widely investigated marker for colorectal cancer is CEA. In colorectal cancer, both the proportion of patients with elevated levels and the extent of elevation are primarily dependent on disease stage. CEA is of little value in the detection of Dukes' A or B colorectal cancer. A further problem with CEA is lack of specificity. This poor sensitivity and specificity when combined with the low prevalence of colorectal cancer in asymptomatic populations means that CEA cannot be recommended as a screening test for colorectal cancer in unselected individuals. For similar reasons, CEA cannot be used alone for diagnosing or ruling out colorectal cancer. However, in patients with appropriate symptoms, a grossly elevated value (e.g., more than five times the upper limit of normal) should be considered highly suggestive for cancer in that individual .

Prognosis

While pre-operative levels of CEA are of little value in detecting early colorectal cancer, a number of investigators have shown that patients with either high pre- or post-operative concentrations of the marker have a worse outcome than those with low values CEA may, however, give prognostic information within the Dukes' subgroups. Additional prognostic markers are particularly required for the Dukes' B category. Approximately 40-50% of patients with Dukes' B disease have aggressive disease. Furthermore, recent preliminary data suggest that adjuvant chemotherapy has a modest but detectable beneficial effect on outcome of patients in this subgroup. Rather than administer adjuvant chemotherapy to all patients with Dukes' B disease, it would be desirable to have a marker capable of discriminating between patients with aggressive and indolent disease. The availability of such a marker should aid the selection of

aggressive tumours that could benefit from adjuvant therapy and at the same time avoid giving therapy to patients likely to have a good outcome.

Monitoring

It is generally believed that the main application of CEA is in the monitoring of patients with diagnosed colorectal cancer. In 1980, an NIH Consensus Meeting concluded that CEA was the best available non-invasive test for the follow-up of patients with colorectal malignancy . In 1996, a statement from the American Society of Clinical Oncology (ASCO) concluded that CEA was the marker of choice for monitoring patients with colorectal cancer.

For identifying recurrences in patients with previously diagnosed colorectal cancer, CEA has a sensitivity of about 80% (range 17-89%) and a specificity of approximately 70% (range 34-91%). Early studies showed that serial CEA levels could detect recurrent disease many months (usually 4-10 months) in advance of clinical evidence of disease.

In the follow-up of patients with colorectal cancer, the optimum interval between CEA measurements has not been established. In practice, most clinicians use intervals of 3 months, at least for the first 2 years after the initial diagnosis. The ASCO Panel have stated that "any benefit from postoperative screening requires serum monitoring every 2-3 months".

While CEA is the preferential biochemical test for colorectal cancer, a number of other markers such as CA19-9, CA242 and cytokeratins (e.g., TPA and TPS) have also been evaluated for this malignancy.

Biomarker	Type	Source	Cancer type	Clinical use
α-Fetoprotein	Glycoprotein	Serum	Nonseminomatous testicular	Staging
Human chorionic gonadotropin-β	Glycoprotein	Serum	Testicular	Staging
CA19-9	Carbohydrate	Serum	Pancreatic	Monitoring
CA125	Glycoprotein	Serum	Ovarian	Monitoring
Pap smear	Cervical smear	Cervix	Cervical	Screening
CEA	Protein	Serum	Colon	Monitoring
Epidermal growth factor receptor	Protein	Colon	Colon	Selection of therapy
KIT	Protein (IHC)	Gastrointestinal tumour	GIST	Diagnosis and selection of therapy
Thyroglobulin	Protein	Serum	Thyroid	Monitoring
PSA (total)	Protein	Serum	Prostate	Screening and monitoring
PSA (complex)	Protein	Serum	Prostate	Screening and monitoring
PSA (free PSA %)	Protein	Serum	Prostate	Benign prostatic hyperplasia versus cancer diagnosis
CA15-3	Glycoprotein	Serum	Breast	Monitoring
CA27-29	Glycoprotein	Serum	Breast	Monitoring
Cytokeratins	Protein (IHC)	Breast tumour	Breast	Prognosis
Oestrogen receptor and progesterone receptor	Protein (IHC)	Breast tumour	Breast	Selection for hormonal therapy
HER2/NEU	Protein (IHC)	Breast tumour	Breast	Prognosis and selection of therapy
HER2/NEU	Protein	Serum	Breast	Monitoring
HER2/NEU	DNA (FISH)	Breast tumour	Breast	Prognosis and selection of therapy
Chromosomes 3, 7, 9 and 17	DNA (FISH)	Urine	Bladder	Screening and monitoring
NMP22	Protein	Urine	Bladder	Screening and monitoring
Fibrin/FDP	Protein	Urine	Bladder	Monitoring
BTA	Protein	Urine	Bladder	Monitoring
High molecular weight CEA and mucin	Protein (immunofluorescence)	Urine	Bladder	Monitoring

Table No 6: US Food and Drug Administration-Approved Cancer Biomarkers - source: Nat.Rev.Cancer 2005 Nature Publishing Group

Group of markers:		Individual markers:
Oncofetal antigens	With fetal functions	o CEA o AFP o hCG o SP1
	Carbohydrate (cancer) antigens	o CA 125 o CA 15-3 o CA 19-9 o CA 50 o CA 72-4
Cytokeratinin tumor markers		o TPA o TPS o CYFRA 21.1 o SCC
Enzymes	Proliferative	o Neuronspecific enolase o Thymidinkinase
	Others	o Prostate specific antigen o Prostatic acid phosphatase o Lactic acid dehydrogenase
Hormones	Ectopic sekretion	o Adrenocorticotropic hormone o Antidiuretic hormone o Cortisone o Parathormon o Prolactin
	Tumor produced	o Placentar lactogen o Calcitonin o Parathormon o Prolactin
Receptors		o Estrogen o Progesteron
Other nonspecify substances		o Ferritin o β_2-mikroglobulin o Imunoglobulins

Tab. No 7: Tumor markers and their functions

Carcinoembryonic Antigen

Tumor marker, **CEA**: Carcinoembryonic antigen (CEA) is a protein found in the developing fetus and cells of many types of tumors . CEA is tested in blood. The CEA was one of the first oncofetal antigens to be described and exploited clinically. It is a complex glycoprotein of molecular weight 20,000, that is associated with the plasma membrane of tumor cells, from wich it may be released into the blood.

Although CEA was first indentified in colon cancer, an abnormal CEA blood level is specific neither for colon cancer nor for malignancy in general. Elevated CEA levels are found in a variety of cancers other than colonic, including pancreatic, gastric, lung, and breast. It is also detected in benign conditions including cirrhosis, inflamatory bowel disease, chronic lung disease, and pancreatitis. The CEA was found to be elevated in up to 19 percent of smokers and in 3 percent of a healthy control population. Thus, the test for CEA cannot substitute for a pathological diagnosis.
As a screening test, the CEA is also inadequate, unacceptably low positive predictive value, with excess false positives. Also, since elevated CEA occurs in the advanced stage of incurable cancer but is low in the early, curable disease, the likelihood of a positive result affecting a patient's survival is diminished.
The CEA has been sugested as having prognostic value for patients with colon cancer, CEA has been used to monitor recurrence. Determinations of CEA should be done frequently: at a minimum of every 3 months. Elevations above baseline should be verified rapidly to exclude laboratory error.
The CEA is of some use as a monitor in treatment. Usually the CEA returns to normal within 1 to 2 months of surgery, but if it returns elevated persistent disease may be indicated. The test is not infallible in patients treated with radiotherapy and chemotherapy but can be useful in those whose tumor is not measurable.
The CEA is often positive in benign disease or in malignancies other than colonic (Table No 8) and can be used to monitor the progress of disease or response to treatement.

Malignant diseases	Benign diseases

• gastrointestinal
  o gastric cancer
  o colon cancer
  o rectal cancer
• lung cancer
• gynecological
  o breast cancer
  o ovarian cancer
  o endometrial cancer
• prostate cancer
• thyroid cancer
	• smokers (up to 5 ng/ml)
	• chronic renal failure
	• gastroinestinal and hepar diseases
	  o IBD
	  o intestinal polyposis
	  o chronic hepatitis
	  o hepatic cirrhosis
	  o chronic pancreatitis
	• lung inflammatory diseases
	  o pneumonia
	  o bronchitis chron.
	  o lung TBC
	• mucoviscidosis
	• autoimmune diseases
	• breast adenomas
	• in the breast and ovarial cysts liquid
	• in the articular synovia in chronic rheumatoid arthritis

Table No 8: Increased CEA values

CA19-9

CA19-9 is a monoclonal antibody generated against a colon carcinoma cell line to detect a monosialoganglioside found in patients with gastrointestinal adenocarcinoma. It is found to be elevated in 21 to 42 percent of cases of gastric cancer, 20 to 40 percent of colon cancer, and 71 to 93 percent of pancreatic cancer.

Malignant diseases	Benign diseases

• gastrointestinal carcinomas
  ○ pancreatic cancer
  ○ gallbladder and bile duct cancer
  ○ primary hepatic cancer
  ○ gastric cancer
  ○ colorectal cancer
• breast cancer
• ovarian cancer (especially mucinous)
• endometrial cancer
• metastases (of all above mentioned)
	• hepatic and bile duct diseases
	  ○ hepatic cirrhosis
	  ○ primary billiary cirrhosis
	  ○ acute hepatitis
	  ○ toxic hepatitis
	  ○ chronic hepatitis
	  ○ cholecystitis
	  ○ cholangitis
	  ○ bile duct obstruction
	• acute and chronic pancreatitis
	• benign gastric and intestinal diseases (especially inflammatory)

Table No 9: Increased CA 19-9 values

TPA and TPS

Tissue polypeptide specific antigen was first defined by Bjorklund in 1957 and is the specific M3 epitope cytokeratin 18-associated marker. TPA is a pan-carcinoma marker. TPA is now known to belong to a class of cytoskeletal proteins called cytokeratins or intermediate filaments. Cytokeratins 8,18,and 19 react with anti-TPA antibodies. These cytokeratins are cytoplasmic proteins and are found in all normal epihelial cells, and cells lining the ducts and their sacs[24]. Thus various tumors arising from different organ sites are known to express TPA, which is also released into the serum by cell destruction. TPA assays represent the first generation cytokeratin tumor marker tests. TPS measures fragments of cytokeratin 18 and the TPA test estimates cytokeratin 8 and 18[25].
The cytoskeleton is responsible for the physical three-dimensional architecture of the cell. During cell division the cytoskeleton assumes a crucial , dynamic, functional role. The precise function of individual cytokeratins is yet to be fully understood but as an intermediate filament it has an obvious role in defining the structure of the cytoskeleton and its dynamics during cell division[26].

Cytokeratin markers are indicator of cell proliferation. Many carcinoma patients have elevated TPA/TPS levels in their serum and the magnitude of the elevation correlates with tumor progression. The widespread distribution of this marker is in some respects similar to CEA. Some experts recommend that these two analytes be measured in combination. Among the types of cancer that show increases in TPA levels are breast, digestive tract, lung prostate, and ovarian. TPA is particularly useful as very sensitive marker for confirmation of the diagnosis of transition cell carcinoma of the bladder in its early stages. TPS appears to be sensitive and specific for breast cancer than CEA and CA 15-3.
TPA has a half-life of 7 days in circulation and a stable level is reached in 3-4 weeks after treatment of the cancer. Serum TPA levels are altered in relation to the proliferation of tumors. Thus it is likely that a tumor without significant cell division and growth should not result in an increased level of TPA in the serum[27].
TPS and TPA are increased in malignant as well as in some benign diseases[28, 29]. The level of tissue polypeptide specific antigen in serum is related mostly with proliferation capacity rather than with tumor mass and cell necrosis [30 , 31].

Limitations

- Cytokeratin markers are not suitable for diagnosis of carcinoma but are used to monitor patients, often along with other organ-specific tumor markers.
- Elevation in TPA are seen in the last trimester of pregnancy and in various benign diseases of the lung, liver stomach and pancreas.
- Monitoring of patients during therapy with cytokeratin markers is more complex than using other markers. Further wirk is needed to resolve the nature of these soluble serum fragments of cytokeratin parent molecules, which are more insoluble by nature.
- Transient increase of cytokeratins can occure in response to therapy.

CA242

The CA-242 is a sialylated carbohydrate antigen present on mucinous type of glycoproteins in carcinomas of many organs. The CA-242 antigen is shedded from the tumor and the CA-242 can be detected in serum from patients with carcinomas.
In the normal healthy subjects and subjects with benign diseases, the CA-242 levels are low, while elevated levels are commonly found in patients with gastro-intestinal cancer. By identifying the colo-rectal cancer patients at an early stage of the disease, primary diagnosis, often relies on occult blood testing, and on radiological endoscopic examination of the large bowel. CEA is widely used for the monitoring and prognostic assessment of patients with colo-retal cancer while the clinical utility of CEA is limited due to the low sensitivity in early stages of cancer. CEA showed higher sensitivity for rectal cancer than for colonic cancer, while the opposite was true for CA-242. However, a combination of CA-242 with CEA will improve with higher sensitivity for both rectal and colonic cancer.
CA-242 is better than CA-19-9 in the diagnosis of pancreatic cancer because of its higher specificity, and it may be useful in the screening of localized or respectable tumors.

ICAM, VCAM

Cell adhesion molecules were first identified through their ability to allow cells to adhere to each other and to the extracellular matrix. We now know, however, that this group of cell surface receptors not only promotes adhesion but also allows cells to interact and communicate with each other and their environment and, in doing so, regulates a range of cell functions, including proliferation, gene expression, differentiation, apoptosis, and migration. There are at least five groups of cell adhesion molecules: integrins, selectins, adhesion molecules belonging to the immunoglobulin superfamily, cadherins, and the CD44 family. All cell adhesion molecules bind to other cells or matrix components through their interaction with appropriate counter-structures, referred to as a ligands. In some cases the ligands are themselves adhesion molecules, as is the case with the selectin family, whose ligands are members of the immunoglobulin superfamily, and vice versa.

Cell adhesion molecules are critical to many normal physiological processes. During embryogenesis, for example, the differential expression of adhesion molecules is responsible for the selective association of embryonic cells into specific tissues, and in the immune system adhesion molecules mediate the migration and homing of lymphocytes to specific tissues. Given their widespread importance it is not surprising that cell adhesion molecules have also been

implicated in many diverse pathological processes such as inflammation and wound healing, septic shock, transplant rejection, cancer, and atherosclerosis.

Recently, an understanding of the role of cell adhesion molecules in these processes has suggested their use as either diagnostic or prognostic markers, or as potential targets for therapeutic intervention. This is best exemplified in cancer. Loss of cell-cell adhesiveness contributes to the process of metastasis, whereby tumour cells can invade surrounding tissues and disseminate to distant organs. The cell adhesion system mediated by E (epithelial) cadherin has been shown to be critical to maintaining cell-cell adhesion and is often inactivated in epithelial cancers. This inactivation may result from mutations that directly affect the genes for E-cadherin or may occur in those genes that code for the catenins, a group of molecules that connect cadherins to actin filaments and establish firm cell-cell adhesion. In fact, loss of E-cadherin expression is an adverse prognostic indicator in several carcinomas, including those of the colon, stomach, prostate, and breast. In some situations, as in the development of oesophageal cancer, temporal changes in adhesion molecule expression correlate with tumour progression. Abnormalities in the CD44 cell adhesion molecules have also been intensively investigated in many types of cancer. Variants of the CD44 protein may be created by a process known as alternative splicing. Expression of certain CD44 variants (CD44v) by cancer cells is associated with the ability of these cells to metastasise and with a poor prognosis. Also, soluble forms of CD44 (sCD44) may be detected in the serum of patients with cancer and in some settings correlate with clinical markers of disease. In non-Hodgkin's lymphomas, for example, high serum levels of sCD44 at diagnosis are associated with a high international prognostic index score, poor response to treatment, and an unfavourable outcome. The possible use of CD44 as a diagnostic marker is emphasised by the detection of CD44 variants in exfoliated cells in urine, which correlates with the presence of urogenital malignancies[32], and in faecal samples from patients with colorectal cancer[33].

One of the most important events in the reaction to all forms of injury is the adhesion of leucocytes to endothelium, which precedes their emigration to the tissues and is central to the processes of inflammation and immune reaction. Leucocyte adhesion to the endothelium is mediated by adhesion molecule pairs, principally the selectins (E, L, and P), members of the immunoglobulin superfamily (ICAM-1 and VCAM-1), and the integrins. The importance of these adhesion molecules in lymphocyte recruitment has been shown in several pathological processes, including transplant rejection, septic shock, atherosclerosis, and late phase hypersensitivity and in reperfusion injury.

Alternatively, lymphocytes could be programmed in vitro to express receptors that would target specific tissues[34].

VCAM-1 belongs to the immunoglobulin super family group of adhesion molecules, and is one of the most important adhesion molecules. VCAM-1 is an 110 KDa glycoprotein that is constitutively expressed on tissue macrophage, dentric cells and epithelial cells, as well as on the surface of stimulated endothelial cells. Thus, VCAM-1 is a widely distributed protein. It is possible that VCAM-1 is a candidate for mediating tumor cell adhesion to vascular endothelial

cells and promoting the metastatic process. Recent reports have shown that angiogenesis favors tumor growth and facilitates entry of cells into the circulation[7,8]. Vascular cell adhesion molecule1 (VCAM-1) is expressed on endothelial cells as a result of vascular endothelial growth factor (VEGF) stimulation[35].

Cancer cells without ICAM-1 expression possibly escape from the immune surveillance system of the host. Cancer cells without ICAM-1 expression can grow without recognotion and cell lysis by lymphocytes, and may survive when metastasize. Such impairment of the immune survillance system may contribute to tumor metastasis and poor clinical outcome. ICAM-1 expression may be a useful indicator of prognosis in patients with colorectal cancer[36].

Adhesive molecules	
Group	**Molecule**
Integrins	β_1-β_8
Selectins	E-selektin, P-selektin, L-selektin
Imunoglobulins	Intercellular adhesion molecules: ICAM-1, ICAM-2, ICAM-3 Vascular cell adhesion molecules: VCAM-1
Kadherins	E-kadherin, P-kadherin, N-kadherin
CD 44 molecules	

Table No 10: Adhesive molecules list

TK- thymidine kinase

The human cytosolic thymidine kinase, TK1 is a key enzyme in the salvage synthesis of TMP from thymidine. TK1 is a cell cycle-regulated enzyme. Its activity fluctuates with DNA synthesis, being high in dividing and malignant cells and low in quiescent cells[37]. The expression of human thymidine kinase 1 is highly dependent on the growth states and cell cycle stages in mammalian cells. The amount of hTK1 is significantly increased in the cells during progression to the S and M phases, and becomes barely detectable in the early G_1 phase by a proteolytic control during mitotic exit. The enzyme is not set free from cells undergoing normal division where the cells have a special mechanism to degrade the proteins no longer needed after the cell division. In normal subjects the amount of thymidine kinase in serum or plasma is therefore very low. Tumour cells release enzyme to the circulation, probably in connection with the disruption of dead or dying tumour cells. The thymidine kinase level in serum therefore serves as a measure of malignant proliferation, indirectly as a measure of the aggressivity of the tumour.

Insulin-like growth factor

The insulin-like growth factors (IGFs) are polypeptides with high sequence similarity to insulin. IGFs are part of a complex system that cells use to communicate with their environment. The IGF has been shown to play roles in the promotion of cell proliferation and the inhibition of apoptosis. IGF-II is thought to be a primary growth factor required for early development while IGF-I expression is seen in later life. The Insulin-like Growth Factor (IGF) signaling system plays a central role in cellular growth, differentiation and proliferation. IGFBP-3 is the most abundant IGF binding protein in human serum and has been shown to be a growth inhibitory, apoptosis-inducing molecule, capable of acting via IGF-dependent and IGF-independent mechanisms. Insulin-like growth factor 1 (IGF-1) is mainly secreted by the liver as a result of stimulation by growth hormone (hGH). IGF1 plays an important role in anabolic effects in adults. Almost every cell in the human body is affected by IGF-1, especially cells in muscle, cartilage, bone, liver, kidney, nerves, skin, and lungs. In addition to the insulin-like effects, IGF-1 can also regulate cell growth and development, especially in nerve cells, as well as cellular DNA synthesis. The effect is the promotion of cell growth and multiplication.

Over the last decade, several clinical studies have proposed that individuals with IGFBP-3 levels in the upper range of normal may have a decreased risk for certain common cancers. This includes evidence of a protective effect against breast cancer, prostate cancer, colorectal cancer, and lung cancer. In addition, a series of in vitro studies and animal experiments point towards an important role for IGFBP-3 in the regulation of cell growth and apoptosis. Several epidemiological studies have found that high levels of plasma insulin-like growth factor (IGF)-I and low levels of IGF-binding protein (IGFBP)-3 are related to an increased risk of colorectal cancer or late-stage adenomas. IGFs vary

substantially between individuals, and a large component of this variation may be determined by genetic factors. Insulin-like growth factor IGF-I and its main binding protein, IGFBP-3, modulate cell growth and survival, and are thought to be important in tumour development.

A variety of tumor systems including colon cancers demonstrate altered expression of the IGF-I and IGF-II and their principle receptor, IGF-IR. Colorectal carcinomas have a 10–50-fold increase in the levels of IGF-I and IGF-II when compared with adjacent uninvolved colonic mucosa[38].

Leptin

Leptin is a 16 kDa protein hormone that plays a key role in regulating energy intake and energy expenditure. The Ob(Lep) gene is located on the 7th chromosome in humans. Leptin is produced by adipose tissue and interacts with six types of receptor (LepRa to LepRf). Although leptin is a circulating signal that reduces appetite, in general, obese people have an unusually high circulating concentration of leptin. These people are said to be resistant to the effects of leptin, in much the same way that people with type 2 diabetes are resistant to the effects of insulin. Leptin is also strongly linked with angiogenesis, increasing VEGF levels.
Obese people have many fat cells, and they generally make lots of leptin. Therefore, obesity results more often from a failure to respond to leptin than from an absence of leptin.
The adipocytokines leptin and adiponectin participate in body weight regulation. In response to weight loss, adiponectin levels increase and leptin decreases. Cancer cachexia is a complex metabolic state, characterized by loss of muscle mass and adipose tissue together with anorexia. Results suggested a gender-dependent attenuation of expected physiologic responses to weight loss among cancer cachexia patients. Results Wolf I. et al impaired response of adiponectin and leptin may play a role in the pathogenesis of cancer cachexia syndrome.

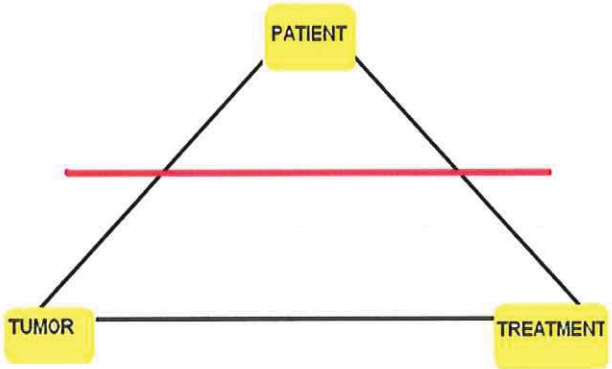
Leptin is derived from adipocytes and appears to play a role in the regulation of ghrelin, a peptide derived from the stomach and small intestine that stimulates appetite and weight gain. In addition to these metabolic changes, there are other anatomical alterations that may indirectly predispose to cancer, including the predisposition of obesity to gastroesophageal reflux and, possibly, oesophageal cancer. Other mechanisms may involve adipocyte-derived cytokines, or adipokines, that may serve as signalling devices in the pathogenesis of cancer. Finally, pharmacologic and surgical avenues available for treatment of obesity, including lipase inhibitors and gastric or jejuno-ileal bypass procedures may set the stage for subsequent gastric or intestinal tract cancer.

Adiponectin

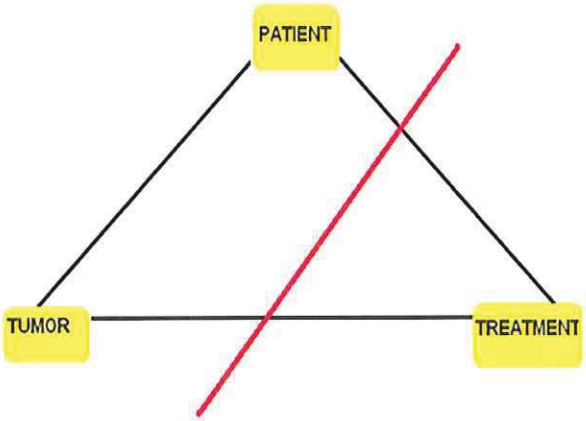
Adiponectin is a new member of an ever increasing family of adipocytokines and is also referred to as ACRP-30 (adipocyte compliment related protein-30), Adipo-Q, and APM-1 (adipose tissue most abundant gene transcript-1). Adiponectin is a predominant secretory protein from adipose tissue and circulates in micro-gram/ml quantities and has a structural homology with the type VIII collagen and hibernation specific protein, C1q. In contrast to the majority of secreted proteins from adipose tissue, which are elevated in obesity, adiponectin appears to be either decreased or unaltered with degree of adiposity. More intriguingly, adiponectin seems to ameliorate the obesity related risk factors unlike other adipose tissue secretory proteins which contribute toward the health risks associated with obesity. Adiponectin also has an insulin sensitizing effect making it an excellent candidate in drug development for obesity and diabetes. Circulating adiponectin levels seem to be an excellent biochemical marker for improved insulin resistance in obese and diabetic states. Results Tamakoshi suggest that leptin most likely increases the risk of female colorectal cancer substantially independent of BMI, Leptin, the product of the ob gene, has been suggested to increase the risk of colon cancer. However, we have shown that although leptin stimulates epithelial cell proliferation it reduces the development of carcinogen induced preneoplastic lesions in the rat colon. Obesity, a risk factor for colorectal cancer, is associated with elevated serum levels of leptin, the adipocyte-derived hormone, and insulin. Experimental and epidemiologic studies have indicated a role for insulin in the pathogenesis of colon cancer, and recent experimental studies have suggested a similar role for leptin.

PROGNOSTIC FACTORS IN COLORECTAL CANCER

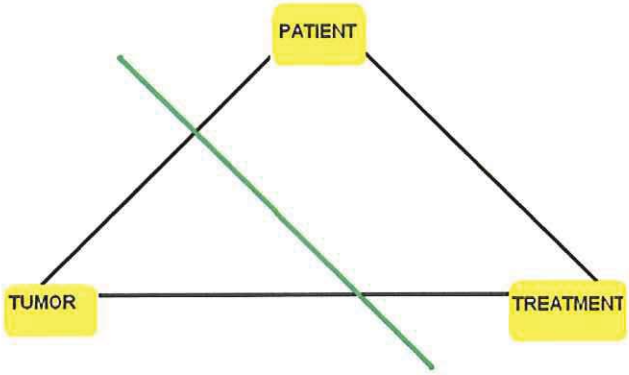
Prognosis depends on factors related to the patient, treatment, and tumor, and the expertise of the treatment team is one of the major determinants of outcome.



Picture No 3: Prognosis in case of inadequate treatment



Picture No 4: Treatment did not enter the fight between patient and the disease



Picture No 5: Treatment helping the patient to improve prognosis by „giving a chance for cure"

At present, prognosis is based predominantly upon the pathological stage of the disease. The molecular biology and genetics of cancers have been studied extensively, hundreds of reports on prognostic markers have been reported, but clinical applications are currently only few.
Prognosis is determined by the presence or absence of metastatic disease that is often microscopic. Molecular staging strategies have applied molecular biology techniques to identify the presence of tumor cells. Molecular characterization of the metastatic phenotype is another strategy that has been employed to attempt to identify prognostic markers.
Current state of knowledge regarding pathologic prognostic[39] factors was evaluated under the auspices of the College of American Pathologists. A multidisciplinary group of clinical (including the disciplines of medical oncology, surgical oncology, and radiation oncology), pathologic, and statistical experts in colorectal cancer reviewed all relevant medical literature and stratified the reported prognostic factors into categories that reflected the strength of the published evidence demonstrating their prognostic value.

category I:

the local extent of tumor assessed pathologically - the pT category of the TNM staging system

regional lymph node metastasis - the pN category of the TNM staging system

blood or lymphatic vessel invasion

residual tumor following surgery with curative intent -the R classification of the AJCC/UICC

preoperative elevation of carcinoembryonic antigen

category IIA

tumor grade

radial margin status (for resection specimens with nonperitonealized surfaces)

residual tumor in the resection specimen following neoadjuvant therapy (the ypTNM category of the TNM staging system of the AJCC/UICC)

category IIB

histologic type

histologic features associated with microsatellite instability (MSI) (ie, host lymphoid response to tumor and medullary or mucinous histologic type)

high degree of MSI (MSI-H)

loss of heterozygosity at 18q (DCC gene allelic loss)

and tumor border configuration (infiltrating vs pushing border)

category III

DNA content (all other molecular markers except loss of heterozygosity 18q/DCC and MSI-H)

perineural invasion

microvessel density

tumor cell–associated proteins or carbohydrates

peritumoral fibrosis, peritumoral inflammatory response, focal neuroendocrine differentiation, nuclear organizing regions, and proliferation indices

category IV

factors included tumor size and gross tumor configuration

Tumor invasiveness and the development of metastases are the most important factors, besides the quality of primary surgery, in determining the prognosis of patients with colorectal carcinomas[40].

Group	Name	Expression	Function
Integrins	VLAs LFAs gp	Colonocytes, EC, fibroblasts, leukocytes, platelets	ECM-cell adhesion, leukocyte–EC adhesion, platelet–EC adhesion, gut homing receptor
Immunoglobin gene superfamily	ICAM VCAM PECAM (CD31)	EC, fibroblasts, leukocytes	Antigen recognition, leukocyte adhesion and trafficking
	CEA (CD66)	Colonocytes	Homotypic cell adhesion
	Cadherins	Colonocytes	Homotypic cell adhesion
Lectins	sLe^x sLe^a (CA 19-9) sLe^c Galectin-3	Colonocytes, EC	ECM–cell adhesion, heterotypic EC adhesion
Selectins	E-, P-, L-selectin	EC, leukocytes, platelets	Leukocyte–EC adhesion, platelet–EC adhesion
Growth factor receptors	EGF-R TGF-R IGF-R VEGF-R PD-ECGF c-Met bFGF	All cells	DNA synthesis, growth, motility, protein secretion
Proteoglycan receptors	CD44	Colonocytes, EC	Hyaluronate adhesion
Protease receptors	MT-MMP uPA-R	Stromal cells, EC, colonocytes	Protease activation
Sex hormone receptors	Androgen receptor Progesterone receptor Estrogen receptor Prolactin receptor	Stromal cells, EC, colonocytes	Growth regulation
Apoptosis receptor	APO-1 (CD95)	All cells	Apoptosis regulation
Cytokine receptors	TNF-R IL-2R IL-6R	Colonocytes, EC, fibroblasts	Cell activation

VLA, very late antigens; LFA, leukocyte function-associated antigens; gp, platelet glycoproteins; ICAM, intercellular adhesion molecules; VCAM, vascular cell adhesion molecule; MAdCAM, mucosal addressin cell adhesion molecule; PECAM, platelet endothelial cell adhesion molecule; CEA, carcinoembryonic antigen; EGF-R, epidermal growth factor receptor; TGF-R, transforming growth factor receptor; VEGF-R, vascular endothelial growth factor receptor; IGF-R, insulin-like growth factor receptor; PD-ECGF, platelet-derived endothelial cell growth factor; c-Met, hepatocyte growth factor/scatter factor receptor; bFGF, basic fibroblast growth factor; MT-MMP, membrane-bound matrix metalloproteases; uPA-R, urokinase-type plasminogen activator receptor; TNF-R, tumor necrosis factor receptor; IL, interleukins; ECM, extracellular matrix; EC, endothelial cells.

Table No 11. Surface molecules with potential prognostic properties in colorectal carcinomas[41].

Regional lymph node involvement
More than 4 involved regional lymph nodes
Tumor penetration through the bowel wall
Poorly differentiated histologic findings
Tumor adherence to adjacent organs
Bowel perforation
Obstruction
Venous invasion
Preoperative elevation of carcinoembryonic antigen level to > 5.0 ng/ml
Increased DNA content (aneuploidy) of malignant cells
Allelic loss of chromosome 18q

Table No 12: Indicators of Poor Prognosis for Colorectal Cancer[42]

The highest category of local extent is pT4, which includes both extension into adjacent organs or structures (pT4a) and penetration of the parietal peritoneum with or without involvement of an adjacent structure (pT4b). A free perforation of a colorectal carcinoma into the peritoneal cavity is also classified as T4b. Among the features that define T4 tumors, serosal penetration is the most dire. A number of large studies have evaluated serosal penetration as a separate pathologic variable and have demonstrated by multivariate analysis that it has independent adverse prognostic significance[43.] The median survival time following surgical resection for cure is significantly shorter for pT4 tumors that penetrate the visceral peritoneum compared with pT4 tumors without serosal involvement, with or without distant metastasis. A careful pathologic study of local peritoneal involvement by Shepherd et al[44] suggested that the prognostic power of this feature may supersede that of regional lymph node metastasis (N category). Despite its biologic importance, serosal involvement is often underdiagnosed by pathologists. Documentation of peritoneal involvement by tumor demands meticulous pathologic analysis and may require extensive sampling and/or serial sectioning.

SURGICAL TREATMENT OF COLORECTAL CANCER

Surgery still remains the principal treatment for patients with colorectal cancer until some more causal therapy appears.
Curative treatment means R0 operation. Oncological standards of curative surgery for colorectal cancer include en bloc radical resection of the tumour bearing colon, primary ligation of the vessels and systematic lymphadenectomy. Resecting all resectable metastatic lesions.
The lymphatic dissection determines the extent of colonic resection and carcinoma located between two drainage areas make extended hemicolectomies or subtotal colectomy with systematic dissection of two lymphatic drainage areas mandatory.
The **surgeon** is an important variable influencing oncological outcome, including local recurrency and survival, **and prognosis** for the patient[45].
One of the basic conditions of successful curative R0 surgical treatment is to approximate to an ideal of correct preoperative staging. Tumor markers are integral part of it.

Surgical principles : colon cancer

(Colected from: H Nelson, N Petrelli, A Carlin, J Couture, J Fleshman, J Guillem, B Miedema, D Ota, D Sargent: Guidelines 2000 for Colon and Rectal Cancer Surgery, Journal of the National Cancer Institute, Vol. 93, No. 8, April 18, 2001)

- extent of a bowel resection is defined by removing the blood supply and the lymphatics at the level of the origin of the primary feeding arterial vessel (apical nodes) to achieve an appropriate lymph node resection
- the lymph node resection should be radical and the lymph nodes should be removed en bloc
- length of bowel resected and the extent of lymphadenectomy performed are closely associated
- when the primary tumor is equidistant from two feeding vessels, both vessels should be excised at their origin
- the inferior mesenteric artery (IMA) should be excised at its origin. (With priority to autonomic nerve preservation) PHOTO No 1.
- 5 cm of normal bowel on either side of the primary colon tumor appears to be adequate to minimize anastomotic recurrences
- the length of ileum resected does not appear to affect local recurrence.
- tumor free margin: 3 margins must be considered for optimal pathologic assessment of lateral, radial, and circumferential margins of resection, since margins positive for disease have been clearly associated with an increased rate of local and distal treatment failure. The local recurrence rate after resection of a rectal cancer was 29%, 78%, and 85% for cases with margins positive for disease compared with 3%, 8%, and 10%, respectively, for cases with margins negative for disease[46, 47, 48]
- locally advanced cancer**:** adherence to adjacent intraabdominal organs or structures is encountered in 15% of patients with colorectal cancer[49]. This type of tumor is characterized by aggresive local behavior in the form of invasions of adjacent organs or stuctures and low propensity to metastasis.

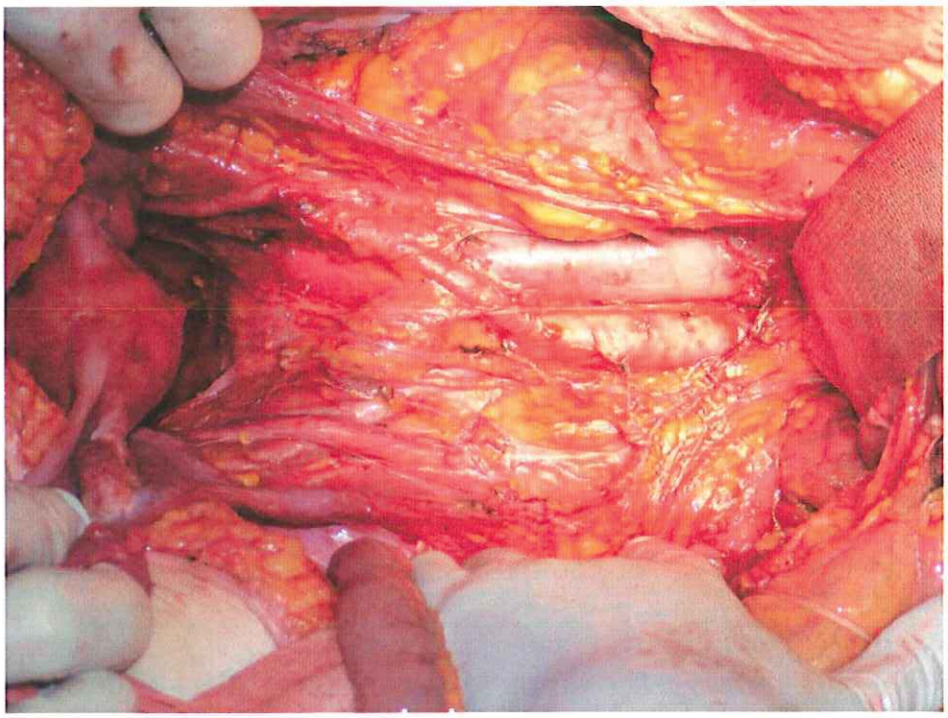
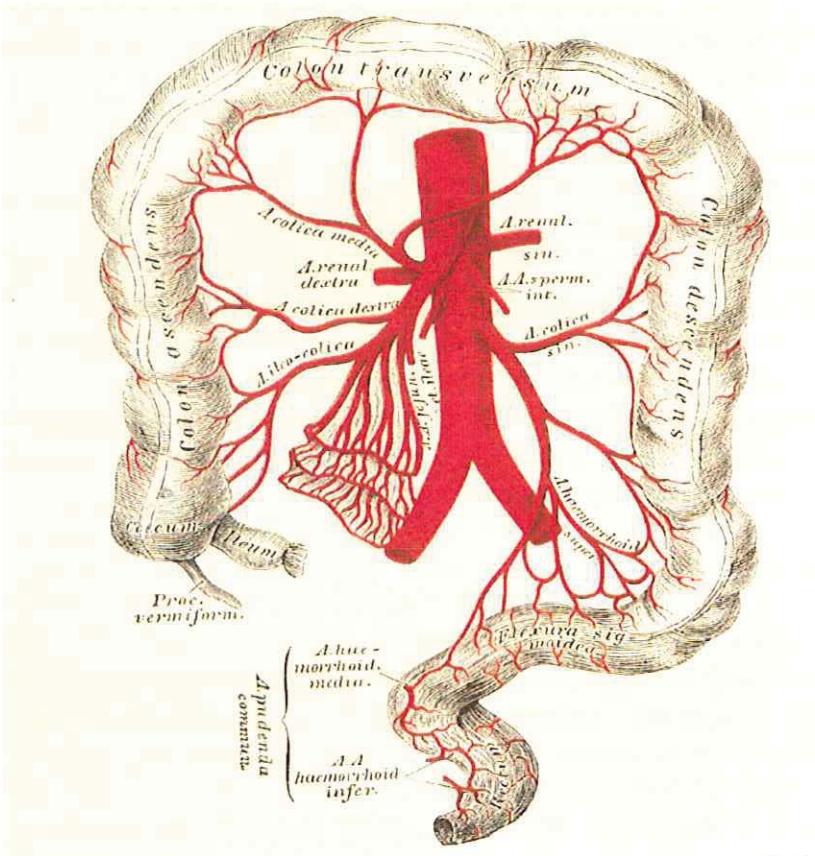


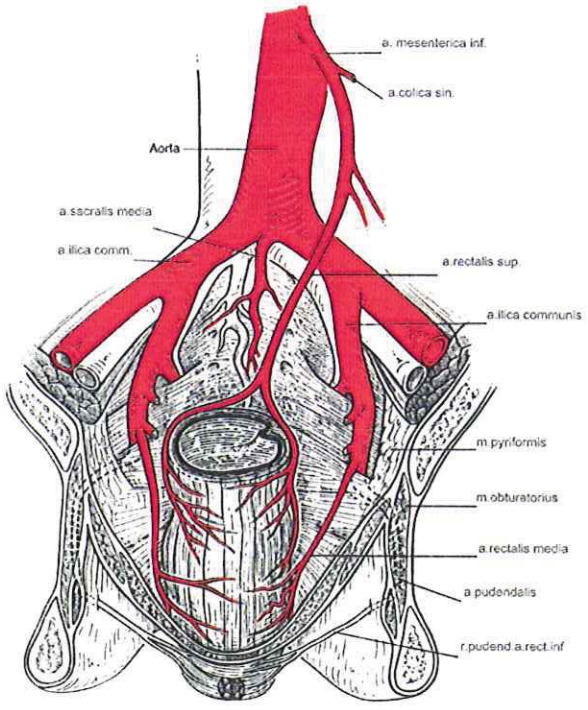
PHOTO No 2 : Low anterior resection with autonomic nerves preservation
(Surg.Dpt.Thomayer Teaching Hospital Prague)



Picture No 6: Colon blood supply

Surgical principles : rectal carcinoma

- the recommended level of proximal vascular ligation for rectal cancer is at the origin of the primary feeding vessel (superior rectal artery)
- there is a lack of evidence about the benefit of ligating the IMA at its origin (high ligation)
- wide anatomic mesorectal excision includes presacral dissection under direct visualization, preservation of mesorectal fascia propria integrity, at least 4-cm clearance of attached mesorectum distal to the tumor, and pathologic confirmation of mesorectum attached to the bowel, distal to the tumor
- in addition to longitudinal lymphatic spread along the colon, tumors in the upper rectum spread mostly to the superior pedicle and tumors in the lower rectum spread superiorly and laterally
- involved lymph nodes can be detected along the aorta and superior rectal vessels, as well as along iliac, hypogastric, sacral, and inguinal nodal sites and laterally along the middle hemorrhoidal and lateral ligaments
- the number and location (i.e., lateral or apical) of lymph nodes positive for disease influence outcomes, especially 5-year survival, and have served as the basis for recommendations for extended or lateral node dissection
- extended lateral pelvic lymph node dissection
- the ideal distal margin length is 2 cm or greater from the transected mucosal edge to the distal edge of the primary tumor
- for tumors of the distal rectum (<5 cm from the anal verge), the minimally acceptable length of the distal margin is 1 cm in the fresh, anatomically restored ex vivo condition



Picture No 7: Blood supply of rectum

Rectal cancer most often involves the uterus, adnexa, vagina, prostate, seminal vesicules and urinary bladder. In addition, 30-40 % of all patients originally resected for cure will develop a local recurrent disease.
En bloc multivisceral resection including total pelvic exenteration is the ideal surgical method to manage locally advanced, adherent colorectal tumors[50]. This type of resection can achieve survival rates similar to those of patients with tumors that do not invade an adjacent organ. Separating involved organs in order to confirm the infiltration impairs results. When adherent organs were separated, local recurrency rates were 69% versus 18%. Incidence of histologically proved malignant adhesions is 49%–84%[51].
After inadvertment rupture of the tumor, 5-year survival is only 17% vs 49%[52].
In the case of advanced pelvic cancer a pelvic exenteration with en bloc resection of involved organs and structures including portions of bony pelvis is indicated[53]. PHOTO No 1 and 2.
Local recurrence rates following primary operation for rectal cancer are in literature ranging from as low as 4% to as high as 55%.

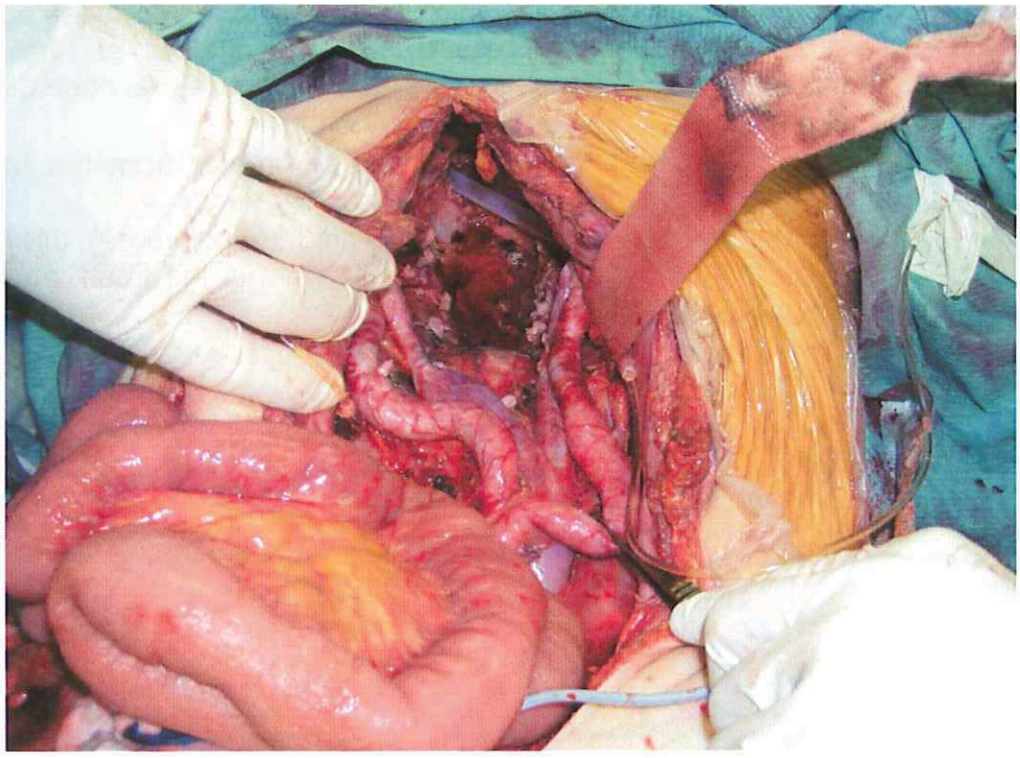


PHOTO No 3: Total proctocolectomy with total pelvic exenteration with resection of internal iliac vessels (Surg.Dpt.Thomayer Teaching Hospital, Prague)

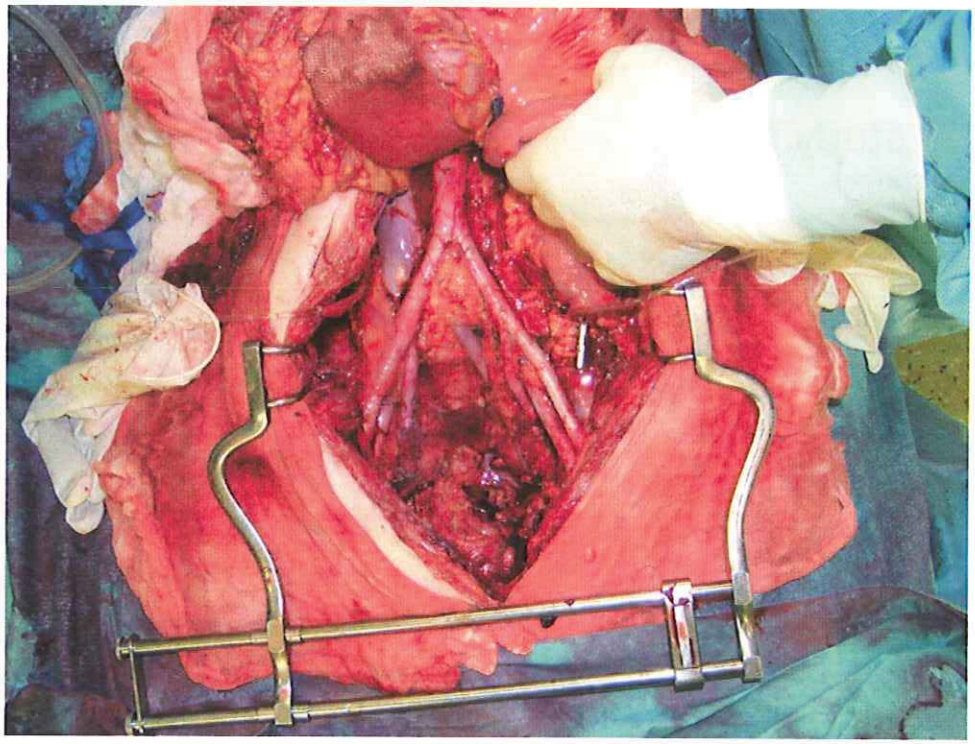


Photo No 4: Sphincter saving total pelvic exenteration without resection of internal iliac vessels (Surg.Dpt.Thomayer Teaching Hospital, Prague)

AIMS OF THE STUDY

Aims of study I to investigate patients before primary operation for colorectal cancer

Aims of study II to investigate patients during follow up after operation for colorectal cancer

Study I.

1:

To investigate the clinical significance of serum tumor markers and biological activity markers - oncofetal tumormarker CEA, mucin tumormarkers CA19-9, CA242, proliferative tumor markers Thymidine kinase, soluble cytoceratines fragments TPS, TPA, adhesive moleculs ICAM - 1, VCAM -1, IGF-1, and adipocytokinins Adiponectin, Leptin in patients with colorectal cancer before primary operation

1.1:

To determine relations of above stated markers with the earliest and the most advanced stages of colorectal cancer .

1.2:

To select from the above mentioned markers those, with statisticly significant differences between levels in early and advanced stages of colorectal cancer .

1.3:

To correlate these selected markers with TNM stages of colorectal cancer.

1.4:

To identify the optimal combination of serum tumor markers in colorectal cancer staging

1.5

To suggest markers for preoperative staging of patients with colorectal cancer.

Study II.

2:

To assess the clinical significance of oncofetal tumormarker CEA, mucin tumormarkers CA19-9, CA242, proliferative tumor markers Thymidine kinase, soluble cytoceratines fragments TPS, TPA, adhesive molecules ICAM - 1, VCAM -1, IGF-1, and adipocytokinins Adiponectin, Leptin in patients during follow up after radical surgery for colorectal cancer.

2.1:

To determine correlations of above stated markers with the remission or relapse of colorectal cancer .

2.2:

To mark out of the above mentioned markers those, with statisticly significant differences between levels in remission or relapse.

2.3:

To identify the optimal combination of serum tumor markers for follow up of colorectal cancer.

2.4:

To suggest markers for postoperative follow up of patients with colorectal staging.

METHODOLOGY

Both study I and study II are presenting prospectivelly colletced data concerning patients with colorectal adenocarcinoma verified by biopsy. Positive biopsy was the **inclusion criterion**.
For study I was second criterion status of the patient - before primary operation.
For study II was the second criterion status of the patient – follow up after the primary operation.
Exclusion criterion: absence or lack of blood sample.

Study I.

Patients with histologically verified colon or rectum adenocarcinoma were enrolled in the study I. **before the primary operation**. Standard preoperative staging according to local (Dept. of Surgery Thomayer Teaching Hospital) protocol was performed (see below). Before urgent operations patients had limited staging according to the individual conditions.
Periferal **blood samples** were collected in the period of 3 weeks prior to the operation and the day of surgery. The blood was centrifuged and the serum samples were stored under temperature of minus 70° C.
The analysis was done in Dept. of Oncology, Immunoanalytical laboratory, University Hospital Pilsen - the methodology vide infra.
All the patients in study I were operated at the Surgical Department of Thomayer Teaching Hospital and the1st Faculty of Medicine Charles University Prague.

The operation strategy

- intraoperative ultrasound examination of the liver is obligatory
- radical lymphadenectomy up to the level of apical nodes at the root of the feeding artery for the affected part of the bowel is a standard part of operation with curative intent
- high ligatures of IMA (inferior mesenteric artery) is performed in case of sigmoid or rectum cancer, most of them with autonomic nerve preservation
- total mesorectal excision is a standard procedure in lower rectum and in middle and upper rectum subtotal mesorectal excision respectively
- in rectal cancer, 2 cm distal margin is considered safe
- in selected cases of T3 and T4 lower rectal cancer, lateral pelvic lymphatic dissection is performed

- locally advanced cancer is considered for multiorgan resection including total pelvic exenteration
- synchronous liver metastases, if assessed resectable, are preferred to be operated simultaneously with the primary tumor of colon or rectum
- pulmonary metastases are resected in the second stage procedure.

In emergency operations of colorectal cancer with curative intent, the same principles of radicality as in elective procedures are followed. Advanced large bowel obstruction caused by the stenosing tumor in the left part of the colon is treated with subtotal or total colectomy with ileorectal anastomosis, or with Hartmann procedure. In acute radical procedures a lymphadenectomy is performed according to the patient´s condition.

Histological specimens were evaluated at the Department of Pathology Thomayer Teaching Hospital. Lymph nodes are retrieved by surgeons from the native specimen.

Stage of the cancer used in statistical analysis in the study was according to the UICC TNM classification 1997 and was based on clinical data, imaging results, operation records and surgical specimens.

For **N status** description at least 12 lymphonodes must be histologically examined, otherwise stated as NX stage.

Standard **preoperative staging** protocol includes

- total colonoscopy (barium enema)
- biopsy of the tumor
- ultrasound of the abdomen
- PET/CT if available (the operation must not be postponed due to PET waiting list)
- CT of the abdomen and pelvis if PET/CT is not performed
- chest x-ray
- CEA, CA19-9

Ad 1.1:

patients were devided according to stage of the disease in three groups. In the first group patients with T category Tis and T1 were included, so that there was the highest probability of non metastatic tumor. In the second group there were patients with evident disseminated disease- N and M positivity, in the third group there were non-early non-metastatic patients. Levels of CEA, CA19-9, CA242, Thymidine kinase, TPS, TPA, ICAM, VCAM, IGF-1, Adiponectin, Leptin were compared between the two groups.

Ad 1.2:
Markers with statistically significant differences between levels in early and metastatic stages were identified.

Ad 1.3:
Selected markers were stratified according to TNM stage

Ad 1.4:
Correlations between selected markers calculated

Materials - STUDY I

The study included 142 patients between the ages of 35 - 89 years.
Operated XI.2003 – III.2006

The only exclusion criteria was if the blood samples were not send to laboratory or stored.

	No
Males	90 patients
Females	52 patients
Colon cancer	72 patients
Rectal or rectosigmoid cancer	70 patients
TNM I	26 patients
TNM II	42 patients
TNM III	31 patients
TNM IV	43 patients

Table No 13: Study I – group characteristics

Study II

Patients after primary operation for colorectal cancer were **followed up** according to standard protocol used at the Surgical Dpt.Thomayer Teaching Hospital, see below. Additional patients from other hospitals referred to our Surgery for operation of relapse were also added in the study. In those, restaging was completed up to the standard.

Periferal **blood samples** were collected during the follow up visits together with regular CEA and CA19-9 controles-see the protocol.

In case of relapse, the blood samples were taken with restaging procedures.

Mode of relapse diagnosis: relapse was confirmed in 11 patients by resection of the recurrence or metastasis, in 7 patients by biopsy without operation, in 5 patients by PET/CT and in 2 patients only CT.

Storage and analysis methods were the same as in study I.

Follow-up was carried out in accordance with the department's protocol and based on periodic evaluations of the patient.

	Months after operation														
	3	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**	**54**	**60**	**72**	**84**	**96, 120**	**108, 144, 166**
Anamnesis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical investigation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CEA, CA19-9	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ultrasonography	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CT			X		X		X		X		X	X	X	X	X
Coloscopy			X		X		X		X		X	X	X	X	X
X ray of chest					X				X			X		X	
PET, PET/CT	If the markers are elevated and other results are negative and/or before indication for reoperation														

Table No 14: Regular follow up protocol at Dpt.of Surgery Thomayer Teaching Hospital

Markers were evaluated in the same manner as in study I, comparing levels of markers in patients with remission and with relapse.

Materials - STUDY II

The study included 158 patients between the ages of 35 - 89 years.
Operated XI.2003 – III.2006

The only exclusion criteria was if the blood samples were not send to laboratory or stored.

	n
Males	93 patients
Females	65 patients
Colon cancer	84 patients
Rectal or rectosigmoid cancer	74 patients

Table No 15: Study II – group characteristics

Analysis of samples

For the tumor marker assessment venous blood from the cubital vein was sampled in standard conditions between 7 and 9 a.m. The serum acquired through centrifugation was stored until laboratory analysis at a temperature of -70°C. Tumor markers were assessed with commercial laboratory kits, in accordance with the manufacturers' recommendations. The following tumor markers were assessed: CEA and CA 19-9 (MEIA, AxSYM Abbott), TPA (IRMA, Byk Sangtec), TPS (IRMA, Beki), TK (REA, Immunotech), CA 242 (ELISA, CanAg Diagnostics AB). ICAM-1 and VCAM-1 were assessed by multiplex analysis (LUMINEX, Linco Research), Adiponectin, Leptin and IGF-1 were assessed by isotopic methods. All the patients were also clinically examined by an surgeon during every blood sampling for tumor markers.

Statistical analysis

Statistical analysis of the data was performed by using the S.A.S program, version 6.12 and the Statistica program. Descriptive statistics (average, median, standard deviation, maximum, minimum) were calculated for the whole group of patients, as well as for individual subgroups. Comparison of the groups according to different criteria was made with the Wilcoxon non-pair test. The values equal to or less than 0.05 were considered as significant. The data were also analyzed using the Spearman correlating coefficient; the values equal to or less than 0.05 were considered as significant.

RESULTS

Study I – preoperative

Ad 1:

Marker	N	Median	Lower Quartil	Upper Quartil	Minimum	Maximun
CEA	19	1,50	1,00	3,60	0,30	12,90
CA 19-9	20	10,75	7,45	19,70	4,00	30,00
CA - 242	16	6,75	4,50	9,40	1,80	22,80
TK	17	3,60	2,70	5,60	1,00	13,90
TPA	17	31,00	0,00	44,00	0,00	159,00
TPS	20	30,00	3,50	52,50	0,00	179,00
ICAM	15	131,00	112,00	161,00	78,00	248,00
VCAM	15	610,00	439,00	1038,00	274,00	1266,00
Adiponectin	11	24,70	20,60	34,60	13,40	62,90
Leptin	11	4,80	3,20	15,40	1,90	32,50
IGF 1	19	274,10	213,40	389,90	150,50	695,30

Table No 16: Descriptions of markers in Study I, early stage

Marker	N	Median	Lower Quartil	Upper Quartil	Minimum	Maximun
CEA	91	3,20	2,00	13,70	0,30	585,00
CA 19-9	90	15,45	8,90	61,00	0,00	2842,00
CA - 242	84	10,15	5,40	36,10	0,70	150,00
TK	76	5,65	3,80	8,55	0,40	295,00
TPA	76	51,50	28,50	97,00	0,00	1266,00
TPS	80	53,00	21,50	131,00	0,00	1544,00
ICAM	72	142,00	117,00	206,00	70,00	655,00
VCAM	72	801,50	498,00	1121,00	222,00	1752,00
Adiponectin	58	23,05	16,40	32,35	7,30	72,10
Leptin	58	5,30	2,95	10,20	1,10	73,10
IGF 1	87	223,60	157,20	277,90	93,50	478,20

Table No 17: Descriptions of markers in Study I, metastatic stage

Marker	N	Median	Lower Quartil	Upper Quartil	Minimum	Maximun
CEA	21,00	2,20	1,30	5,00	0,00	85,00
CA 19-9	21,00	8,10	5,90	13,40	0,70	104,80
CA - 242	17,00	6,40	3,70	10,00	0,10	98,70
TK	14,00	5,15	3,70	6,80	1,00	80,00
TPA	14,00	41,50	27,00	52,00	0,00	94,00
TPS	16,00	36,50	17,00	56,00	3,00	687,00
ICAM	16,00	130,50	107,50	170,50	94,00	463,00
VCAM	16,00	845,50	543,00	1148,50	277,00	1608,00
Adiponectin	13,00	26,10	18,30	35,80	6,00	66,70
Leptin	13,00	7,00	5,70	40,60	2,30	51,80
IGF 1	17,00	237,90	170,80	393,50	125,10	679,10

Table No 18: Descriptions of markers in Study I, non-early non-metastatic stage

Ad 1.1 :

Marker	Statistically significant differences p <		
	1 versus. 2	1 versus 3	2 versus 3
CEA	**0,0005**	0,1834	0,6290
CA 19-9	0,08	0,1952	**0,0520**
CA - 242	**0,04**	0,8722	**0,0340**
TK	**0,0058**	0,1933	0,4060
TPA	**0,0084**	0,4409	0,6780
TPS	**0,0157**	0,3148	0,1990
ICAM	0,2900	1,0	0,3770
VCAM	0,4320	0,4021	0,7916
Adiponectin	0,3502	0,9315	0,4420
Leptin	0,5395	0,2947	0,0810
IGF 1	**0,0112**	0,3810	0,2700

Table No 19: Comparison of early stage (1) versus metastatic stage (2) versus non-early non-metastatic stage (3)

Comments:
Significant differences in serum levels of markers of biological activity:

were between early stage colorectal cancer (1) and colorectal cancer of metastatic stage (2) in CEA, CA242, TK, TPA, TPS and IGF confirmed

were between early stage colorectal cancer(1) and non-early non-metastatic stage (3) not confirmed in any of the markers

were between non-early non-metastatic stage (3) and metastatic stage (2) confirmed in CA 19-9 and CA 242 only (CEA, TK,TPS,TPA were not significant)

Ad 1.2 :

CEA, CA19-9, CA242, TK, TPA, TPS and (IGF.1) were selected for further investigation.

Ad 1.3 :

CEA	0.47921
	<.0001
CA 19-9	0.35074
	<.0001
CA – 242	0.30089
	0.0013
TK	0.27791
	0.0045
TPA	0.34815
	0.0003
TPS	0.37325
	<.0001
ICAM	0.11006
	0.2832
VCAM	0.09944
	0.3325
Adiponectin	-0.13053
	0.2228
Leptin	-0.12949
	0.2265
IGF 1	-0.18850
	0.0409

Table No 20: Spearman Correlation Coefficients between TMN stage and markers

Comments: statistically significant correlation between TNM stage and markers are in CEA, CA 19-9, CA 242, TK, TPS, TPA, IGF 1

Marker	**Statistically singnificant differences p <**					
	TNM I vers TNM II	**TNM I vers TNM III**	**TNM I vers TNM IV**	**TNM II vers TNM III**	**TNM II vers TNM IV**	**TNM III vers TNM IV**
CEA	**0,034**	**0.0329**	**0,0001**	0.7762	**0.0001**	**0.0003**
CA19-9	NS	0.3059	**0.0023**	0.0667	**0.001**	**0.0053**
CA242	NS	0.1714	**0.0018**	01629	**0.0067**	**0.00818**
TPS	0.2658	0.4210	**0.0002**	0,8014	**0.0007**	**0,014**
TPA	0.5399	0.1121	**0.0037**	0,2835	**0.0041**	**0, 0522**
TK	**0,034**	**0.0302**	**0.0030**	0.7619	0, 3473	0, 2158

Table No 21: Comparing levels of selected markers in TNM stages

Comments:
Significant differences in serum levels of markers of biological activity:

were between TNM stage I colorectal cancer and stage IV in all selected markers

were between TNM stage II and stage IV in CEA, CA19-9, CA242, TPS and TPA

were between TNM stage III and stage IV in CEA, CA19-9, CA242, TPS and TPA

were between TNM stage I and stage II in CEA and TK

were between TNM stage I and stage III in CEA and TK

none of the selected markers showed statistically significant difference comparing levels in TNM II and III stage

Ad 1.4:

The differences among behaviors of particular markers throughout examined groups were noticed and so we were interested if some fix context can be detected or if each marker is independent. That is why correlations in all groups were performed.

Marker	**CEA**					
CA 19-9	r = 0.46459					
	p < 0.0001	**CA 19-9**				
CA 242	r = 0.48894	**r = 0.73556**				
	p < 0.0001	**p <.0001**	**CA 242**			
TK	r = 0.14749	r = 0.35168	r = 0.13867			
	p < 0.1314	**p < 0.0003**	p < 0.1689	**TK**		
TPA	r = 0.34573	r =0.26601	r = 0.14216	r =0.26892		
	p < 0.0003	p < 0.0063	p < 0.1583	**p < 0.0051**	**TPA**	
TPS	r = 0.28978	r =0.27910	r = 0.09568	r = 0.42998	r = .54810	
	p < 0.0017	**p < 0.0028**	p < 0.3269	**p < 0.0001**	**p< 0.0001**	**TPS**
IGF – 1	r = - 0.1795	r = - 0.2310	r = - 0,2855	r = - 0,4530	r =- 0.2593	r = -0.2913
	p < 0,0478	**p < 0.0111**	p < 0, 7640	**p < 0,0001**	p < 0.0052	**p < 0.0016**

Table No 22: Spearman Correlation Coefficients in all groups

Marker	CEA					
CA 19-9	r = 0.61884					
	p < 0.0047	CA 19-9				
CA 242	r = 0.78293	r = 0.60383				
	p < 0.0003	p < 0.0133	CA 242			
TK	r = 0.01627	r = 0.33374	r = 0.12555			
	p < 0.9523	p < 0.1905	p < 0.6689	TK		
TPA	r = 0.31348	r = 0.07028	r = 0.24531	r = 0.14002		
	p < 0.2371	p < 0.7887	p < 0.3979	p < 0.5920	TPA	
TPS	r = 0.31468	r = 0.13042	r = 0.19779	r = 0.24261	r = 0.15889	
	p < 0.1895	p < 0.5837	p < 0.4628	p < 0.3481	p < 0.5425	TPS
IGF 1	r =-0,27329	r =-0,28609	r =-0,08214	r =-0,13991	r =0,3560	r =-0,21626
	p <0,2725	p <0,2351	p <0,7710	p <0,6053	p <0,1762	p <0,3739

Table No 23: Spearman Correlation Coefficients in group 1 (early)

Comments: there are correlations between CEA and CA19-9, CEA and CA242. Suprisingly TK, TPS, TPA do not correlate with any other marker.

Marker	CEA					
CA 19-9	r = 0.48335					
	p < 0.0001	CA 19-9				
CA 242	r = 0.45679	r = 0.7432				
	p < 0.0001	p < 0.0001	CA 242			
TK	r = 0.0680	r = 0.3311	r = 0.08750			
	p < 0.5594	p < 0.0042	p < 0.4617	TK		
TPA	r = 0.40848	r = 0.3230	r = 0.13882	r = 0.29777		
	p < 0.0002	p < 0.005	p < 0.2415	p < 0.0090	TPA	
TPS	r = 0.24148	r = 0.3070	r = 0.0566	r = 0.41023	r = 0.55836	
	p < 0.0309	p < 0.0066	p < 0.6270	p < 0.0002	p < 0.0001	TPS
IGF -1	r =-0,19651	r =-0,17542	r =0,03094	r =-0,55117	r =-0,29177	r =-0,29161
	p <0,0681	p <0,1105	p <0,7826	p <0,0001	p <0,0105	p <0,0067

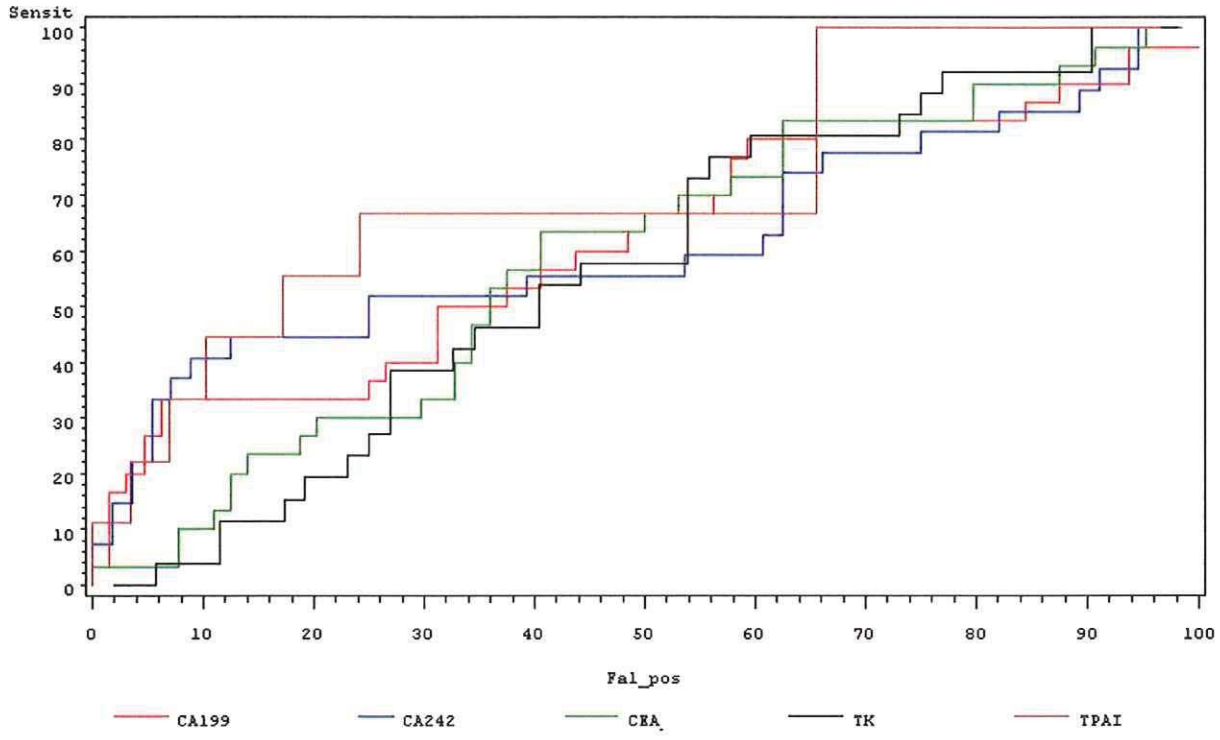
Table No 24: Spearman Correlation Coefficients in group 2 (metastatic)

Comments: TK correlates with TPA, TPS and CA19-9. TK does not correlate with CEA and CA242. TPA correlates with TPS, CEA and CA242. TPA does not correlate with CA242. TPS correlates with TPA, CEA and CA19-9; does not correlate with CA242. CA 242 correlates with CEA, CA19-9 but does not correlate with TPS, TPA and TK.

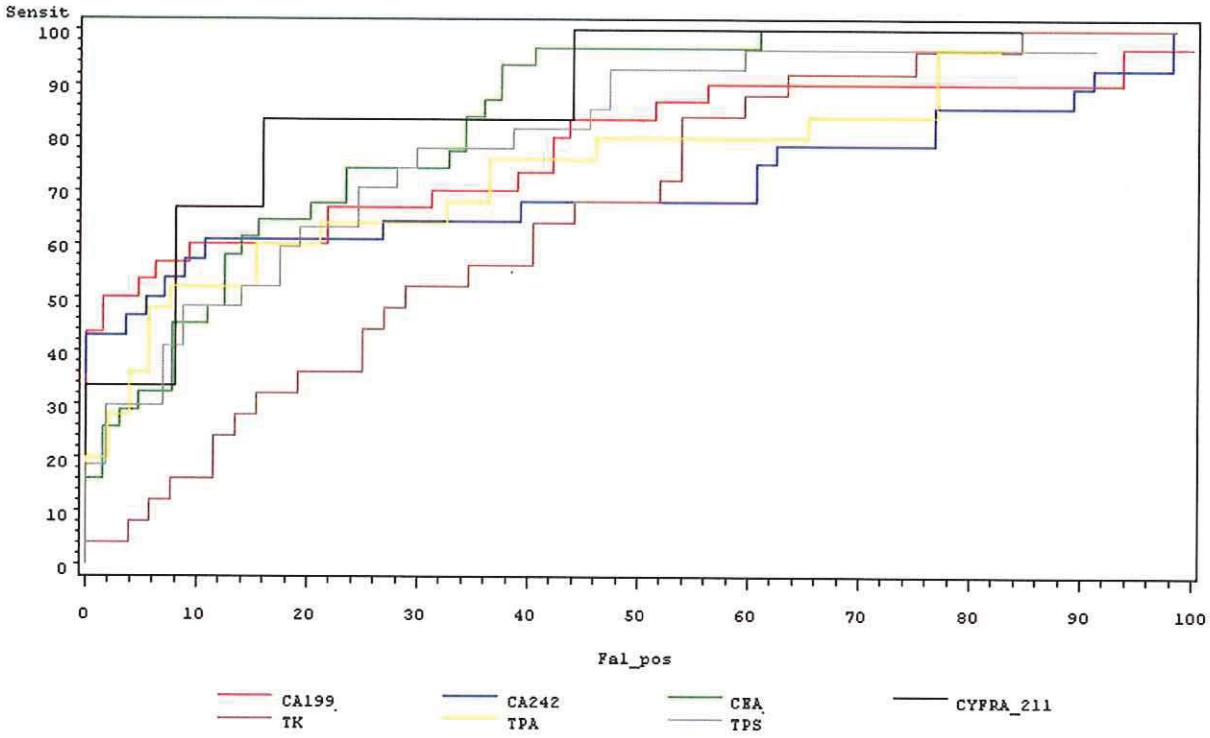
Marker	CEA					
CA 19-9	r = 0.19233					
	p < 0.4036	CA 19-9				
CA 242	r = 0.44758	r = 0.74156				
	p < 0.0716	p < 0.0007	CA 242			
TK	r = 0.02857	r = 0.20242	r = 0.27785			
	p < 0.9228	p< 0.4877	p < 0.3580	TK		
TPA	r =-0.43158	r = 0.18584	r = 0.10249	r = 0.07956		
	p < 0.1612	p < 0.5247	p < 0.7390	p < 0.7869	TPA	
TPS	r = 0.13991	r = 0.18805	r = 0.06088	r = 0.50165	r = 0.50000	
	p < 0.6053	p < 0.4855	p < 0.8294	p < 0.0676	p < 0.0687	TPS
IGF 1	r =0,42402	r =-0,22454	r =-0,00442	r =-0,31429	r =-0,26962	r =-0,26118
	p <0,0898	p <0,3863	p <0,9871	p <0,2738	p <0,3512	p <0,3471

Table No 25: Spearman Correlation Coefficients in group 3 (non-early non-metastatic)

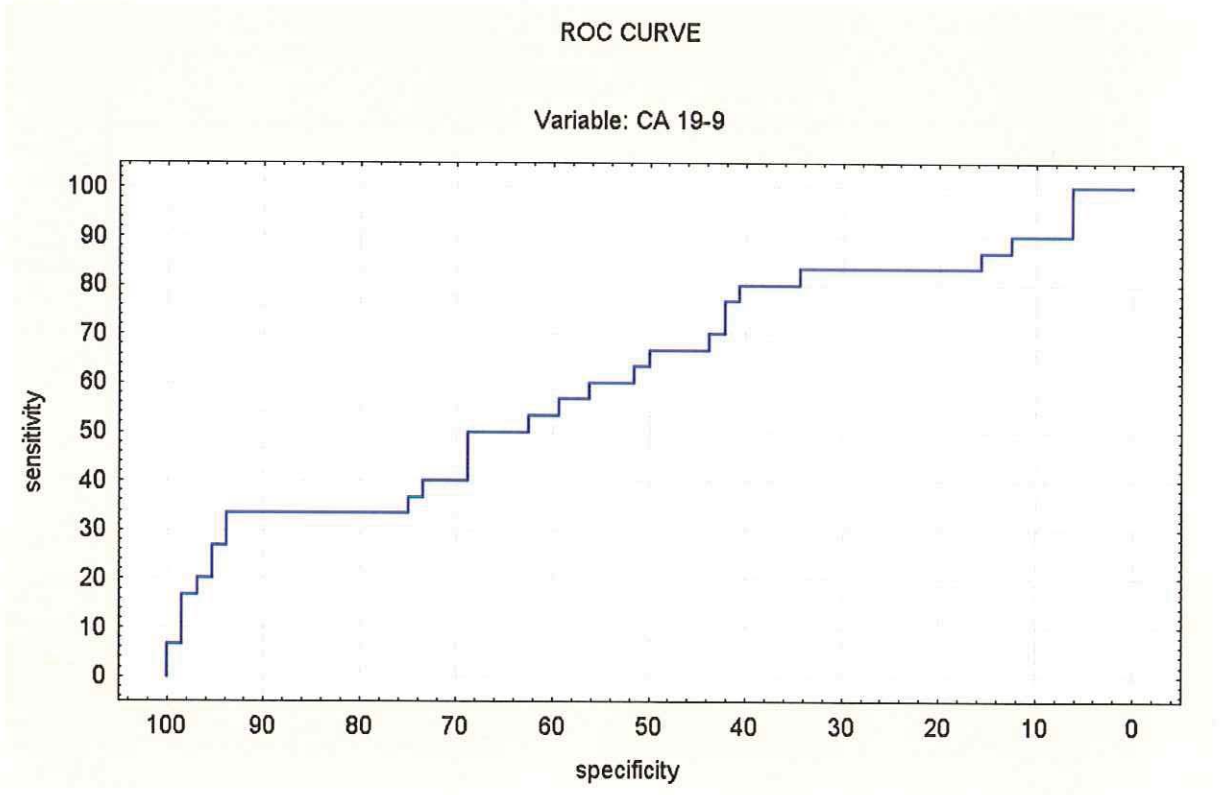
Comments: CEA correlates with TPA in a negative way, CEA does not correlate with CA19-9, CEA does not correlate with CA19-9,TPS. CEA correlates withCA242. CA19-9 correlates with CA242 and with nothing else.



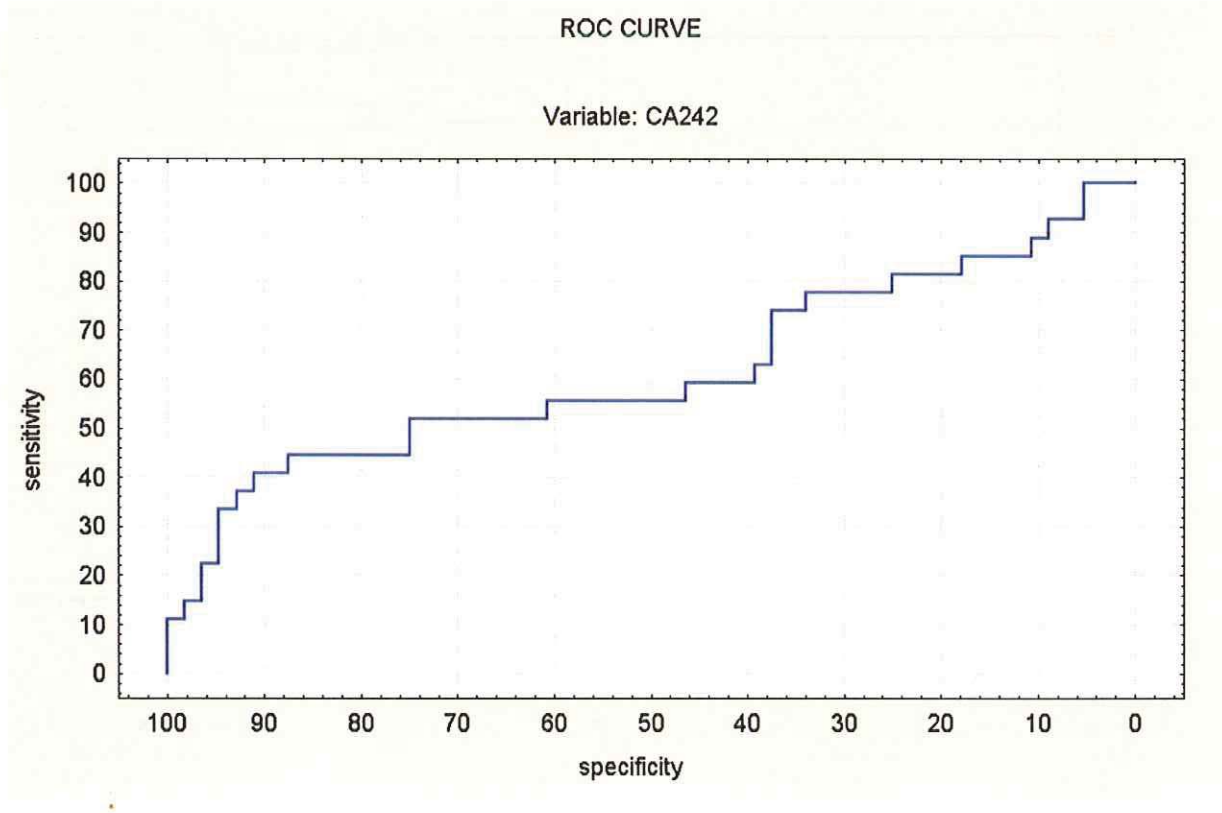
Graph No 11: ROC curve: TNM I + II vers. TNM III – all markers



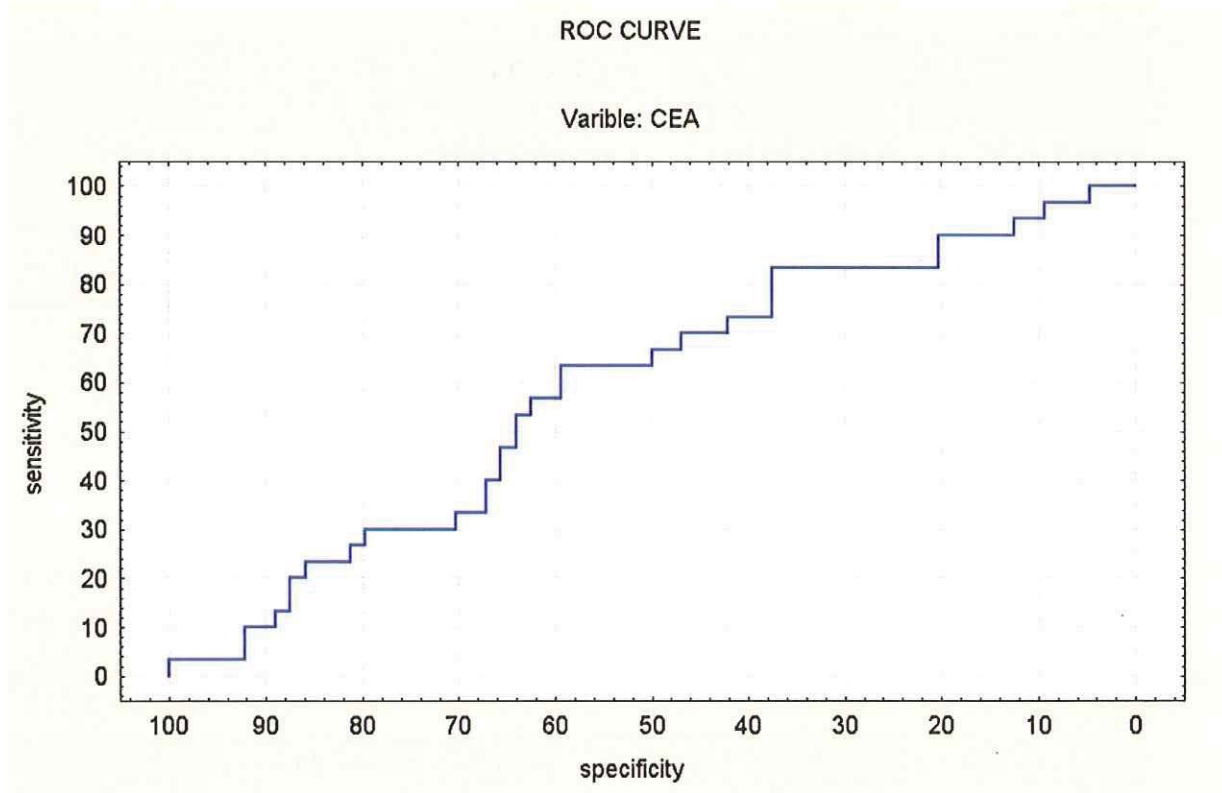
Graph No 12: ROC curve: TNM I + II vers. TNM IV – all markers



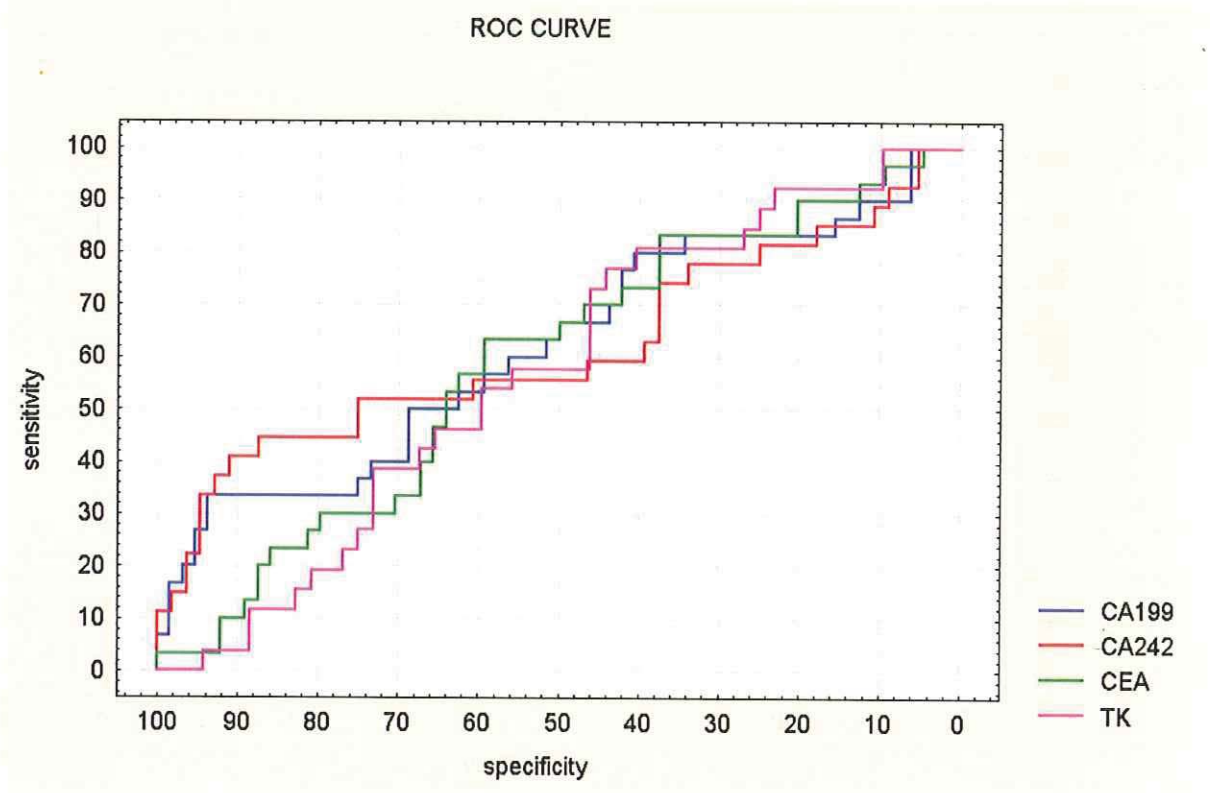
Graph No 13: ROC curve: TNM I+II vers. TNM III – CA 19-9



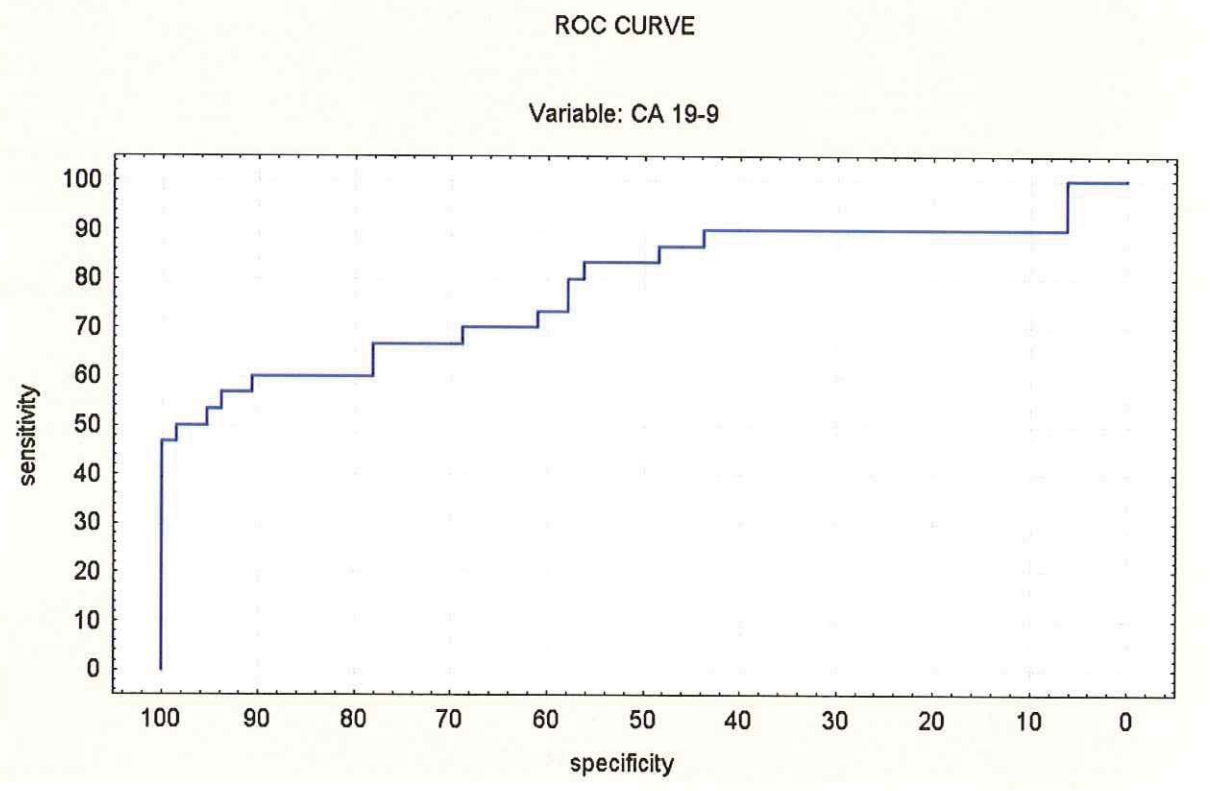
Graph No 14: ROC curve: TNM I+II vers. TNM III – CA242



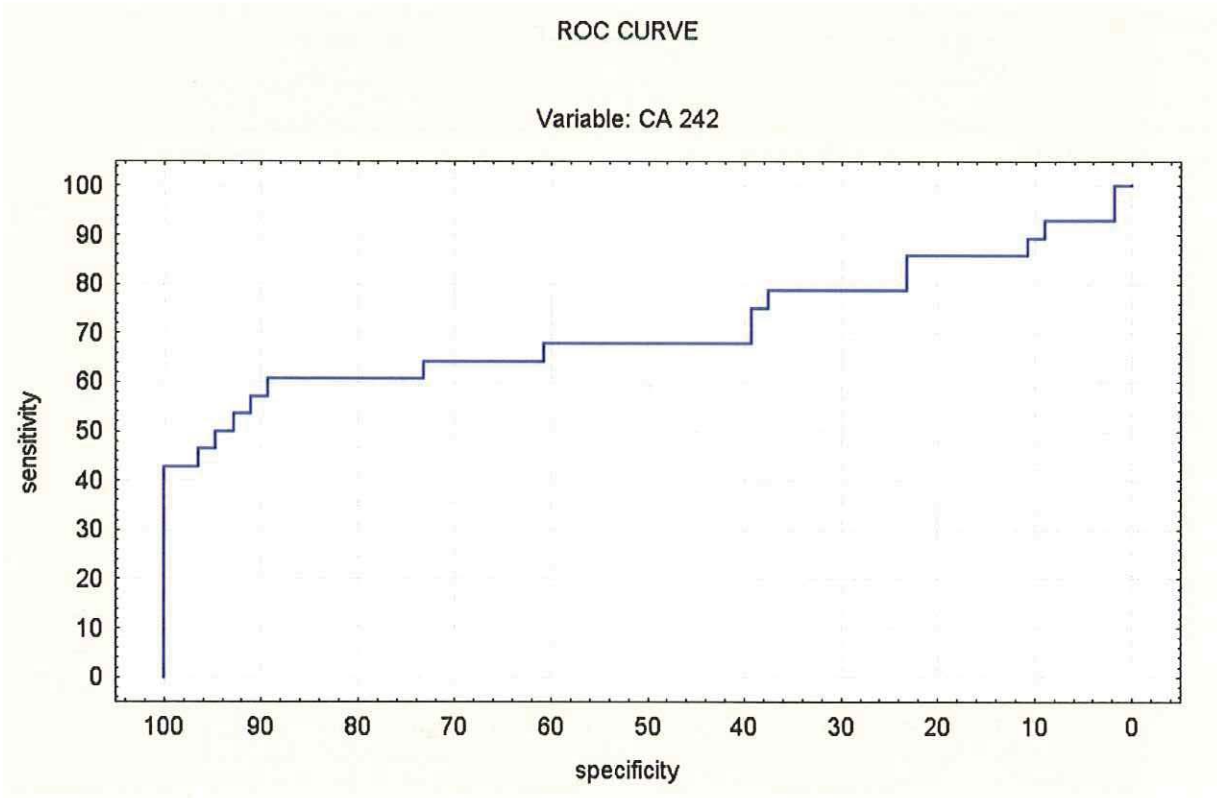
Graph No 15: ROC curve: TNM I+II vers. TNM III - CEA



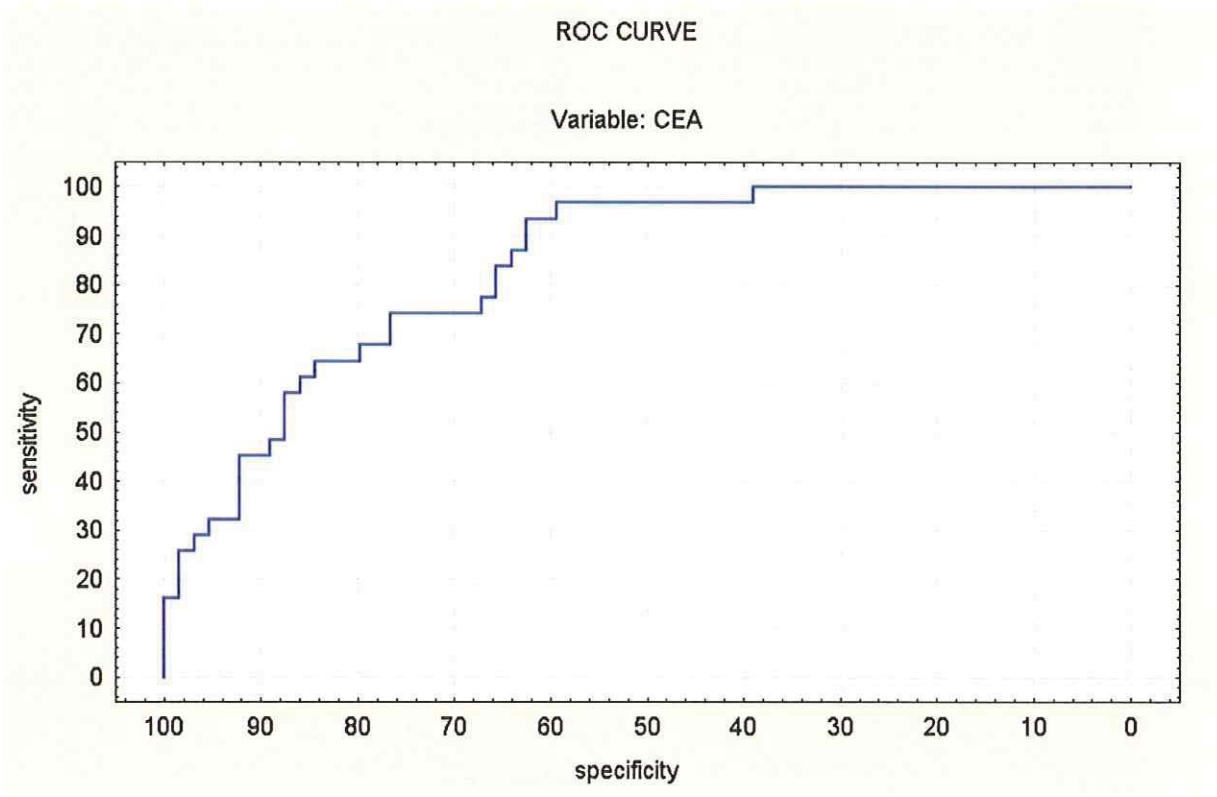
Graph No 16: ROC curves: TNM I+II vers. TNM III – selected markers



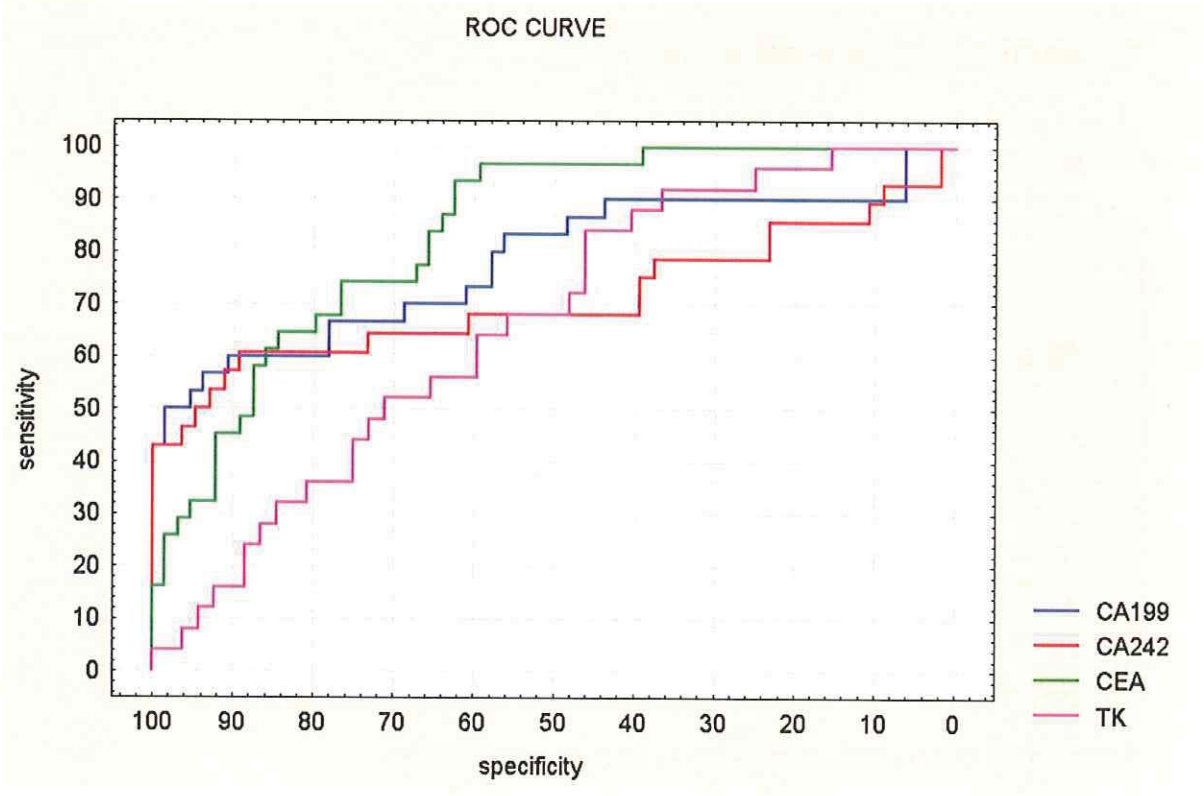
Graph No 17: ROC curve: TNM I+II vers. TNM IV – CA 19-9



Graph No 18: ROC curve: TNM I+II vers. TNM IV – CA 242



Graph No 19: ROC curve: TNM I+II vers. TNM IV - CEA



Graph No 20: ROC curves: TNM I+II vers. TNM IV – selected markers

Study II – postoperative follow up

Ad 2:

Marker	N	Median	Lower Quartil	Upper Quartil	Minimum	Maximun
CEA	25	10,70	4,40	50,50	1,20	466,00
CA 19-9	25	26,80	11,40	106,20	0,60	370,90
CA - 242	22	25,00	8,30	60,99	0,30	150,00
TK	19	5,50	3,80	11,00	2,70	101,50
TPA	19	114,00	49,00	246,00	0,00	1045,00
TPS	19	133,00	35,00	268,00	16,00	708,00
ICAM	12	129,50	114,50	162,50	92,00	259,00
VCAM	12	1083,50	522,00	1293,50	182,00	1523,00
Adiponectin	18	19,80	12,60	30,00	7,00	52,60
Leptin	18	8,90	6,10	18,30	2,50	28,40
IGF 1	28	226,30	202,55	269,15	106,10	436,60

Table No 27: Descriptions of markers in Study II, relapse stage

Marker	N	Median	Lower Quartil	Upper Quartil	Minimum	Maximun
CEA	114	2,35	1,40	3,30	0,20	17,50
CA 19-9	114	10,85	6,50	21,20	0,60	428,30
CA - 242	52	5,65	2,20	10,50	0,00	22,30
TK	42	7,30	5,50	9,90	1,80	79,20
TPA	42	32,50	16,00	55,00	0,00	111,00
TPS	42	30,50	13,00	66,00	0,00	221,00
ICAM	4	151,50	126,50	186,00	121,00	201,00
VCAM	4	406,50	317,00	962,50	228,00	1518,00
Adiponectin	31	30,80	16,80	38,20	9,50	114,30
Leptin	31	10,90	6,10	17,90	1,70	75,80
IGF 1	108	231,95	188,00	292,35	105,80	632,10

Table No 28: Descriptions of markers in Study II, remission stage

Ad 2.1:

Marker	Statistical significance
CEA	**0,0001**
CA 19-9	**0,0007**
CA - 242	**0,0003**
TK	0,2204
TPA	**0,0004**
TPS	**0,0007**
ICAM	0,5145
VCAM	0,2573
Adiponectin	0,0638
Leptin	0,7807
IGF 1	0,9550

Table No 29: Comparision of relapse(4) versus remission (5)

Comments: Five markers show statistically significant difference between levels in relapse or remission

Ad 2.2:
CEA, CA19-9, CA242, TPA, TPS were selected for further investigation.

Ad 2.3:

Marker	**CEA**				
CA 19-9	0.50231				
	0.0105	**CA 19-9**			
CA 242	0.55452	0.92665			
	0.0074	<.0001	**CA 242**		
TK	-0.17244	0.35667	0.22462		
	0.5231	0.1751	0.4209	**TK**	
TPA	0.33701	-0.02355	0.11300	-0.17363	
	0.2018	0.9310	0.6884	0.4772	**TPA**
TPS	0.30294	0.20000	0.16129	0.22134	0.70588
	0.2541	0.4577	0.5658	0.3625	0.0007

Table No 30: Spearman Correlation Coefficients in group 4 (relapse)

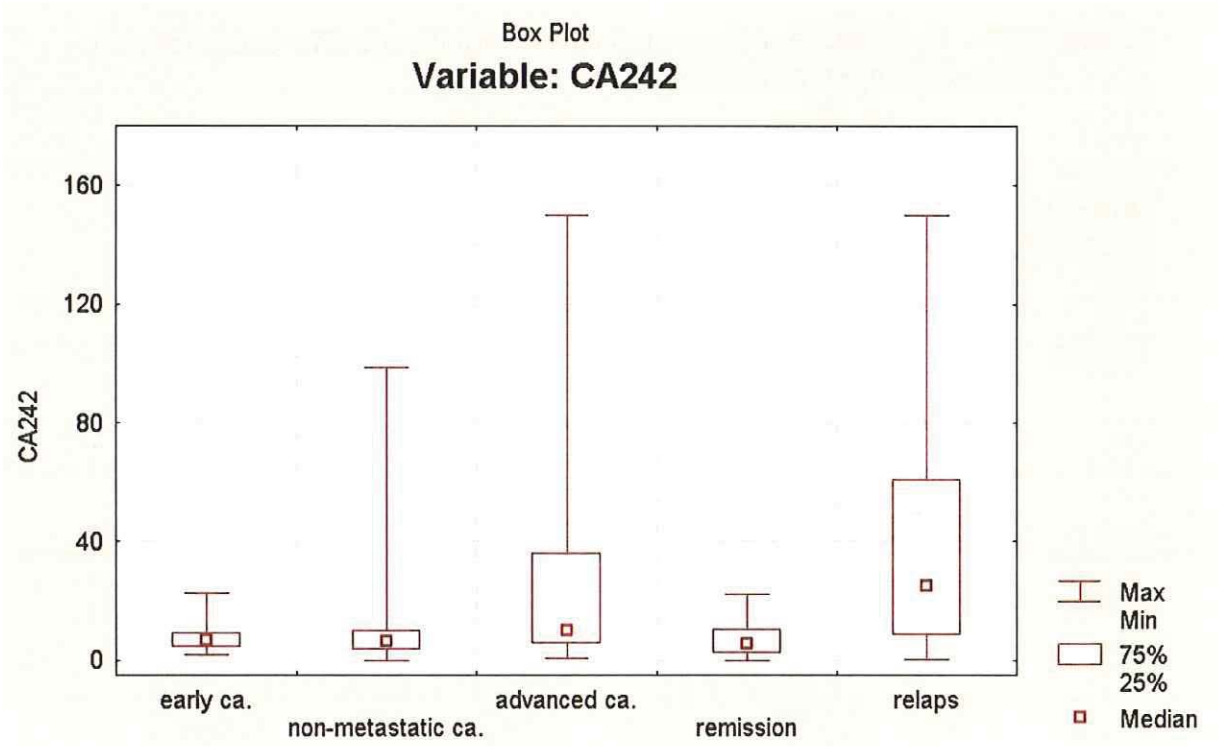
Comments: High correlation between TPA and TPS, potential replacement of TPA,TPS

CA19-9 and CA242 correlate as well, both of them correlate with CEA, but correlation coefficient is low, thus it does not allow to interchange CA19-9 or CA242 with CEA. There is also no correlation coefficient between CEA and cytokeratines and CA242 and cytokeratines.

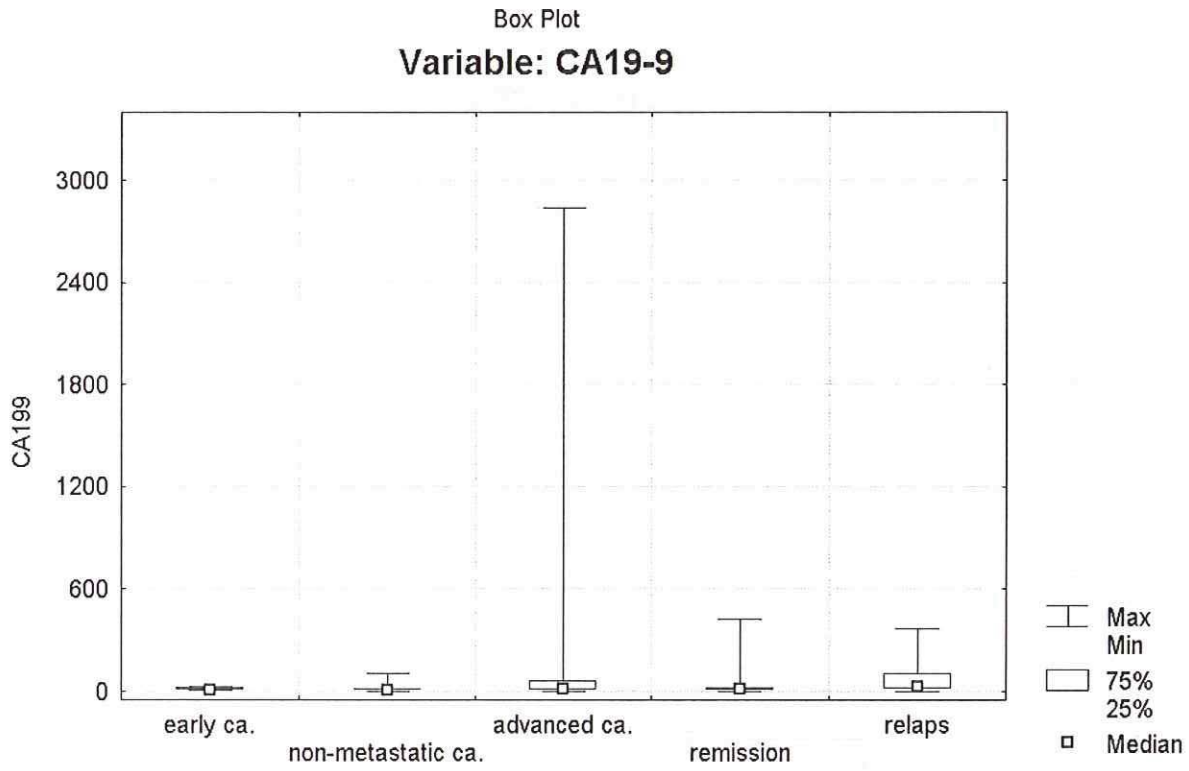
Marker	CEA				
CA 19-9	0.41739				
	<.0001	CA 19-9			
CA 242	0.29386	0.72705			
	0.0215	<.0001	CA 242		
TK	-0.05915	-0.06359	-0.08386		
	0.7243	0.7005	0.6118	TK	
TPA	0.16830	0.23668	0.15243	0.00409	
	0.3125	0.1469	0.3542	0.9795	TPA
TPS	0.13852	0.38008	0.19623	-0.04083	0.72591
	0.4069	0.0170	0.2901	0.7974	<.0001

Table No 31: Spearman Correlation Coefficients in group 5 (remission)

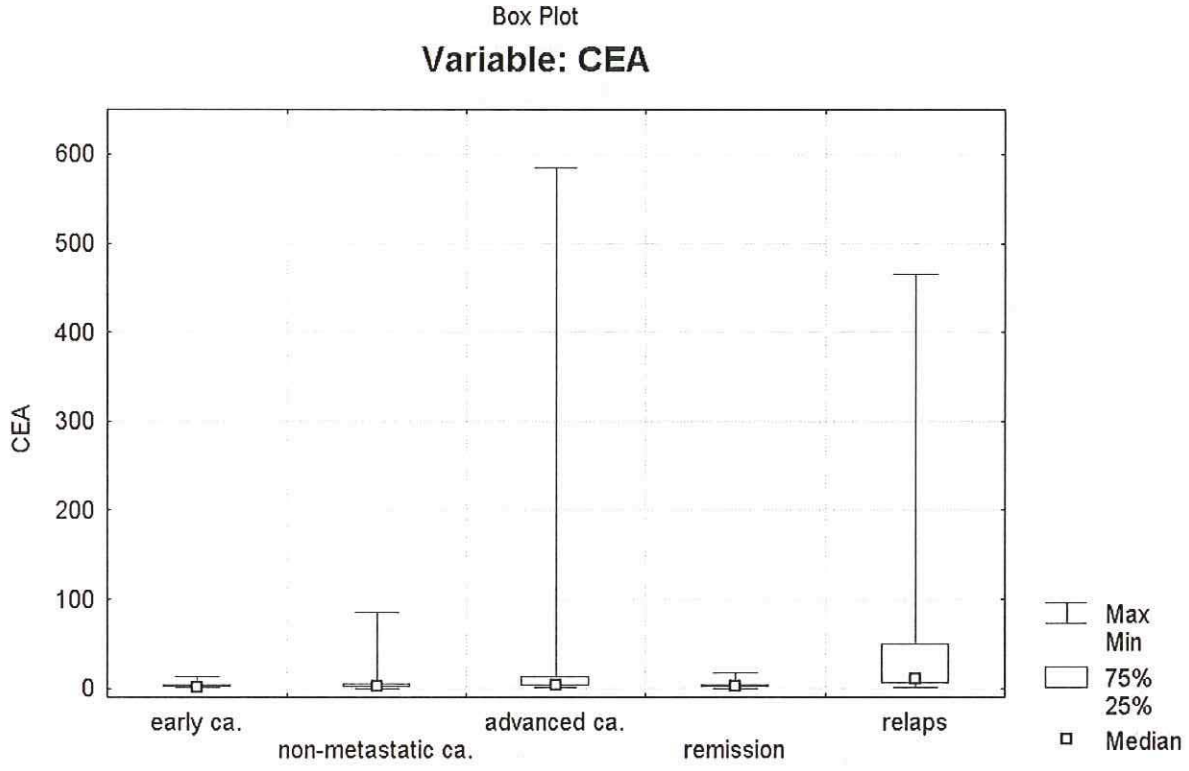
Ad 2.4:
For follow up it seems optimal to use 3 markers: CEA and CA19-9 or CA242 and TPS or TPA.



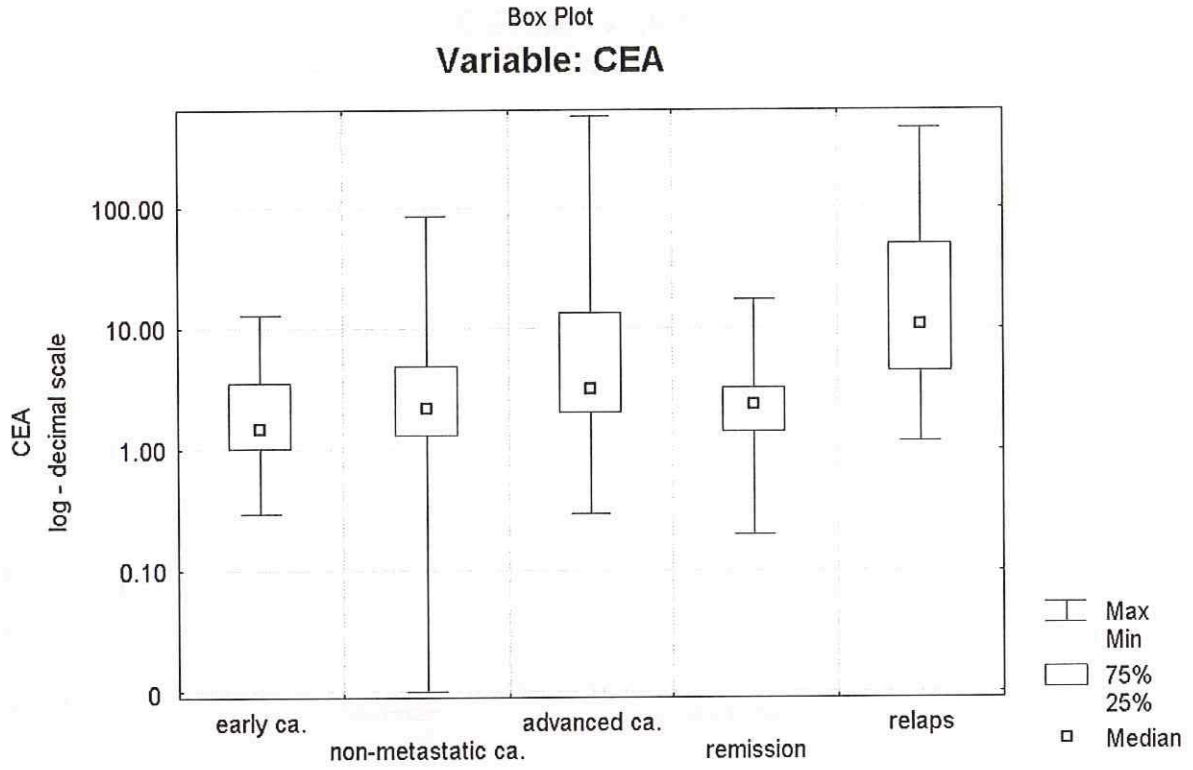
Graph No 21: Box Plot CA 242



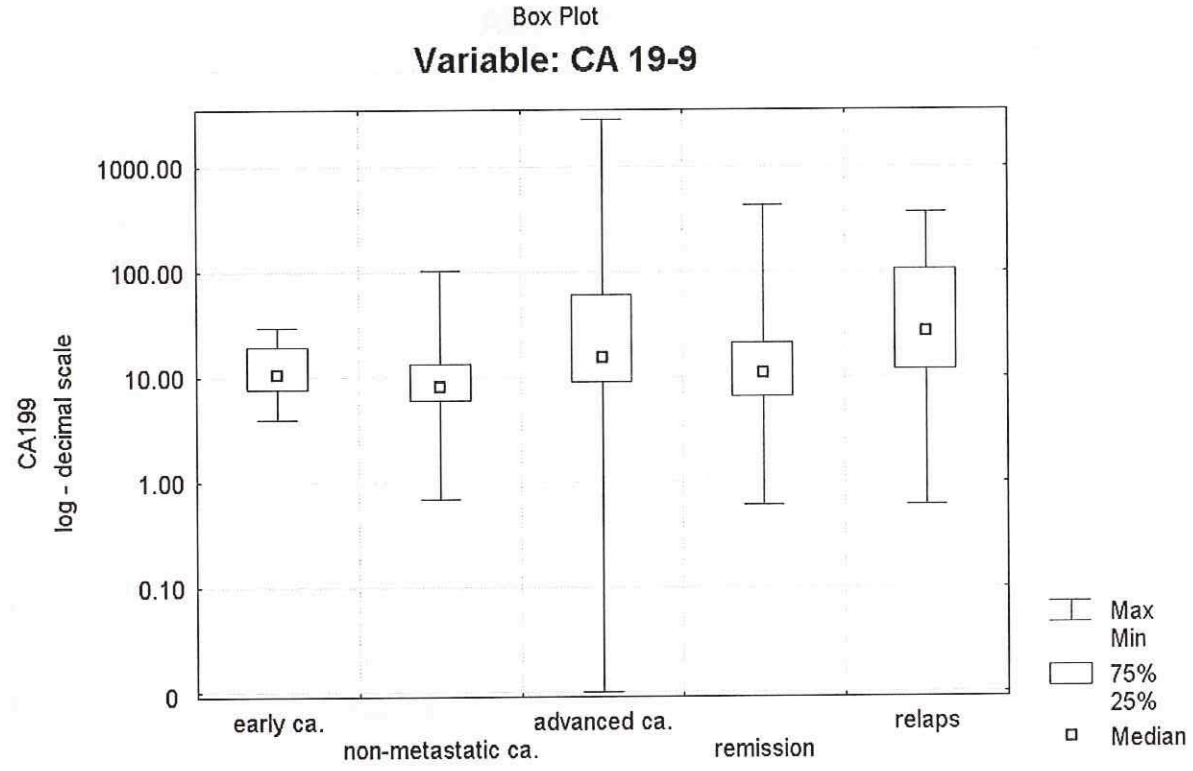
Graph No 22: Box Plot CA 19-9



Graph No 23: Box Plot CEA



Graph No 24: Box Plot CEA – log-decimal scale



Graph No 25: Box Plot CA 19-9 – log-decimal scale

DISCUSSION

The aim of this thesis was to evaluate the role and help of markers of biologic activity of colorectal cancer in oncosurgical practice. There are two main topics – preoperative staging and restaging during follow up. They have mutual similarities and differences and so the patients and results were devided in two studies. For this intention three groups of biological factors were chosen. Different types of tumor markers- CEA, mucin markers, markers of cytoskeleton etc., adipocytokines, which are known risk factors for colorectal carcinogenesis and adhesive molecules, known for relations with metastatic process.

Marker	Number of citations in Pub Med	
	cancer	colorectal cancer
TK	129	3
CA 242	28	9
Adhesive molecules	15	7
Adiponectin	80	25
Leptin	553	28
CEA	8555	2833
CA 19-9	1953	366
IGF 1	3302	183
TPS + TPA	804	67

Table No 32: Numbers of papers published in literature on the topics, source Pub Med

During the last 10 years there were about 240 papers concerning **adiponectin, leptin** and especially **IGF**. These articles dealing mostly with cancerogenesis of colorectal cancer with links to metabolic syndome X, hypertension, diabetes mellitus, where IGF is studied as one of potential risk factors. This presented thesis is one of few which is monitoring levels IGF, adiponectin and leptin according to the stage of colorectal cancer. It seems that IGF, leptin and adiponectin play an important role in genesis of colorectal cancer, but as they are locally active substances, it is questionable if they can in periferal blood reflect the stage of tumor. In our study there was no statistical significance in differnces of **leptin** levels between patients in early stage (n = 11) and metastatic stage (n = 58) (4.3 ng/ml vs 5.3ng/ml, p< 0, 5). As well as there was no correlations between leptin and any other presented parameter of biological activity. The same results presented Arpaci et al. in [54] in a group of 36 patients with colorectal cancer. Serum leptin levels of early-stage patients (n = 15) did not differ from those of advanced-stage patients (n = 21) (7.74 vs 9.54 ng/mL, P = 0.542) and there was no correlation in cancer patients between serum leptin levels and CEA or CA19-9 (r = 0.015, P = 0.929 and r = 0.097, P = 0.574). Bolukbas et al.in 2004 presented serum leptin concentrations in group of advanced cancer patients (gastric n=29 and colorectal n =17). Independently from the involved site of the gastrointestinal tract, serum leptin

concentration in advanced gastrointestinal cancer was lower than in healthy controls. But relations to TNM stage of cancer were not calculated[55] Impaired response of adiponectin, ghrelin, and leptin may play a role in the pathogenesis of cancer cachexia syndrome[56].

Adiponectin and IGF are mentioned in a series of papers published by Giovannucci et al. as risk factors in etiopathogenesis ef colorectal cancer[57] , but in literature we did not find a study concerning adiponectin levels related to colorectal cancer stage in the same way as it is in this thesis.

In total we have found seven papers on **adhesion molecules** and colorectal cancer, in these, in agreement with our experience, is significant difference between patients with colorectal cancer and a controle group. There are still controversies in literature regarding correlations of levels of adhesion molecules and stage of colorectal cancer.

In a work of Alexiou and col.[58], there was, a significant association between the serum levels of ICAM and VCAM, colorectal disease stage and the presence of both lymph node and distant metastases in 63 patients with colorectal cancer.

The sE-selectin levels in the cancer patients with liver metastasis were significantly higher than the levels in patients without liver metastasis, and were also higher than control levels as presented Uner [59].

Benoliel[60] described ICAM and VCAM as a prognostic factor of relapse following colorectal cancer surgery.

Velikova[61] in 1998 investigated the concentrations of the soluble adhesion molecules in 48 patients with colorectal cancer before treatment, levels of circulating ICAM-1 and VCAM-1 were increased both in patients with local and those with metastatic disease. In our results neither ICAM nor VCAM levels correlated with dissemination of colorectal cancer when compared early stage and metastatic stage of the disese.

In pub-med search have been found only three articles concerning **thymidine kinase** and colorectal cancer. In 1994 Tanigawa studied in 127 patients with colorectal cancer, the relations between thymidine uptake by cancer cells in semi-solid media, their clinico-pathologic features and survival times. In 1995 Thomas presented a study on TK concerning patients with asymptomatic colorectal carcinoma (n = 21) and patients known to have hepatic metastases from colorectal tumours (n = 33).The TK activity in patients with asymptomatic cancer (median 1.85; range 1.00-4.50 pmol/ml/h) was lower comparing to TK activity in patients with metastatic disease (median 4.23; range 2.03-14.12 pmol/ml/h) and the difference was statistically significant. In our study the same result was confirmed- the difference between levels of TK in patients with colorectal carcinoma stage TNM I versus TNM IV was statistically significant. Moreover there was statistically significant difference between stage TNM I versus TNM II and the same in TNM I versus TNM III. Only the stage TNM II versus TNM III has no statistically significant difference in levels of TK, similarity with other markers will be shown below. In a study published by Topolcan[62] in 2005 thymidine kinase seems to be a suitable parameter for monitoring the effect of adjuvant and palliative chemotherapy in colorectal cancer.

Literature data on TK and follow up of colorectal carcinoma are inconsistent.

The changes of cytokeratine markers in colorectal cancer were in previous literature often discussed and most of the authors came to the cunclussion, that **TPA and TPS** are of none or diminished prognostic significance. In Study I, we have found statistically significant differences between early and metastatic colorectal carcinoma, but there were no significant differences between "non-metastatic non-early " and metastatic stages or between "non-metastaic non-early" and early stages. It seems that TPS and TPA is able to confirm advanced disease, but comparing to routinelly used CEA and CA19-9, cytokeratines do not provide any additional information. On contrary, in Study II during follow up, TPS and TPA was significantly elevated in case of relapse of colorectal cancer and the elevation did not correlate at all or only minimally with CEA, CA19-9 or CA242 in relapseing CRC. Therefore TPS and TPA seem to be independent prognostic factors in follow up of colorectal carcinoma.
TPS and TPA are regarded to be proliferative markers[63]. TPS is a useful marker in postoperative monitoring of patients with colorectal cancer. The evaluation of TPS concentration allows to diagnose the recurrence of colorectal cancer erlier than by using burden markers CEA etc. The increase of TPS concentration may be ahead of relapsee symptoms at about 2-6 months. Common evaluation of TPS and CEA increase sensitivity in detection of relapsee in patients with colorectal cancer[64]. The study of Kornek et al. suggests, that proliferation marker TPS appears to be a very useful biochemical marker in that it is elevated in more than 90% of the patients with advanced colorectal cancer, and has a very good predictive value[65]. Plebani et al. reported that TPA and TPS are very sensitive indices in early detection of relapse of colorectal cancer during follow up. TPA was the most sensitive index in identifying early or well- differentiated colorectal cancers. The sensitivity was enhanced when this marker was determined in combination with CEA, in diagnosing both advanced and early colorectal tumours[66].Cytokeratines seem to represent a sensitive, clinically relevant and specific marker of proliferative activity in gastrointestinal cancer especially in advanced tumours of colon and rectum.

Papers concerning **CA242** are published predominantly in Scandinavia and China. In the years 1991-1994 most of the articles presented results of investigating CA242 in relations to diagnosis and monitoring of colorectal cancer[67,68,69]. A longitudinal evaluation of serum CA242 levels demonstrated that this marker was indicative of the status of colorectal cancer disease in a paper of authors from Rome[70]. After surgery of colorectal cancer, CA 242 emerged as a significant predictor of survival, in multivariate analysis, entering the tumour markers as continuous variables, Dukes' stage was the strongest prognostic factor, followed by CA 242 , whereas age, gender, CEA and TPA were not.[71] In 2004 the same authors publishid a paper discussing the same markers in relation to recurrence of colorectal cancer. CEA had the highest diagnostic accuracy in detecting recurrent colorectal cancer. Inclusion of CA 19-9, CA 242, CA 72-4 or hCGbeta in the model did not improve the accuracy, although CA 72-4 approached borderline significance ($p = 0.053$). Thus, CEA seems to retain its position as the surveillance marker of choice for patients surgically treated for colorectal cancer[72]. In our currently presented thesis the results confirm experience different from the above mentioned. CEA is an excellent marker of relapse, but it does not correlate with CA242 or CA19-9. On contrary these two mucin markers correlate very well between each other. These conclusions

implicate that combination of CEA and one of the mucin markers could increase sensitivity to relapse. Simmilar results were published by Spila[73].

The issue of **CEA and CA19-9** is well known from literature and our current results endorse these findings. We have confirmed that CA19-9 is besides CEA an important marker in colorectal cancer. Comparing CA19-9 and CA242 in preoperative staging, CA242 is more specific. Correlation coeffitients between CA19-9 and CA242 are about 0,7 thus these markers are not identical, but in colorectal cancer is the advantage of CA242 over CA19-9 not so evident as in cancer of pancreas. Regarding results of study I, CA242 seems to be more sensitive in preoperative staging than CA19-9, especially comparing early and metastatic stages.
In the follow up, there seem to be three independent groups of markers: 1. CEA, 2. CA19-9 and CA242 and 3. TPA and TPS. Between CA19-9 and CA242 and in the same way between TPS and TPA is a very high correlation and so the replacement is possible.

From the clinician point of view is the comparison of marker levels in TNM stage II and III very interesing. None of the used markers was able to distinguish stage II and III, in other words to identify patients with infiltration of lymph nodes. This fact is very important in our aspirations to find which marker from periferal blood could help to poit out patients in risk of lymphatic infiltration and to indicate these patients for adjuvant therapy. As well interesting is, that the same comparison of the same markers in stage TNM I and III is suggesting two markers - TK and CEA. In this consequence it is necessary to mention issue of N understaging despite of meticulous effort to identify and examine the lymphonodes from the specimen as described above in methodolgy. It is possible that some patient with infiltrated lymph nodes are hidden in the TNM stage II. That is the reason why at our department we indicate patient pT3 and pT4, N0, M0 for chemotherapy.

CONCLUSIONS

1. Two groups of patients were tested : 142 patients before primary operation for colorectal cancer, included in Study I and 158 patients during the regular follow up, included in Study II.
2. Following markers and biologic factors were examined: CEA, CA19, CA242, thymidine kinase (TK), TPA, TPS, ICAM -1, VCAM, leptin, adiponectin, IGF-1.
3. In Study I statistical significant difference between early and metastatic stage of colorectal cancer was not confirmed in markers: ICAM-1, VCAM, adiponectin, leptin.
4. In Study I statistical significant difference between early and metastatic stage of colorectal cancer was confirmed in markers: CEA, CA19-9, CA242, TPS, TPA, TK, IGF-1.
5. In Study I (preoperative) correlations between levels of markers and TNM stage were confirmed
6. In Study I correlations of selected markers against each other are stated.
7. In Study II during the follow up correlations between relapse and markers ICAM-1, VCAM, TK, leptin, adiponectin and IGF-1 were not confirmed.
8. In Study II during the follow up, correlations between relapse and markers CEA, CA19-9, CA 242, TPS and TPA were confirmed.
9. Combination of CEA and either CA19-9 or CA242 can be recommended for preoperative investigation. CA 242 in this study seems to have slightly better results in preoperative staging.
10. Combination of CEA and either CA19-9 or CA242 and either TPS or TPA can be recommended for postoperative follow up.

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