

Prognostic and predictive factors in colorectal cancer

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Abstract

Aim: The aim of the thesis was to study prognostic and predictive factors in patients treated for colorectal cancer (CRC).

Method: In paper I, a retrospective comparison was made between the patients treated in 1999 (n=180) with those treated in 2004 (n=175). During the period, a multidisciplinary team conference and an improved cooperation with the pathologists had been initiated. The focus of interest was the lymph node assessment, its' development and how this affected clinical staging and treatment. In paper II, the lymph node diagnostics were studied in patients with stage III colon cancer 1999-2003 (n=265). Prognostic markers were evaluated along with the use of the lymph node ratio as a prognostic indicator to differentiate the risk assessment within the stage group. In paper III, single nucleotide pair (SNP) gene analyses was made for the metylenetetrahydrofolate reduktase (MTHFR) gene polymorphism C677T in patients treated for colorectal cancer 1999-2006 (n=544). The functional polymorphisms were then correlated to pathology, stage, outcome and side effects of chemotherapy. Comparisons of genotype prevalence were made against a cohort of 299 blood donors as well as the pathology data of the other 1256 patients treated during this period. In paper IV, the presence of cyclin E in both tumour and mucosa was studied in 114 patients with stage I/II colon cancer treated 2003-2007. The expression was analyzed in both tumour and adjacent mucosa and the results were correlated to pathology, staging and prognosis.

Results: In paper I, an improved lymph node assessment was shown to lead to stage migration and thus an increase of patients with stage III disease. A highly variable outcome in stage II associated to an inadequate assessment was also found. In paper II, stage III disease was found to have heterogeneous survival prognosis and the lymph node ratio was a significant marker for the outcome ($p < 0.001$). In paper III, no correlations between polymorphism genotype and the risk of cancer or cancer stage were found. There was a significant correlation to the risk of suffering side-effects ($p < 0,05$) and to the outcome in stage III colon cancer ($p < 0,003$). In paper IV, cyclin E was found to be expressed in both full length form and shorter isoform in both tumour and adjacent mucosa. A high total expression of cyclin E correlated significantly to the risk of tumour recurrence ($p < 0,0063$).

Conclusion: The lymph node assessment is a key factor in CRC pathology and of importance for both clinics and research. Additional prognostic information can be gained in stage III colon cancer by use of the lymph node ratio. The function of the folic acid metabolism can affect the risks associated with 5-fluorouracil treatment and also the outcome in stage III colon cancer. Cyclin E is expressed in both tumour and mucosa and could be an independent prognostic factor in stage I/II colon cancer.

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List of abbreviations

AJCC	American Joint Committee on Cancer
ACF	Aberrant Crypt Foci
CEA	Carcinoembryonic Antigen
CIN	Chromosome Instability
CK	Cytokeratin
CRC	Colorectal Cancer
CRM	Circumferential Resection Margin
CSS	Cancer-specific Survival
CT	Computer Tomography
DFS	Disease Free Survival
FAP	Familial Adenomatous Polyposis
FLV	5-Fluorouracil and Leucovorin
GCP	Good Clinical Practise
GLP	Good Laboratory Practise
HNPCC	Hereditary Non Polyposis Colorectal Cancer
IHC	Immunohistochemistry
LMW	Low Molecular Weight
LNR	Lymph Node Ratio
MDT	Multidisciplinary Team
MRI	Magnetic Resonance Imaging
MSI	Microsatellite Instability
MTHFR	Methylenetetrahydrofolate reductase
OS	Overall Survival
PET	Positron Emission Tomography
PFS	Progression Free Survival
PCR	Polymerase Chain Reaction
PS	Performance Status
SNP	Single Nucleotide Polymorphism
TEM	Transanal Endoscopic Microsurgery
TME	Total Mesorectal Excision
TS	Thymidylate Synthase
TTP	Time to Tumour Progression
UICC	Union Internationale Contre le Cancer

Introduction

Aim

The aim of this thesis was to study clinical and pathological factors related to the outcome of patients with colorectal cancer (CRC). The first specific aim was to explore how the clinical situation could be influenced by the pathology quality standards and their implementation. The aim in the second paper was to explore the possibility of finding more prognostic information for the risk assessment, within the limits of the TNM system for classification of malignant tumours, for patients of stage III disease. The third aim was to evaluate the possible role of a functional folate-associated gene polymorphism in relation to both pathology and treatment in CRC. Lastly, we wanted to explore if the expression of a cell regulatory protein in both tumour and mucosa related to pathology and outcome in early colon cancer.

Definitions

Cancer (medical term: malignant neoplasm) is a class of diseases in which a group of cells display the traits of uncontrolled growth (growth and division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood). These three malignant properties of cancers differentiate them from benign tumours, which are self-limited and do not invade or metastasize. Further, the subject of this thesis is CRC, where the rectum is defined as the distal 15 cm of bowel, measured from the anal verge. The colon is located from the terminal ileum and the ileocecal valve to the beginning of the rectum [1]. The cancers of the anus, which often emanates from squamous epithelia, are a separate entity and will not be further discussed in this thesis.

History

Today, the Greek term carcinoma is the medical term for a malignant tumour derived from epithelial cells. It was Celsus who translated carcinos into the Latin word cancer, also meaning crab. Galen used "oncos" to describe all tumours, the root for the modern word oncology. The name comes from the appearance of the cut surface of a solid malignant tumour, showing the veins stretched on all sides just as the feet of a crab. He later added the suffix -oma, Greek for swelling, giving the name carcinoma. Treatment was then based on the humor theory of four bodily fluids (black and yellow bile, blood, and phlegm). According to the patient's humor, treatment consisted of dietary restrictions, blood-letting, and/or laxatives. Through the centuries it was discovered that cancer could occur

anywhere in the body, but humor-theory based treatment remained popular until the 19th century with the discovery of cells.

Our oldest description of surgical treatment of cancer was discovered in Egypt and dates back to approximately 1600 B.C. The Papyrus describes eight cases of ulcers of the breast that were treated by cauterization, with a tool called "the fire drill." The writing says about the disease, "There is no treatment." Another very early surgical treatment for cancer was described in the 1020s by Avicenna in The Canon of Medicine. He stated that the excision should be radical and that all diseased tissue should be removed, which included the use of amputation or the removal of veins running in the direction of the tumour.

With the widespread use of the microscope in the 18th century, it was discovered that the 'cancer poison' spread from the primary tumour through the lymph nodes to other sites. This view of the disease was first formulated by the English surgeon Campbell De Morgan. There was also parallel evolution of surgical understanding. An early written example is by baron Larey in the Napoleonic era, who formulated details on how to suture a bowel. The use of surgery had poor results due to problems with hygiene and the abdominal region was even worse. The renowned Scottish surgeon Alexander Monro saw only two breast tumour patients out of 60 surviving surgery for two years. In the 19th century, asepsis improved surgical hygiene and as the survival statistics went up, surgical removal of the tumour became the primary treatment for cancer. We shall also acknowledge the important progress made in other medical areas such as anaesthesiology, radiology and the development of antibiotics. Without their evolution we would not have the colorectal cancer surgery of today.

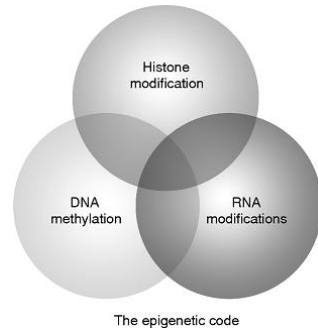
Carcinogenesis

Cancer is, ultimately, a disease of genes. In order for cells to start dividing uncontrollably, genes which regulate cell growth must be damaged. Proto-oncogenes are genes which promote cell growth and mitosis, while tumour suppressor genes discourage cell growth, or temporarily halt cell division to carry out DNA repair. Typically, a series of several mutations in these genes are required before a normal cell transforms into a cancer cell [2]. A mutation limited to one oncogene would be suppressed by normal mitosis control and tumour suppressor genes as suggested in the Knudson two-hit hypothesis. Also the microenvironment can affect the neoplasms as the cells compete for space and resources in a form of Darwinian clonal evolution [3]. A special interest is in the environment at the border of the tumour or its near surroundings [4].

Yet another factor to consider is the regulation of the genetic expression called epigenetics [5, 6]. By acting through the intra-cellular processes of histone

modification, DNA methylation and RNA modifications the expression and function of the genome can be altered.

The loss of genomic stability appears to be a key molecular and a pathogenetical step that occurs early in the carcinogenesis process[7]. At least three forms of genomic instability have been identified in colon cancer: microsatellite instability (MSI), chromosome instability (CIN, i.e. aneusomy, gains and losses of chromosomal regions), and chromosomal translocations. MSI occurs in approximately 15% of colon cancers and results from inactivation of the mutation mismatch repair (MMR) system by either MMR gene mutations or hypermethylation of the MLH1 promoter. This instability, often referred to as high-frequency MSI (MSI-H), is caused by defects of the mismatch repair system, which is involved in repairing DNA errors that arise during DNA replication. MSI promotes tumourigenesis through generating mutations in target genes that can possess coding microsatellite repeats or disturb the expression by a frame-shift mechanism. CIN is found in the majority of colon cancers and leads to a different pattern of gene alterations that contribute to tumour formation. CIN appears to result primarily from deregulation of the DNA replication checkpoints and mitotic-spindle checkpoints.



A rather new theory is the cancer stem cell paradigm that proposes some or all cancers to arise from transformation of adult stem cells [8]. These cells persist as a subcomponent of the tumour and retain key stem cell properties. Furthermore, the relapse of cancer and the emergence of metastasis are also attributed to these cells. The cancer stem cell hypothesis does not contradict earlier concepts of carcinogenesis. It simply points to adult stem cells as the site where the process begins. It is impossible to tell the initial cause for the specific cancer. However, with the help of bio-molecular techniques, it is possible to characterize the mutations or chromosomal aberrations within a tumour. For CRC the hypothesis is that the tumour development starts in the crypts of the bowel mucosa. In the aberrant crypt foci (ACF) there are morphologic and genetic abnormalities when compared to the normal tissue. Several of the genetic disorders later seen in the adenomas and cancers can be detected in the ACF. The molecular characterizations have led to theories proposing the heterogeneity of CRC into several different entities, each with specific features[9, 10]. Although the Vogelstein model of the adenoma-carcinoma sequence still is considered valid, it has been challenged and does not serve as a full explanation to the development of bowel carcinomas.

Incidence and risk factors

Colorectal cancer is one of the most common forms of neoplastic diseases in Sweden as well as in the rest of the Western World. In Sweden there are approximately 3500 new cases of colon cancer and almost 2000 of rectal cancer annually [11]. There is also some evidence that the incidence is increasing in Sweden [12]. Although the cause of CRC is unknown there are factors that can affect the risk. The role of some, such as smoking and diet, are not yet established. Recent suggestions include those of dietary fibre content and the nitrous oxide in red meat. The main known risk factors for developing CRC are listed below.

- Age: The risk of developing colorectal cancer increases with age. The median age for CRC diagnosis is around 70 years, while cases before age 50 are uncommon unless a family history of early colon cancer is present.
- Polyps of the colon: Particularly adenomatous polyps are considered a risk factor for colon cancer. The removal of colon polyps at the time of colonoscopy can reduce the subsequent risk of colon cancer.
- History of cancer: Individuals who have previously been diagnosed and treated for colon cancer are at risk for developing colon cancer in the future. Women who have had cancer of the ovary, uterus, or breast are at higher risk of developing colorectal cancer.
- Heredity: Family history of colon cancer, especially in a close relative before the age of 55 or multiple relatives, Familial adenomatous polyposis (FAP) carries a near 100% risk of developing colorectal cancer by the age of 40 if untreated. Hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome are also strongly associated to a risk of CRC.
- Inflammation: a chronic inflammation in the bowel mucosa is associated to an increased risk of CRC. An example is a long-standing ulcerative colitis or Crohn's disease of the colon and especially if the entire colon is involved.

Clinical picture

CRC can often be asymptomatic, especially in the early stages of the disease. When symptoms appear they can vary with the cancer's size and the location in the intestine. Common signs of CRC include changes in the bowel habits, including diarrhoea or constipation, and anaemia. Among the other symptoms are fatigue, weakness and unexplained weight loss as well as persistent abdominal discomfort or bleeding. In some instances the cancer manifests with acute symptoms and then presents with perforations, fistulas or complete bowel obstruction. It then requires a handling for the emergency situation that can be different from the normal elective treatment routines.

Diagnostics

A clinical consciousness and a readiness to act on suspicious findings in patient history or tests are needed to find the CRC. Quite often anaemia is the only initial lead and then in several instances associated with fatigue. The work-up is commenced by taking the patient history, including heredity, followed by a physical exam including a digital rectal exam. A faecal occult blood test can confirm GI bleeding. The bowel can then be examined by barium enema x-rays or colonoscopy. An advantage of the latter is the possibility of taking biopsies or making smaller interventions. Whilst virtual colonoscopy is being developed we should not forget the simplicity of the rectoscopy, which also is a necessity to measure the level and height of the tumour in rectal cancer.

After the diagnosis additional investigations are made preoperatively for the purpose of cancer staging and operation planning. Routinely we examine the lungs, by x-ray or CT, and the liver, by usg or CT/MRI. For rectal cancer the MRI is used for the pelvis assessment, sometimes also with the endorectal ultrasonography. The abdominal CT is now also increasingly used to identify possible advanced local growth preoperatively. When there are uncertainties about the properties of detected lesions, the PET/CT-PET modality can be used for further clarification. To ensure optimal possibilities of a successful cancer treatment, an assessment of the patients' general condition is also done including optimization of other coexisting conditions.

Histology

It is often common practice to take a tissue biopsy during the endoscopy. The histopathology does not only reveal the cell origin of the neoplasm but also the differentiation grade. The cell type can in itself affect which treatment and follow-up that will be most appropriate. More than 95% of CRC are a typed as adenocarcinomas [13]. The adenocarcinomas emanates from cells that form glands (adeno) that make mucus to lubricate the inside of the colon and rectum. Other, less common types of tumours may also develop in the colon and rectum. These include: carcinoid tumours, which develop from specialized hormone-producing cells of the intestine, and gastrointestinal stromal tumours (GISTs) which derive from specialized cells in the wall of the colon called the "interstitial cells of Cajal." Among other rare tumours found are cancers of immune system cells, lymphomas, and melanomas. Many of these uncommon tumours have a different treatment algorithm and will not be further discussed in this thesis. It is also important to define the anal carcinomas originating from squamous epithelia since they are treated in a different manner and respond well to radiotherapy. Thus it is especially important to verify the cell origin for the

rectal tumours before starting treatment as it often includes preoperative radiotherapy.

Biopsies can also be taken from metastasis or tumours of unknown origin. A malignant lesion in the liver is statistically most likely to be a metastasis from the gastrointestinal tract. The origin should be found before starting the treatment. The analysis of the biopsy can be an aid. However, the microscopic differentiation of metastatic colorectal adenocarcinoma from those arising from other sites can be challenging. Other tests like IHC of cytokeratins (CK) can then provide additional information. Two examples of tumour with similar appearance but with different profiles of CK 7 and CK 20 are endometrioid-type carcinomas and pulmonary adenocarcinoma [14].

Treatment modalities

Surgery is the common treatment for most stages of CRC. In cases where the cancer is found before the development of metastasis, a surgical removal of the tumour can lead to a cure. Details of the CRC surgery are further described below. A special form of treatment is the prophylactic surgery which can be used in certain cases of FAP, HNPCC (Lynch syndrome) and strong hereditary factors. These conditions are associated with very high risks of cancer and for optimal planning and outcome they should be handled by selected surgeons in cooperation with clinical geneticists.

Chemotherapy utilizes cytotoxic agents to target dividing cells. The intention is to treat cancer cells outside of the area of surgical excision. The cornerstone in CRC is 5-Fluorouracil (5-FU), discovered 50 years ago. 5-FU is usually combined with Leucovorin into what is known as Nordic FLV. In more recent time it is frequently combined with Oxaliplatin (FOLFOX) or Irinotecan (FOLFIRI). There are per oral regimes but normally the chemotherapy is given as infusion and then often in treatment cycles. As the agents target all dividing cells there are also risks of adverse effects. Common side effects include nausea, vomiting and fatigue. Blood disorders like leucopenia can lead to secondary infections. The degree of side effects can be severe and can cause both morbidity and mortality. Monoclonal antibodies for targeting cancer cells or the vascular components needed for tumour growth is a more recent addition. Although not cytotoxic agents in themselves, they are normally given as a treatment regime combined with chemotherapy. Their use is still mainly in the palliative regime spectrum.

Radiotherapy is mainly employed for rectal cancer. In Sweden we use a preoperative short course treatment with 5x5 Gray, meaning 5 Gray locally applied each day during the week before the operation. The intention is to reduce the risks of a local recurrence. A more long-term radiotherapy is used in case of locally advanced growth such as a T4 tumour. The therapy is then conducted for at least one month (2x25 Gray) and often combined with chemotherapy. The idea is to shrink the lesion, either as palliation or to facilitate a later surgical resection. The risks with radiotherapy include fatigue and collateral damage to adjacent organs.

Surgery for Colon Cancer

The main aim in performing surgery is a curative and radical removal of the cancer [15]. Occasionally, the cancer may be limited to a portion of a polyp. The patients can then be cured by endoscopy polyp removal alone. In most instances the surgery is more extensive and involves removal of the segment of the colon that contains the tumour as well as supplying vessels and the regional lymph nodes. The extent of bowel resection is often governed by the involved vessels and the placing of the ligatures. A common principle is to remove one vascular arcade both proximal and distal to the tumour. It will improve the chances of removing any affected lymph nodes and also having them assessed by the pathologist. Regarding the bowel length it is normally preferred to get a resection margin of 5-10 cm at each end. In concordance with the evolution of the TME techniques for rectal surgery, the same manner of dissection along the embryonic planes is also used in colon surgery. It aids in minimizing blood loss and also for the radicality of the procedure.

In case of a locally advanced tumour growth any adjacent organs can be involved. Examples are the overgrowth from the left flexure into the spleen or from the sigmoid into the bladder. An en-bloc removal is indicated if it can lead to a radical tumour removal, sometimes in after neoadjuvant therapy. The procedure can be performed in cooperation with other specialities. The individualized actual limits should strive for curative surgery but should be balanced by the risk of complication and morbidity due to host factors and the possibilities of healing. A trend in colonic surgery is to make larger resections. It could be beneficial and does increase the removal of nodes and vessels. However, the clinical benefit is contested and the gain remains to be proven. In current practise most colon resections are done by laparotomy. The use of laparoscopy has been proven to be feasible and oncologically safe and it is now being more widely used.

Surgery for Rectal Cancer

The surgery for rectal cancer is often more complex than for colon cancer. Whilst the intentions are the same there are anatomical and physiological differences. The location in the pelvis and proximity to sensitive structures, like nerve bundles, has specific demands. The surgical resection margins are not only at the ends of the bowel specimen but also the circumferential margin is of importance. The aim of radical surgery is balanced by the aim to retain a good quality of life. The sensory and executive rectal functions are impaired by the surgery. However, the risks of damaging the nerves that are involved in sexual and urinary function should be minimized.

In the last decades the implementation of total mesorectal excision (TME) surgery has decreased the risks of local recurrence together with the preoperative radiotherapy [16]. The rationale behind the TME concept is to follow the embryonic layers which contain structures like blood vessels and often limit the cancer processes. An important parameter in rectal cancer is tumour height, meaning the distance from the anal verge to the lower neoplastic limit. Normally, a distal resection margin of at least two cm is desirable. Inter-sphincteric dissections and very low anastomosis are feasible but has not yet given desired functional results. Therefore, for low rectal cancers at 6-7 cm height, it is common in Sweden to perform an abdominoperineal resection. The procedure is often named an amputation as the low placement leaves no margin for anastomosis and thus requires a terminal colostomy.

For the mid-range tumours, at 8-13 cm height, the TME anterior resection is the standard. There is a risk of poor healing and leakage for a low anastomosis and thus the patient usually gets a temporary diverting stoma. The very high tumours bordering to the rectosigmoid colon can often be treated with the anterior TME technique but with a possibility of limiting the dissection of the distal rectum. It can then mean a better functional result and with less trouble in anastomosis healing a temporary stoma is not considered mandatory.

A possible option for small and early cancers with no or very limited risk of lymph node involvement is the transanal endoscopic microsurgery (TEM) procedure. TEM is a modality of minimal invasive surgery which can be defined as laparoscopy through the anus. The total surgical trauma is small in comparison to an anterior resection and the recovery is quicker. Therefore the procedure is feasible also for the patients with more co-morbidity. There are some studies of a non-operative treatment of rectal cancer where a combination of radiotherapy and chemotherapy is used [17]. It means less to heal but also the drawback from a lack of pathology information and the impaired post-radiation anorectal function.

Preoperative assessment

After establishing the cancer diagnosis, the further work-up is directed at a preoperative cancer staging. It is aimed at finding or excluding any metastasis and also at identifying possible locally advanced disease. The findings from the preoperative work-up are normally discussed in a multidisciplinary treatment (MDT) conference. The conference is an important tool for communication and cooperation with other specialities including oncologists and other specialized surgeons. The presence of metastasis means an advanced stage of disease and does often radically change the treatment strategy. The patient data are evaluated and a decision is made if a curative approach is possible and if a neoadjuvant treatment can improve the chances of successful surgery. Whilst the use neoadjuvant therapy is at an experimental stage in colon cancer, radiotherapy is frequently being used for rectal cancer.

Postoperative treatment and follow-up

After the completion of the intended surgery and the pathologists assessment of the specimen new data will be available [18]. The analysis of this data is the foundation for cancer staging and thus also for the decision on further treatment. This is usually again discussed in a MDT setting. As discussed in paper I the participation of the pathologists is here of importance. Many patients will be free of all cancer following surgery and thus cured. However, in some patients, there can be residual microscopic tumour cells that were not detectable before or during surgery. As a result, many patients with stage III disease, the cancer has spread to the lymph nodes, will receive chemotherapy in addition to surgery [19]. Such "adjuvant" therapy increases the chances for a complete cure by destroying microscopic accumulations of cancer cells before they have an opportunity to grow to larger tumours. The effect of the treatment at a group level is documented to improve survival by 15-20% [20]. Whilst the stage III patients are the foremost recipients of adjuvant chemotherapy it is also considered in some high-risk cases. It is often suggested in case of locally advanced tumours or if the lymph node assessment was inadequate. Since there is no postoperative growth to be seen the adjuvant treatment lines can be difficult to monitor and evaluate. The balance between a beneficial effect and the eventual suffering through side-effects is then important to assess. Some aspects of the risk evaluation within stage III is discussed in paper II.

The adjuvant treatment is normally continued for at least 6 months [1]. The patient is monitored by the oncologists and checked for possible recurrence with CT scans. There is no solid evidence of how the further follow-up should be conducted. Therefore the later follow-up for these patients, as well as for those who did not need adjuvant chemotherapy, can vary with region and tradition.

The algorithms for follow-up normally contain some liver exams to find or exclude metastasis. Blood samples and use of markers, as CEA, has been suggested but their actual use is contested. Also suggested is a new bowel exam at some point to find any meta-chronous cancer. The question about the follow-up has become more important since the outcome has improved during the last decades.

Treatment of advanced disease

The term adjuvant treatment is used when there is no remaining visible tumour lesion to target after the surgery. If there is, then the term first line chemotherapy treatment is used instead. If the first line has to be abandoned, due to disease progression or adverse effects, it can be followed by the second and third etc. The terminology is important since it reflects on the intention and thus how the therapy is evaluated and the results. The first line treatment is often associated with a palliative strategy and often indicated in metastatic disease. The chemotherapy is often the cornerstone of the palliative treatment. It is monitored by radiological response and clinical tolerability. The role of surgery is more directed at relieving obstruction or preventing profuse blood-loss than being a chance for a cure. In some instances the problems can be solved without surgery. An example is when relieving an obstruction by the use of stents.

All patient care should be discussed in a MDT setting when planning the strategy of treatment. In some selected cases there can be an option of trying for a curative strategy even in stage IV disease. A careful assessment of all available clinical data must be done, not to overestimate the potential of cure and thus changing the risk-benefit balance. The plans should include preoperative treatment, timing and aim of surgery and the possibilities of metastasis removal. Liver metastases are the most common manifestation of visible metastatic disease in patients with CRC. About 15-20 % of patients have liver metastases at the time of diagnosis. Also, up to 50 % of the patients in stage III disease will later develop liver metastases. Some of these patients can be treated by surgically removing a part of the liver. Other equivalent options include cryosurgery and radio-frequency ablation. Surgery may also be done to remove metastases in the lungs or for local recurrences.

Prognosis

It is the best thing, in my opinion, for the physician to apply himself to the art of foreknowing.

-Hippocrates

Background

History

Since ancient times, sick people have in many cultures been preoccupied by their prospects for recovery. In early history it was often a question of using magic and omens to foretell the outcome. In an evolutionary process the understanding of the human physiology along with illness and disease has developed throughout the centuries. The Semiotic prognostics, which are based on clinical findings and still in practice, can be traced back through the civilizations to the Sumerian culture 2000 B.C. One of the best known references is to Hippocrates, who defined and translated prognosis to the art of foreknowing.

Though recognizing complex patterns in prognostic purpose they did go straight from symptoms to prognosis rather than through diagnostics as in modern medicine. As the art of medicine has evolved two other cornerstones in medicine has strengthened, namely diagnosis and treatment. Still, whilst the diagnosis is an abstract notion of the disease that provides a guide for treatment, the prognoses describes the probable course of the illness. This also responds to the patients need and desire for information about the future. This fundamental search and desire for additional knowledge of what is about to happen have remained rather untouched by scientific progress.

The reason for prognosis

A prognosis in medicine has been widely defined as “a reasoned forecast concerning the course, pattern, progression, duration and the end of the disease”. It is also a dynamic process that changes as events unfold; meaning that new information often affects the prognosis. Since this thesis is written on CRC the prognostic process discussed is focused on the field of oncology even if some of the discussion can be applied to a more general setting. The prognostics are often used almost synonymously with survival in oncology. This is appropriate at some cancer stages whereas in advanced disease other points as quality of life or function could be better parameters. There are many different reasons to why

the prognostic information is important. It is good to be aware of some of these perspectives as they in some way often can affect the clinical practise and implementation. Some of the reasons are listed below and it should be remembered that they also often are interrelated.

- Personal decision-making. The most important question for many cancer patients is that of their chance of cure. Simultaneously, they need the information to make decisions about other aspects of their lives including a wide spectrum of vital things ranging from economy to travelling and housing.

- Medical decision-making. The clinical settings and treatment recommendations are often founded on prognostics. Knowledge of which patients that can benefit from a specific therapy is important and for more risky or side-effect heavy ones it can be crucial. This applies also to the planning of follow-up and the eventual adjuvant therapies.

- Medico-legal perspectives. As of today, the modern medical ethics stress the patients' right to self-determination and thus an increasing involvement in their choice of therapy. A prerequisite is good information on what is about to happen concerning risks and possible benefits of possible treatments.

- Health policy perspectives. This aspect includes optimizing the use of resources within the healthcare system. Whilst expensive treatment in desolate cases should be questioned as a possible economic waste, the potential cure could in the end save both money and suffering. As the resources in many economic systems are limited it is often a question of a fair allocation decision where solid prognostic data can be an aid.

- Research and cooperation. A uniform classification system facilitates the possibility of comparing the results and exploits of different research centres and countries. It also makes the cooperation easier in both research and clinical practise.

Definitions

Whilst there are several words in the English language indicating and directing at probabilities in the future, some of the commonly used can be defined along with their meaning in medical science. A risk factor is a clearly defined occurrence or characteristic that has been associated with an increased rate of a subsequently occurring disease. In contrast, a prognostic factor refers to a probability of future events in patients who currently have a disease. It then provides information about the patients overall cancer outcome, regardless of therapy. The information is usually directed at a group or population level. The

presence or absence of such a prognostic marker can be useful for the selection of patients for a certain treatment, but does not predict the response to this treatment. The prognostic factors should in some instances be distinguished from those of predictive value. The predictive factors give information on the effect of a therapeutic intervention in a patient. In other words the prognosis for a measurable response to a given or intended specific therapy. In contrast to the prognostic factors the predictive ones are by necessity directed at the individual level. Some examples from this thesis would be the prognostic interests of the lymph node assessment vs. the possible predictive value of the MTHFR polymorphism C677T for the chemotherapy. In some instances a factor can serve both of these functions, or either one depending on the clinical situation. However, to facilitate discussions and proper evaluation of research advances in this field it is important to develop the use of a functioning terminology.

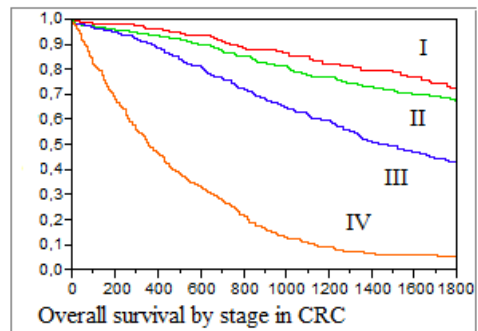
What makes a factor?

According to a NIH Consensus Conference, a clinically useful prognostic factor must be a proven independent, significant factor, which is easy to determine and interpret and which has therapeutic consequences. There are several obstacles to override before establishing a new factor that fits this pure definition. One is the question of independence as most of the known and used factors actually interrelate in some way. The independence could also be interpreted that it should carry information that either is new or facilitates clinical practice. Significance statistically is often easier to attain but for consolidation of the factor it should be verified in more and larger studies, on less selected patient populations and so on. Next, the question of analysing and determining the marker must be addressed. This can include subtle weaknesses and difficulties. A good example is the lymph node assessment which in the end is susceptible to the focus of the assistant making the preparation of the specimen. Standards are also set by the concepts of good clinical and laboratory practice (GCP/GLP). The definition ends with a statement about demanding therapeutic consequences, meaning the knowledge of this marker should in some way affect the line of treatment. As the modern world grows more complex we do rely less on single factors and rather lean on multi-factorial algorithms. This does not lower the requested standards in searching for and proposing new factors. It rather makes it more difficult as the basic known parameters should, in most cases, be a foundation for further analyses. As discussed in paper I, there are difficulties and obstacles in performing a proper staging process. An inadequate staging can then later lead to difficulties in evaluating markers in such a clinical material. There are innumerable possible factors that have been suggested for use in CRC. Unfortunately it is not possible to relate them all in this thesis, instead a selection of the most important or widely used are presented.

Measuring outcome

The end-point parameters should be defined when discussing prognostics and outcome data. As different treatments can be directed at various patient groups there can be a need for evaluation by their own specific parameters not to miss potential benefits. Some end-points are obvious as for example to relate hernia surgery to recurrence rather than survival. A parallel in CRC is the risk of local recurrence in rectal cancer which can be seen as a surgical quality marker and often does correlate to survival. Also to be considered are the possible surrogate markers as they in themselves are strongly associated to the specific requested event. Examples of surrogate markers include the local recurrences, as mentioned above, and the intra-operative blood loss. The most commonly used terms are described below.

- Overall survival (OS) is a term that denotes the chances of staying alive for a group of individuals suffering from any disorder such as a cancer. It denotes the percentage of individuals in the group who are likely to be alive after a particular duration of time. At a basic level, the overall survival is representative of cure rates.



- Cancer-specific survival (CSS) is the probability of surviving the cancer, and not considering other causes of death. It is a measure that is not influenced by changes in mortality from other causes. It is of importance also since it then also takes the age of the patient into consideration. Important is also the validity in the registration of the cause of death. It can be affected by environment factors like the autopsy rates and the willingness to make extensive investigations among the actual population. The difference to OS will also be stage dependant as the risk of a cancer death increases by stage.

- Disease Free Survival (DFS) is usually used to analyze the results of the treatment for the localized disease which renders the patient apparently disease free, such as surgery or surgery plus adjuvant therapy. In the DFS, the event is relapse rather than death. The patients who relapse are surviving but are no longer disease-free. Because the patients survive for at least some time after the relapse and the ensuing therapy, the curve for the actual survival will look better than DFS curve. It has been shown that the 3-year DFS correlates well to the 5-year CSS and DFS is thus a functional surrogate survival parameter[21, 22]. An advantage, though mainly limited to stage III disease, is the shorter observation time needed for evaluation of treatment and studies.

- The Progression Free Survival (PFS) is usually used when analyzing the results of the treatment for the advanced disease. The end-point for the PFS is that the disease gets worse or progresses. Sometimes the associated term response duration is used. This endpoint involves selecting a subgroup of the patients. It measures the length of the response in those patients who responded. Another associated parameter is the Time to Tumour Progression (TTP) which denotes the time in days until the event of clinical or radiological findings of progressive disease and thus is equivalent to PFS.

There are some occasions where the survival-related parameters could be inappropriate. Other means to evaluate the possible beneficial effects of a treatment can then include functional scores and quality of life (QoL) assessments [23]. One example in CRC is when an elder is diagnosed with a rectal tumour. If the patient is healthy, the treatment is along normal routines but when hampered by age and co morbidity other options could be considered. In this case a TEM procedure could be more appropriate even for a T2 or T3 since it could reduce the local symptoms without the risks of an anterior resection. In such a setting it is also doubtful if the finding of a positive lymph node would change either treatment or outcome. Any advantage with such an approach would not be seen when assessing by survival alone.

The nature of the survival parameters makes them best suited for the prognostic setting. In the evaluation of predictive factors there is more of a focus on the risk for the individual. The risk could be expressed either in percentages or as relative risks or odds ratios. The ratios could then also be calculated not only for the risk of, for example, the adverse effect of nausea but also the risk of the event being of a greater severity grade. For the patient it is potentially more acceptable with a high risk of a low grade side effect than the opposite.

Prognostic factors

Cancer pathology and the anatomic extent of the disease are well associated to the possibilities in outcome [24, 25]. Thus, the prognosis is quite often equated with the tumour characteristics. However, there are several other factors that can also affect the course of the disease and the outcome. As cancer is a disease which develops over time, there are usually a number of occasions where the prognostic factors are re-evaluated. A re-evaluation is often triggered by an event during follow-up like recurrent disease, new tumours or metastasis progression that requires a possible redirection in the line of therapy.

The concept of cancer staging is meant to facilitate the clinical practice through creating boundaries and ramifications for the specific disease. The aim is to acquire stage groups that correspond to disease severity, suggested levels and modalities of treatment and also toward prognosis. One of the first to be commonly used in CRC was created for rectal cancer by Dukes in 1932. He made a classification of the rectal cancers from A to C by local factors with the C reserved for regional metastasis. This system was later modified, by him and others, through the years. The stage D was added for advanced disease and it was extrapolated to incorporate colon cancers. One of the later versions was the Dukes-MAC meaning modified by Astler-Coller. During the same period Union Internationale Contre le Cancer (UICC), a cancer organization founded in 1948, started to support the development of another staging system. The aim was to incorporate more parameters into the algorithm and make it more sophisticated. The result was the TNM-system. Through the years it has been edited and revised and currently the 6th edition is used [26]. By cooperation between the American Joint Committee on Cancer (AJCC) and the UICC the TNM-system is now worldwide standard.

Different factors account for various perspectives of the patient, the disease and the treatment. The factors can be categorized in several different ways. One possibility is to classify them by the different scale levels which they cover. The factors can then be liberally grouped into four categories. They range from the uttermost miniscule level of laboratory factors, through tumour and host related factors to the more strategic levels of environmental factors. At times the last three are referred to as clinical factors. A common denominator for the clinical factors is that they can be assessed by either clinical examination, radiology or through the microscope. This is then opposed by the laboratory factors that require more advanced techniques in their assessment.

1 Tumour-related factors

The pathology report

After the operation the removed specimen is subject to a thorough examination by the pathologists. They assess tumour anatomy as well as histology and differentiation grade. The procedures and their standards are regulated by the pathologists' guidelines, which in Sweden are referred to as KVASt documents, currently in version 3.1 [27]. The reporting itself has to some extent been standardized with beneficial results [28, 29]. The results are then compiled into the pathology report which is used in the multidisciplinary, postoperative staging process. In this chapter several of the factors that can be found in the report will

be described. The histology characteristics are normally included but have been previously discussed.

TNM-staging

The anatomical extent of the disease is the single most important prognostic factor of today [30]. Although most components can only be revealed postoperatively under the microscope, the staging begins at the time of diagnosis. As far as possible, the patients should have the clinical stage determined before any treatment commences. In CRC it means a preoperative use of radiology to identify the patients with advanced disease which can lead to an altered treatment strategy [31]. The staging is then continued intra-operatively by the surgeons' assessment and the possibility of gathering samples and specimens. It is then completed by the pathology report even though it later can be reassessed as the events unfold and new data becomes available.

The anatomically-based TNM classification uses the local, regional and distant extent of the cancer to describe the disease [25]. The pre-treatment extent of disease is determined clinically (cTNM), with information collected from clinical examinations, laboratory tests, radiological imaging and biopsy samples. Additional information obtained from surgical excision and pathological examination of the entire primary tumour allows for a detailed post surgical pathologic TNM classification (p TNM). The TNM system thus allows an integrated classification of two distinct systems, the clinical TNM and pathological TNM. Clinical TNM is used to determine the initial treatment strategy, while pathological TNM is used to determine the requirement for post-surgical adjuvant therapy and follow-up. The letter y denotes autopsy data. The three main components of the TNM system are described below together with some other features that at times are presented in the pathology report. The results of each component are also put together into an overall cancer stage. Although the components in many instances are discussed separately, the clinically used treatment algorithms are commonly built around the overall cancer stage.

A T – local tumour stage

The T category in CRC describes the extent of spread through the layers that form the wall of the colon and rectum. There can also be letters added to the description for a further sub-classification (example: T4b). This practise has mainly been used for research purpose rather than formally being incorporated into the staging system. The T4 sub-classification is described below. For T2-3 it is suggested to partition the layers into thirds (a-c) or quartiles (a-d) whilst the SM sub-grading is evaluated for the T1 tumours. The T stages are as follows:

T_x: No description possible due to incomplete information.

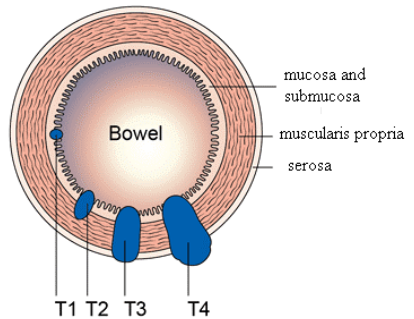
T_{is}: The cancer involves only the mucosa. It has not grown beyond the muscularis mucosa.

T₁: The cancer has grown through the muscularis mucosa and extends into the submucosa.

T₂: The cancer has grown through the submucosa and extends into the muscularis propria.

T₃: The cancer has grown through the muscularis propria and into the subserosa but not to any neighboring organs or tissues.

T₄: The cancer has grown through the wall of the colon or rectum and into nearby tissues or organs. T_{4a} means growth into other organ whereas T_{4b} is growth through the serosal layer.



B N – regional node stage

The N category indicate whether or not the cancer has spread to regional lymph nodes and, if so, how many lymph nodes that are involved. The location of the primary lesion affects which node stations are to be considered as regional and which are referred to as distant spread.

N_x: No description possible due to incomplete information.

N₀: No lymph node involvement is found.

N₁: Cancer is found in one to three regional lymph nodes.

N₂: Cancer is found in four or more regional lymph nodes.

C M – distant metastasis

The M category indicates whether or not the cancer has spread to distant organs, such as the liver, lungs, or distant lymph nodes. The metastasis data is often included into the pathology report but the information is normally acquired by the preoperative radiological exams. It can also be affected by intra-operative findings, gathering of samples and a later verification of the disseminated disease by the pathologist.

M_x: No description possible due to incomplete information.

M₀: No distant spread is detected.

M₁: Distant spread is present.

Differentiation grade

The differentiation grade is a description of how closely the cancer resembles normal colorectal tissue when looked at under a microscope [13]. In CRC where the adenocarcinomas are predominating, the pathologists consider the percentage of gland formation. The scale used for grading CRC goes from G1 (where the cancer looks much like normal colorectal tissue or >95% gland structure) to G4 (where the cancer looks very abnormal, less than 5% gland structure). The grades G2 and G3 fall somewhere in between (50-95 % and 5-50 % gland structure respectively). The grade is at times simplified to range from "low-grade/well differentiated" through medium to "high-grade/poor differentiation". The histology grade is rather well interconnected with the prognosis as it correlates to the tumour stage and node metastasis rates. In the case of mucinous or signet cell tumours, they are always considered as poorly differentiated. It is also always location in the tumour with the worst findings that will define the overall grade.

Status of the surgical margin

A prerequisite for a curative procedure is a radical removal of the tumour. The denomination is by the letter R in the report with R0 meaning complete and R1 incomplete removal. The examination of the resection margins includes both to the proximal and distal ends of the specimen. Also being examined is the circumferential resection margin (CRM) which is especially important in rectal cancer. A positive margin means that the tumour removal might be incomplete and thus a higher risk of a local recurrence [32-34].

Micrometastasis vs. isolated tumour cells

The sixth edition of the AJCC Staging Manual makes a clear distinction between micrometastasis and isolated tumor cells, and it recommends guidelines for their reporting. Patients whose lymph nodes contain isolated tumor cells (<0.2 mm in diameter) are classified as N0. In the absence of prognostic data, patients with nodal micrometastasis (0.2 to 2 mm) are classified as N1. The mere presence of cytokeratin-positive cells within a lymph node has no known prognostic significance at present. There are no current evidence that the isolated tumour cells affect the prognosis [35, 36].

Lymphatic and venous invasion

The presence of tumour growth into the small vessels is suggested to be of prognostic interest [37, 38]. It can be commented as L/V for lymphatic and

venous respectively and denominated by a 1 for microscopic or a 2 for the macroscopic tumour presence. There are currently no standardized definitions and thus the data on their possible role is difficult to assess. The T-staging is not affected and as N-stage only considers the involvement in the nodes, any proximal growth still only means a N0. The role of the invasion features in the staging and prognostics are yet unclear.

Lymph node location

The importance of the lymph node assessment is by now well known [39, 40]. As a consequence, there has often been an increase in the number of assessed lymph nodes. There is a potential risk of anatomically aberrant node metastasis but the main risk of positive findings should be along the main supplying vessels [41]. The data of these central nodes are at times being reported in a separate line. There is also evidence that the location itself could be of prognostic interest [42]. The overall size of the node as also been suggested to be of prognostic interest but the measurement is not in common practise [43].

Structural properties

There have been suggestions that the macroscopic and microscopic appearance of the tumour could reveal prognostic information. Tumour budding, defined as small clusters of undifferentiated cancer cells at invasive margins could reflect biologic aggressiveness of colorectal cancers. There are some evidence that it could affect the metastatic risks and properties [44]. It could be of prognostic importance but needs further study. Another similar property is the lymphocyte infiltration of the margin which also is suggested to affect the outcome [45]. The actual depth of the tumour invasion has been shown to have importance for the risk of lymph node metastasis [46]. The data has lead to the development of the “sm” sub-classification of the T1 tumours.

Cancer location

The survival prognosis can vary with the location of the tumour [47]. If the difference is related to bio-molecular features, growth patterns or treatments is not yet clear. However, with the development of rectal TME surgery the prognosis for rectal cancer is now better than for colon cancer. Colon tumours located on the left side carries a better prognosis than those on the right. In the material from our hospital, the best prognosis is when the cancer is located in the sigmoid colon. An explanation could also be that they possibly give symptoms earlier and more frequent and thus lead to an early diagnosis.

Stage grouping

Once a patient's T, N, and M categories have been determined, usually after surgery and completed pathology, the information is then combined in a process called stage grouping. The stage is expressed in Roman numerals from stage I to stage IV. It is the common way to present the overall stage and also the form used when incorporated into treatment algorithms. Even though the TNM-system is considered as the standard of today some older labels can still be seen. The stage groups and their correlation to previous staging systems are described in the table below.

UICC/AJCC	AJCC/TNM	TNM	Dukes	Astler-Coller
I	I	T1-2,N0,M0	A	A, B1
II	IIA	T3,N0,M0	B	B2
	IIIB	T4,N0,M0	B	B3
III	IIIA	T1-2,N1,M0	C	C1
	IIIB	T3-4,N1,M0	C	C2, C3
	IIIC	T1-4,N2,M0	C	C1, C2, C3
IV	IV	T1-4,N0-2,M1		D

2 Host-related factors

The host related factors are parameters affecting the prognosis by affecting the possibilities of treatment and how the therapy is tolerated. Included are general demographic factors as age and sometimes gender but also specific parameters such as performance status and co-morbidity. Some of them are difficult to estimate and classify and thus the impact on the treatment and outcome can be hard to assess. The patient's will, attitude and the therapeutic compliance are important in the clinical setting but also form good examples of factors that are difficult to evaluate. The socioeconomic status and educational levels are also suggested to affect the survival, possibly through differences in disease awareness, pattern of information seeking and in when to contact the health care system. Some of the most important host-related factors are commented below.

Age: The risk of developing CRC increases with age as does the risk of suffering from other coexisting diseases. As a prognostic factor, the age itself can be difficult to assess. An older patient is more likely to die, by any cause, in the coming 5 years than a young patient. When looking at the risk of dying from the cancer the equation is reversed and there is some evidence that young patients are more prone to have more aggressive disease. The age is at times associated with the prevalence of co-morbidity and can also affect treatment decisions like the use of adjuvant chemotherapy. However, it has also been shown that the elderly can be surgically successfully treated and now there are also reports showing that chemotherapy can be well tolerated in advanced age [48, 49].

Performance status (PS): The PS is an attempt to quantify the patients' general function and well-being. Among the systems used are the Karnofsky score, ranging from 100 (perfect health) to zero (death), and the Global Assessment of Function (GAF) from the DSM manuals. Most common is the Zubrod score which also is adopted by WHO. It ranges from 0 (asymptomatic), through 2 (ambulatory and capable of all self care but unable to carry out any work activities) to 5 (death). The PS is one of the strongest prognostic factors [50]. It often, but not always, co-varies with age and it affects several treatment decisions such as type of surgery and the use of chemotherapy. As it heavily affects the survival, it can be of importance to choose and assess other outcome parameters than survival to evaluate treatments for the patients with a poor PS score.

Emergency presentation: The tumours that presents with bleeding, perforation or obstruction in a manner that requires emergency surgery is associated to a worse prognosis [51]. The patient is often in a worse general condition and the risk of suffering complications is heavily increased. At the same time it is more common that the cancer has more aggressive properties. The risk of metastasis and is higher than in elective surgery and thus it is more common with worse overall stages. It is likely that there also are bio-molecular differences but their nature is yet unknown. Some measures taken to improve the prognosis include subspecialisation and the increased use of stents as a bridge to elective setting surgery.

3 Environment related factors

The environment related factors affect patient groups or mark regional differences rather than being on an individual level. They constitute a diversity of factors such as medical education and expertise, the health care system levels and their availability. Even the society levels of education and socioeconomics could affect the outcome. The awareness and knowledge in the society of the

disease can affect how people react to symptoms and thus the contacts with the health care system. The use of screening is also a possibility to raise awareness and promote early diagnosis and thus could have a chance to improve the level of outcome. It can be difficult to estimate and measure these kinds of variables as with the host-related factors. Thus, the individual impact of these factors on the outcome can be difficult to assess. Some important environment-related factors are commented below.

The hospital and its volume: There have been several studies trying to link the hospital volume to the patients' outcome but often without clear-cut evidence [52, 53]. However, here also other factors are brought into the equation. A larger centre often has better access to more resources and support in for example pathology service and intensive care. In many instances a larger hospital can also provide better opportunities for sub-specialization and team cooperation rather than the vulnerability of relying on single individuals. An example is the proposed outcome benefit for emergency colorectal surgery when performed by trained coloproctologists.

The pathology service: There is evidence showing that the survival prognosis is better for patients where more lymph nodes have been assessed [54]. A natural cause is the correlation to stage migration and stage specific survival [55]. Still, the example shows the importance of a good pathology service and the compliance to quality documents [56, 57]. The KVASt documents of the Swedish pathologists association has aided in improving the data quality and the consistency of the pathology reports [27]. The standards, fully compatible with the UICC recommendations, have also been implemented into other documents as national or regional treatment guidelines. The presence of the pathologist in the multidisciplinary team conferences has aided in implementing the knowledge and improving the standards.

The radiology service: The detection of metastasis is a key event both in the preoperative staging and during the postoperative follow-up. The availability of radiological equipment and interpretation skills of the images can affect the results of both staging and treatment. The metastases must also be of a certain size to be visible and thus creating a susceptibility to the technical level and development of the imaging machinery. The further progress could result in an earlier finding of metastasis and thus probably a stage migration into stage IV.

The surgeon and the operation: There has been attempts to link also the surgeons operative volume to the outcome but without clear findings [58]. The education and meticulous surgical technique of oncology treatment is of great importance [59]. It is well shown that the dissection should be performed in the embryonic layers and that the vessels should be divided at a proximal level.

Failure to accomplish this can decrease the chance to achieve radical surgery which is a necessity for a curative procedure. For the more locally advanced tumours the surgical preoperative planning is important since en-bloc removal should be attempted. There have also been early attempts to assess the surgeon and the complexity of the procedure as potential factors. Although the concept is interesting there are no scoring systems in current practice.

4 Laboratory factors

Whilst the environmental factors represent the highest or most strategic level the opposite role is taken by the laboratory factors, commonly labelled biomarkers. A biomarker is an objectively measurable or evaluable characteristic that serves as an indication of a biological or pharmacological process, or of a therapeutic intervention [60]. This is not always synonymous with being a prognostic factor. The common goal and intent for the use of biomarkers is to identify high-risk individuals, facilitate screening and early disease detection, as well as to identify new pathways in pathology for drug design and promoting individualized therapies [61]. Several of the laboratory factors have functions of a more predictive sort or even of both purposes. There are several difficulties in isolating true molecular factors. The obstacles include the multitude of covariates in the intrinsic systems and the complexity of the molecular mechanisms. Another way to phrase the challenge is that the multi-factorial pathways of the cancer disease are hard to summarize by a single parameter and then knowing what it really reflects.

There are also markers of a more general kind. They can assist in the overall assessment of the patient. An example is the serum levels of albumin, which can reflect on the nutritional status of the patient and can be affected by generalized or greatly symptomatic cancers [62]. The haemoglobin levels are through anaemia an important diagnostic indicator but have also been described to have prognostic value. Likewise the CRP has been described in a similar manner [63]. They have not been proven in a more general setting and not stage specific. It is more likely that they provide data on the patient's condition and possibly also can be associated to the tumours clinical effect and impact on the individual.

Science and techniques

As new possibilities of analysis have evolved so have the associated sciences, often referred to as different "-omics". The search and its practise have, at least in part, been linked to this development. Although each has a specific target of use they often interlink in the practise. Genomics is the study of genomes and the complete collection of genes that an organism contain. This also includes

important structures within in the genome, such as transcription factor binding domains, regions encoding microRNAs and antisense transcripts, and large, evolutionarily conserved regions. The functional genomics, also known as transcriptomics, attempts to analyze patterns of gene expression and to correlate the patterns with the underlying biology. There is a wide range of techniques used, including DNA microarray analysis and serial analysis of gene expression.

Metabolomics is a large-scale approach used to monitor as many as possible of the compounds involved in cellular processes in a single assay to derive metabolic profiles. The techniques applied to metabolic profiling include nuclear magnetic resonance and mass spectrometry. Proteomic approaches are used to examine the collection of proteins to determine how, when, and where they are expressed. Techniques used in this approach include two-dimensional gel electrophoresis, mass spectrometry, and protein microarrays. Bioinformatics, although not graced with the -omics suffix, remains a key element in collection, management, and analysis of large-scale data sets that are generated.

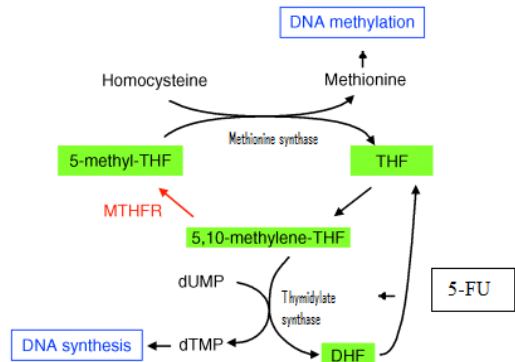
Used and possible factors

A commonly used prognostic factor is the carcinoembryonic antigen (CEA), which can be a marker of disease progression [64]. At some centres, it is used as standard in the postoperative follow-up even though the value of the CEA information has been contested. MSI can be tested and categorized into high or low frequency. There are evidence that the MSI degree could affect prognosis and also response to chemotherapy [65-68]. Mutations in mismatch repair genes are strongly associated with MSI and can also be of interest [69]. Other factors of suggested value are the loss of heterozygosity (LOH) and the associated change in DNA ploidy. An 18q LOH can lead to the loss of a suppressor gene and could thus potentially affect the tumour risk. Mutations in the p53 tumour suppressor genes are involved in the carcinogenesis and have been suggested to carry prognostic value [70, 71]. The MLH 1-6 genes are in some degree associated to the hereditary cancers and can thus be of interest. All these factors have been described and reported from several studies to carry some prognostic value. However, they have not yet reached the levels of evidence to have an established role in the clinical setting. There is also a parallel need to know how to use and implement the data as well as for which group it could be of importance.

While for example the 18q LOH affects a large part of a chromosome, the genetic changes can be more subtle. Single nucleotide polymorphisms (SNP) are small genetic alterations, affecting only a single nucleotide pair. Still, there can have impact on the function and expression of the gene. Such an alteration in a gene involved in DNA repair, cell cycle, invasion pathways or drug metabolism,

could therefore also affect both treatment and outcome. The method of choice for analysis is SNP analysis by RT-PCR. Potential polymorphisms of interest in CRC include those in the thymidylate synthase (TS) and MTHFR genes [72]. Their potential importance derives from their central role in the folic acid metabolism [73].

The folic acid is a vitamin and thus an important dietary component. Folate deficiencies have been associated to several entities such as congenital malformations and cardiovascular disease. There are suggestions of enrichments and supplementations of this acid. The figure provides the outlines of the folate metabolism.



Suboptimal function in the folate metabolism could affect several diverse matter such as the epigenetics through the supply of methyl groups, the function of 5-FU as a cytotoxic agent and possibly also the cell repair efficiency through supply of substrate. Even with folate substitution there could be areas of local depletion in the microenvironment. The possibly lower availability for the folate system could bring negative effects that could be further affected by the enzyme functional levels [74]. TS have been described in several reports as a potential prognostic factor. Still, its use and function in the clinical practise is yet unclear. Concerning the MTHFR, it is further discussed in paper III.

Predictive factors

Several prognostic factors have now been discussed. The prognostic factors should in some instances be distinguished from those of predictive value. As previously discussed, prognostic markers can be useful for the selection of patients for a certain treatment. The predictive factors could complement by giving information on the effect of a therapeutic intervention in a patient. It can also be expressed as the prognosis of a given or intended specific therapy. Whilst the prognostics factors are more oriented at a group or a population level, the predictive factors are more focused on the the individual.

The matter of predictive factors is quite new and undeveloped when compared to the prognostics. It is being developed parallel to the evolution of new analysis methodology and the increasing knowledge on the process of carcinogenesis. A

better possibility of prediction is a necessary step towards a fully individualized and tailored cancer treatment. The inter-individual variability in the efficacy and toxicity of drug therapy is associated with polymorphisms in genes encoding drug-metabolizing enzymes, transporters, or drug targets. At times the term pharmaco-genetics is used, meaning an aim to identify individuals predisposed to high risk of toxicity by determining the genetic makeup of the host. The information could aid the clinician in choosing the right drug at the right dose. It could be of importance since it has been shown that a failure to complete the adjuvant treatment is associated with a worse outcome [75].

The difficulties and obstacles in finding solid bio-molecular prognostic factors could to a large extent be applied to the research of predictive factors. Several factors have been suggested. One of the few markers that currently are being used for its predictive role is K-ras. Mutations in this gene, a Kirsten ras oncogene homolog from the mammalian ras gene family, can affect the clinical response for treatment with specific monoclonal antibodies such as cetuximab. The K-ras mutation is associated with resistance to cetuximab and a shorter survival of EGFR-positive metastatic CRC patients [76]. Thus, the KRAS mutations status might allow the identification of patients who are likely to benefit from cetuximab and avoidance of a costly and potentially toxic administration of this treatment in non-responder patients [77, 78].

Among the multitude of potential predictive markers, only two will be mentioned here. The first, dihydropyrimidine dehydrogenase (DPD), is the initial and rate-limiting enzyme in the catabolism of 5-FU, and it is suggested that patients with a partial deficiency of this enzyme are at risk for developing a severe 5-FU-associated toxicity. Patients with a partial DPD deficiency have been shown to carry an increased risk of developing grade IV neutropenia. In addition, the onset of toxicity occurs twice as fast compared with patients with a normal DPD activity. To date, 39 different mutations and polymorphisms have been identified in DPD. This knowledge can be of importance for future use and dosage of 5-FU [79, 80].

The second marker to be mentioned is thymidylate synthase (TS). This synthase is one of the targets of 5-FU treatment and as such of great interest. The place and function in the folate metabolism of TS has been described earlier. There are several reports on correlations between functional polymorphisms in the TS gene in both a prognostic and a predictive role [81-84]. A difficulty with TS is that there are several possible shifts and different polymorphisms. The heterogenic appearance makes it more difficult to assess and evaluate towards the clinical data. Therefore the use and application of TS analysis has not yet reached the clinical practise. To further complicate matters, the function of MTHFR and other bordering enzymes can affect the overall metabolic function

of folates and thus the results [85, 86]. That the function and status of the folate metabolism is of importance is difficult to dispute. There are interesting correlations to the 5-FU treatment, the substrate availability but also as it could also link to the epigenetics through the methylation processes [87]. More research is needed to understand the complex system of the entire integrated folate metabolism. An individualised assessment of the function of the folate metabolism would probably assist in both choice of regime and regulation of doses and intervals.

Translational research

The concept of translational research first became popular in the 1990s. The goal and intention was to transfer information gathered in the laboratory to the patients' bedside and, conversely, to integrate clinical knowledge into hypotheses to be tested experimentally in appropriate molecular models. The basic research at medical institutions plays a crucial role in this field of research, to create a flow of information from/to the laboratory to/from the patient bedside, and to foster the production of the next generation of medical scientist trainees. The use of multidisciplinary teams is becoming more and more employed in modern clinical practice. The possibility to utilize the specific knowledge and way of reasoning from several different professional fields can improve the overall results. The same principle can be applied to the research process. Creating teams constituted by clinicians, geneticists, statisticians and other specific competences can increase the chance of attaining interesting scientific achievements. The team cooperation, which is necessary for further individual specialization, will affect the research environment and thus also the training of future scientists. Defining translational research is still a complex task. In oncology, translational research implies using basic knowledge learnt from in vitro and in vivo experiments to directly improve diagnostic tools and therapeutic approaches in cancer patients.

Materials & methods

The database

With the restructuring of the hospitals in Gothenburg in 1997, the main part of the colorectal surgery was concentrated to Sahlgrenska University Hospital/Östra (SU/Östra). At the same time the demand for clinical data in oncology research became apparent. There was a need to keep records of clinical data and a solid follow-up to be able to assess treatments. A local CRC registry was started which developed into a clinical database. To begin with the registration was sporadic, but from 1999 and on the inclusion has been consecutive and rather complete. Added to the clinical and treatment information are pathology and follow-up data as well as biological samples from tumour, mucosa and blood.

Validation and weaknesses

To be functional, a database needs to be continuously updated and regularly validated. A record is kept of how the registrations should be made to keep them consistent over time. The database used has been validated in several ways. A control has been made against the regional Centre of oncology for the number of registered cancer patients. Controls have also been made against the hospitals registrations of diagnosis and surgical procedures. In some instances, extra controls have been made of randomly selected patient files to double-check data. The reliability and completeness of the data for the patients treated at SU/Östra is very high. However, the same certainty cannot be applied to the patient data of those treated at the other units. Among the known limitations is the emergency surgery performed at Sahlgrenska Hospital, the early stage colon cancers treated at SU/Mölndal (until 2006) and the disseminated cancers among patients ineligible for oncology treatment. Therefore the data is presented for SU/Östra rather than for the area of Gothenburg even though the vast majority of the patients are treated at SU/Östra. Due to the numbers and proportions of the treated patients it is unlikely that it would affect the study results. An exception could be that the proportion of stage III patients can be higher than expected as the adjuvant chemotherapy regimens mainly are administered at SU/Östra.

Ethical approval

All studies have been performed with full consideration and adherence to a good ethical practice. The local ethics committee was informed and gave its approval before the database was set and before the gathering of material was began. As a part of the clinical routine and with the aid of our study-nurses, the patients were informed of the projects, the collecting of material and the aim and goal of their use. All patients signed a note of informed consent and thereby also have the possibility to decline participation.

Paper I and II

The first two papers are retrospective analyses of database information. In the first paper the lymph node assessment data and staging information of two cohorts are compared. The first cohort consisted of the patients treated for CRC in 1999 (n=180), the second (n=175) of patients treated in 2004 after the implementation of the MDT cooperation and an improved adherence to pathology standards [55]. In the second paper, the OS and DFS of patients with stage III colon cancer treated 1999-2003 (n=265) was assessed [88]. The focus was on lymph node diagnostics and the possibilities of risk evaluation within the stage group. The survival was related to the lymph node ratio (LNR), which was calculated as the quotient of metastasis positive and assessed lymph nodes.

Paper III and IV

These papers are based on biomolecular studies, where the resulting data were associated to the clinical information from the database. The actual methods of analysis are briefly described below. In paper III, PCR was used to study the SNP gene polymorphism C677T in MTHFR in blood samples from 544 patients (a random 30%) treated for CRC in the period 1999-2006 [89] as well as a cohort of 299 healthy blood donors. The genotypes were then correlated to tumour pathology, the treatment and possible response and the survival. The demographics and pathology were also compared to the other 1268 patients treated during the same period to validate a possible extrapolation of the results. In the fourth paper, the focus was on patients treated for early colon cancer, stages I and II, during 2003-2007. Tissue from both tumour and adjacent mucosa from 114 randomly selected patients was analysed for the expression of cyclin E with Western blot technique. The expression of cyclin E in its' different forms were then correlated to both pathology data and the patient outcome.

Experimental methods

PCR: The polymerase chain reaction (PCR) is a technique widely used in molecular biology. It derives its name from one of its key components, a DNA polymerase used to amplify a piece of DNA by in vitro enzymatic replication. As PCR progresses, the DNA thus generated is itself used as template for replication. The selectivity of PCR results from the use of primers that are complementary to the DNA region targeted for amplification under specific thermal cycling conditions. The term real-time PCR notifies the constant reading and registration of the output as opposed to the older method of assessing each cycle on its' own. The RT-PCR (reverse transcription PCR) is a method used to amplify, isolate or identify a known sequence from a cellular or tissue RNA. The PCR is preceded by a reaction using reverse transcriptase to convert RNA to cDNA. Quantitative PCR methods allow the estimation of the amount of a given sequence present in a sample – a technique often applied to quantitatively determine levels of gene expression.

Western Blot: The Western blot is a method of detecting specific proteins in a given sample of tissue homogenate or extract. Western blotting can give information about the size of a protein (with comparison to a size marker or ladder in kDa), and also provide information on protein expression (with comparison to a control such as untreated sample or another cell type or tissue). It uses gel electrophoresis to separate native or denatured proteins by the length of the polypeptide or by the 3-D structure of the protein. Separation of proteins may be by isoelectric point, molecular weight, electric charge, or a combination of these factors. After separation, the proteins are then transferred to a membrane, where they are probed using antibodies specific to the target protein. The result is a pattern of bands formed by the specific protein by their characteristic. The patterns can then be read optically or by further densitometry analysis in an effort to quantify the expression.

Statistical analysis

The SAS/JMP 4.0 statistical software was used for the statistical analysis (SAS Institute Inc/USA). The basic patient demographic data were performed using distribution statistics with T-test/ANOVA for parametric data and Mann-Whitney test for non-parametric variables. The significance level set throughout the studies was 95%. The Kaplan-Meier method was used to calculate cumulative survival and the log-rank test was used to compare survival differences by groups. When a multivariate analysis was requested, this was performed in accordance with the advice from statistical support.

Results

Paper I

Looking at the number of examined lymph nodes a significant increase ($p < 0.05$) was found, from 9 (1-33) in 1999 to 17 (5-36) in 2004. During the same period, the number of assessed positive (metastatic) lymph nodes increased from a mean value of 1.7 to 2.4. Parallel, it also meant an improvement in meeting the 12 node UICC standard from 27% to 67% of the cases (the elective colonic surgery 85%). Simultaneously, there was a tendency of stage migration. While stage IV remained unchanged there was an increase of the proportion of stage III, from 38 to 46%, which was notable but did not reaching significance ($p < 0.10$). Simultaneously there was decline in the proportion of both stage II and importantly also stage I cancers. In the patients with stage II cancers of 1999 there were interesting differences in 5-year survival when related to number of assessed lymph nodes. The low node yield group (1-6 nodes assessed) had a survival prognosis significantly worse ($p < 0.05$) than the high yield group (12+ nodes).

The pathology has since then developed further. In 2007 the median number of assessed nodes had increased to a median of 21 while the number of positive nodes was unchanged. There was only a marginal further stage shift. The data concur with the evidence for assessing at least 12 nodes, but that the optimal number could be higher. A significant improvement in survival in stage II ($p < 0.02$) was also noted during this period.

Paper II

Significant differences in 3-year DFS were found for TNM N-status, tumour differentiation grade and LNR quartile groups. The DFS ranged from 80% in LNR group 1 to less than 30% in group 4 ($p < 0.001$). There were also significant correlations between the differentiation grade and the number of positive nodes ($p < 0.01$) and thus both the N-status and the LNR. A high-risk group could be identified which tended to experience more side effects with adjuvant chemotherapy. The LNR ratio is a computation and thus an indicator rather than a factor itself. It can be used a tool in differentiating the risk assessment within stage III.

Paper III

The group analyzed for MTHFR was representative for the overall patient material and did not differ in stage distribution or demography from the remaining cohort. Neither was there any difference in performed surgery or pathology data such as differentiation grade or lymph node assessment. Nor was there any difference between the MTHFR genotypes regarding stage distribution or basal pathology parameters. For patients with stage III disease, no differences were found in eligibility for chemotherapy or in given regimes. Patients with the MTHFR genotypes CT/TT had a significantly higher risk of requiring a dose reduction ($p<0.05$) compared to patients with the CC genotype. These patients also had a significantly higher risk of suffering side-effects like nausea, leucopenia and paresthesia. In colon cancer, a significant difference ($p<0.003$) in OS and CSS was found with a better prognosis in the CC group. These differences were not observed in rectal cancer. The genotype distribution in the control group was the same as in the cancer group.

Paper IV

Cyclin E was detected in both tumour and adjacent mucosa and in both FL and LMW-forms. FL was present in 29 (25.4%) tumours but only in 3 (2.6%) mucosa samples. The corresponding figures for the LMW-isoforms were 80 (70.2%) and 67 (58.8%), respectively. There was no correlation between the cyclin E expression and gender, age or tumour location. Neither was there any association to the T-stage, node assessment or tumour differentiation grade. There was a significantly higher risk of tumour recurrence for patients with a high expression of both the FL and the LMW forms ($p<0.01$). This risk was also associated with an increased risk of multiple metastasis locations ($p<0.01$), resulting in a significantly worse survival of that group ($p<0.006$). There was no statistical association between the expression of cyclin E in the mucosa and the patient outcome.

Discussion

The factor semantics

In the introduction some definitions of the terminology were stated. A prognostic factor refers to a probability of future events for a group of patients who currently have a disease. It then provides information about the patients overall cancer outcome, regardless of therapy. In contrast, the predictive factors give information on the effect of a therapeutic intervention in a patient. In other words: the prognosis for a measurable response to a given or intended specific therapy. In some instances a factor could have dual roles. An example is the MTHFR results as discussed in paper III. The main prognostic role, and the most well known of today, is taken by the tumour factors and foremost the TNM system. They have often been used for a longer time and thus been established, by accumulation of evidence, and incorporated into different treatment algorithms. Nonetheless, as discussed in paper I, even known factors still need development and also quality surveillance. The host factors are less often consciously considered. The most common occasion is in the preoperative preparations or when appraising the possible eligibility for chemotherapy. Yet, the performance status is one of the strongest known prognostic factors by its very nature. They can also constitute the limitations for the possible treatment options. With an aging population, it is likely that the host factors will have a future important role. The grand scale of the environmental factors has a role, but rarely becomes apparent except when making interregional or international comparisons. Then they certainly could have some impact on the study results. The opposite level of detail is the field of laboratory factors. None has yet reached a prognostic role and they are mainly emerging into a predictive role for chemotherapy. A good example is the K-ras mutations, which now are implemented into clinical practise. The definitions might seem as an arbitrary issue. However, the meaning and intention can affect the choice of end-point or aim of a study. Thus it could have impact on the results and the evaluation of a possible factor. The terms factor and marker could be used almost synonymously even though the latter could have a wider meaning. Concerning the LNR it would be appropriate to label it an indicator rather than a factor as it is a computation of actual factors.

The TNM

Through the work and effort of UICC and AJCC, the TNM classification system has been established as an international standard. The intention was to create a more detailed system to replace the Dukes grading. A uniform standard makes the cooperation, research and result interpretation easier. Among the strengths of

the TNM staging are its' logical structure and well spread use. Concerning its possible weaknesses, the users should be aware of them, as with all systems [90]. Overall, the TNM focuses on anatomy whilst oncology in many instances is more about biology and cell properties. The gradual effort to add the differentiation grade is one step towards compensation for this limitation. Another possibility is the use of markers dependent upon the differentiation grade, as the LNR described in paper II. With time and an increasing understanding of the cancer biology, the anatomy features will decrease in importance and this will affect the staging system.

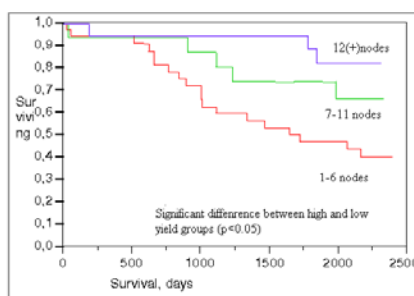
One of the major components of the TNM system is the M-factor. It heavily affects both prognosis and treatment by being the key and denominator to stage IV disease. In the current version of the TNM system the subject of distant metastasis is only addressed in a binary mode. Thus it does not cover the extent of disease in stage IV with a greater level of detail. It has become more apparent in recent years when the therapeutic aims for these patients have been set higher. A recent suggestion has been made to radically expand the description of this category. The purpose is to be able to better distinguish and evaluate the stage sub-categories that are now subject to a more aggressive treatment [91]. The finding of metastases and thus the M-factor is the susceptibility to the medical technology [92-94]. A metastasis growth has to be of a certain size to be visible. The available level of radiological technique can affect the findings and thus affect the staging. Some growth, as peritoneal carcinomatosis, is even more difficult to detect preoperatively and therefore carries a risk of incorrect staging. It can then affect not only the staging but also the classification between adjuvant and first line chemotherapy. As the key to a first line chemotherapy treatment is a visible lesion, the metastasis detection possibilities could therefore affect study and treatment results. With the gradual improvement of the diagnostic possibilities, there could be future stage migration phenomena and then into stage IV.

The next major component of the system is the T factor. It defines the differences between stages I and II as well as between IIIA and B. It correlates to the risk of having lymph node metastasis (N-factor) but also with the differentiation grade. The current development is in subdividing each step, often into thirds. The result can be seen in some reports as an A/B/C sub-grading. The best described issue is the SM grading of the T1 tumours. In clinical practise there are several reports linking the risks of recurrence to the SM grade. It is used mainly in when assessing local tumour excisions, including TEM surgery. A difficulty is the lack of uniform standards. The difference between two steps can be a single layer of cells and then the uncertainty of the most advanced spot was found. The limitations can also be challenged by other features such as the venous invasion which is not accounted for today.

Lymph nodes and Will Rogers

The third component in the equation is the N factor. It can vary with several other factors, including the T-stage and the differentiation grade. Furthermore, it can depend on the number of lymph nodes removed at surgery but also the number of assessed nodes [95]. The standard, by UICC, is set at 12 analyzed nodes. However, even if there is a written document it may take time and effort to implement the standards into clinical use [96]. As discussed in the first paper, this is an important quality issue that does affect the clinical practice, treatment and thus the outcome. Ultimately the prognostics could thus vary with the thoroughness of the pathologists' assistant. The reciprocity of information attained at a MDT conference can assist in improving the quality of the pathology assessment [97]. An improvement of the assessment level can result in a migration from stage I or II into stage III. The prognosis for the individual remains the same but the stage specific survival can be affected. It will improve as the patient with the worst prognosis in the lower stage group suddenly has the best prognosis in the new, higher stage group [98]. The phenomenon is usually named Will Rogers after an American comedian.

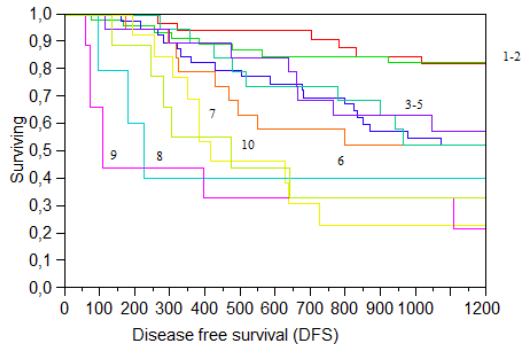
A special aspect on this matter is when evaluating a change in surgical strategies. In some instances it is accompanied by changes in the pathology service. It then becomes very difficult to assess if the improvement is actually gained by the modified surgical procedure or if it is, in part, the result of changes in sorting or stage migration. Another important aspect of the quality issue concerns the research. If the basic staging was not of proper nature then it would be more difficult to assess the results. It especially concerns the assessment of factor in stage I/II as the inherent survival then be to variable as shown in paper I. The figure from the first paper shows a great variability in the survival in stage II cancer with an inadequate node assessment. As a consequence, extra efforts were made in the later papers to minimize the risk that any findings were biased due to this phenomenon. The assessed and positive nodes were controlled by the analyzed factors. A specific reassessment of the study results was also made and then using the patients with an adequate lymph node data.



The next challenge for the N-factor is to decide on the role of micro-metastasis and isolated tumour cells. Another interesting aspect on the lymph nodes is the role of the immune system in malignant disease and the possible protective or therapeutic functions.

Lymph node prognostics

As mentioned above the quality standard for the lymph node assessment is set at 12 nodes. The figure of 12 nodes itself has been contested. Some suggest lower numbers, as 6-9 [99], whilst some request figures of at least 15 nodes for a correct staging [100]. In our data, a stage migration was found until 2004 when the median number of assessed nodes was 17. The further increase to 21 in 2007 was not associated to any significant further changes in the stage proportions. Neither was there any further increase in median number of found positive nodes. Thus, our data supports the requested 12 but a higher count increases the chances of correctly staging category I and II. Another way to phrase it is that 12 negative nodes should be assessed to correctly assume stage I or II as the risk is of under-staging. The difference between stages I and III are but a single node. With very few assessed nodes the actual stage could also be an N2. It could be a part of the explanation for the survival differences in the figure above as well as the suggestion that an increase in node assessment would be beneficial to outcome [101]. Another possibility for the lymph node prognostics is by the lymph node ratio. As the ratio is a computation of factors it could preferably be called a prognostic indicator or marker rather than a factor itself. It was originally shown to be of interest in gastric cancer [102]. In paper II, the prognostic merit was shown and confirmed also in colon cancer. In our opinion it could be a good tool in differentiating the risk assessment in stage III disease.



In the figure above, the DFS in stage III is presented by LNR decentiles. It shows that the ratio is a continuous variable along with the heterogeneity of stage III. The use of groups makes the assessment easier and also provides the possibility to identify high and low risk groups. Noteworthy is also the early presentation of progress in the high risk groups whilst the low risk group could have a survival prognosis fully comparable to that of stage II disease.

In the data, the DFS in stage III can be shown to vary between 20-85% and that the LNR can aid in that differentiation. It is important for the risk assessment in adjuvant chemotherapy and the management of treatment side-effects. It can also show that the difference between stages III and IV are less clear than conceived. It has been shown that the LNR could be of interest as a prognostic tool in stage IV [103], bringing attention to the survival variability within stage IV. An interesting aspect is about the early recurrences which are known to carry a worse prognosis. An explanation could be that the tumour already has spread but it is not yet visible by radiology. A few months later it has passed the minimum visible size. It then means progress and is then a DFS event. The patient is then in the highest LNR group of stage IV and thus of poor prognosis. It is important in the clinical setting but also important for the evaluation of treatment studies.

Outcome parameters

Earlier, the terminology and the choice of end-point were discussed. Each outcome parameter has its own strength and weakness. The overall survival is perhaps the easiest to monitor and especially in countries keeping central registries. The cancer specific survival can often be preferable since the mortality can have other causes and is dependent on time and age. A possible weakness is that there can be uncertainties regarding the cause of death since there no longer is any legislation demanding post-mortem examinations. The DFS is often used in stage III disease and is a surrogate marker for the overall survival. An advantage is the possibility of a shorter observation time. However, it can depend on factors such as the intensity and mean of follow-up. Small details, like the interval of CT scans and certainly the selection of included patients, can affect the data. Also, the “Will Rogers phenomenon” can have an effect with “more healthy” patients adding towards better results. The same possibility of bias applies to studies using the PFS parameter. In the treatment studies, with new regimes, it is often a selection of the healthiest patient of the disease stage that is included and appraised. It is then difficult to extrapolate the results for a more general use, and comparison to historical controls should only be made after careful consideration. The qualitative parameters such as quality of life or functional level are interesting. In some instances, like the palliative setting or patients of advanced age, they could be more appropriate than survival. An example could be to evaluate the use of TEM surgery among our oldest patients.

Treatment aspects

Whilst the M-factor of the TNM classification system is a key question in the preoperative assessment, the T and N factor data are acquired postoperatively

from the pathology report. As previously discussed, the report itself must be of adequate quality including the node assessment. The findings are usually discussed at a MDT conference. One of the main questions is if further treatment is indicated. The risks of chemotherapy are compared to the potential gain. The main factor in the equation is the presence of node metastasis and thus stage III disease. A possibility to differentiate the risk assessment within the boundaries of stage III is by using the lymph node ratio [88, 104]. Other factors such as age and co-morbidity can affect the recommendations by adding to the treatment risks. The risk that the disease already is disseminated though it is not yet visible should also be considered. The risk could be estimated by the LNR as shown above. It is very important in the handling of possible adverse events in the adjuvant treatment.

Currently, there are no predictive factors in clinical use that can guide us in the choice of cytotoxic regime. The only exception is the K-ras which is analyzed to predict the response to treatment with monoclonal antibodies. The cost of treatment did probably aid in the test development and does also motivate the cost of analysis. It would be of great interest to find a corresponding predictor for the more commonly used treatments. It could in the future aid in both the choice of drug and in its' dosage. There are indications that the functional level of the folate metabolism could be of importance for the use of 5-FU as discussed in paper III. However, the role of MTHFR should also be assessed in conjunction with the function of the other associated enzymes. Also, if a solid predictor was found there is a need of several equally good treatment options.

Another matter of discussion is the use of adjuvant chemotherapy in stage II disease [105]. In current practice, the potential benefits do not motivate the risks. However, there are some suggested indications like inadequate node information or a locally advanced disease. Since there is a risk in stage II disease, estimated to 5-20%, of dying from cancer recurrence, there is an interest in finding factors that could identify the high risk individuals. Several different tumour characteristics, such as tumour budding or venous invasion, have been suggested. In paper IV, a regulator protein in the cell division cycle, cyclin E, was evaluated. The idea was inspired from both clinical and laboratory science, also a possibility that could benefit from a translational angle of research. Cyclin E expression has been shown to affect the tumour properties in mice [106]. In clinical settings the cyclin E in breast cancer has been strongly associated to the tumour behaviour and the survival [107]. Cyclin E was found to, independently of the TNM factors, correlate to the risk of tumour recurrence in stage I and II colon cancer. It was also associated to the risk of aggressive, multi-focal recurrences which concur with the preclinical laboratory data. Thus, it could become a part of a future risk assessment instrument. An associated question is also how to adjust the treatment even if such a parameter is known.

What is to be measured?

In the quest of finding new prognostic and predictive factors, there are several obstacles. What from the beginning might seem to be a straightforward connection between for example a gene polymorphism and a clinical result can have a multitude of confounders. In each step from gene to clinical outcome there are possibilities of misinterpretation. Among the first steps are the sequence from a gene presence to its activation and expression. Next, the genetic product must not only exist but also be at a functional state as well as having the proper receptors. The fact that few genes act totally independent only adds more difficulty. A good example is the complexity of the folate metabolism. In a not yet published material on the 3' and 5' polymorphisms in TS, there were no significant results until it was correlated to the functional polymorphisms in MTHFR. The example reveals a difficulty to study each part in itself. When then trying to include more factors there is the problem and risk of mass-significance bias.

An interesting finding could also be clouded by an improper choice of study population or possible end-point. There should be a theoretical reason why a factor could be of importance, and then also for which group. For example, the findings of cyclin E in paper IV could well have been invisible in stage III and IV disease as other factors like metastasis then carry a heavy prognostic weight. Neither should we try to find marker for tumour characteristics that are easier assessed by known and cheaper methods. Unless it then adds new opportunities. Consideration should also be given to the known factors and the current staging system. An example is in paper III, where we tried to show the role of the MTHFR C677T polymorphisms in a structured way along the routes of the TNM-system.

Another interesting issue is where the samples should be taken and what material to use. The most common practice is to take tissue from the tumour. One possible problem could then be the differences and heterogeneity within the tumour and which part that would be the most representative. In paper III, the PCR SNP analyses were instead made from blood samples. It should correspond to the genotype, might be more consistent and perhaps better associate to the following adjuvant chemotherapy. After all, the tumour has been removed and we do not know the possible bio-molecular change that brings the surrounding. Blood can also often be easier to access and monitor in vivo than biopsy samples. A third possibility is the adjacent mucosa. There is evidence that there are abnormalities present also in the macroscopically normal mucosa. The local microenvironment can have alterations. Localized folate deficiencies and genetic changes have been observed the mucosa [108, 109]. There could also be changes in enzyme expression as we found in paper IV. The interesting part is not only

the finding of cyclin E in its' different forms. It also shows a difficulty of knowing what to look for, as in this example the existence of the isoforms [110]. Last, there could be bio-molecular differences related to the tumour location. The growth pattern and prognosis of left and right sided colon cancers are not the same and the definition of the rectum is not fully consistent. It is unclear how the molecular analysis is affected by this.

Study samples

An important part in translational research is connecting the findings to the clinical setting. In order to get valid data from any mean of bio-molecular analysis it is necessary to have a solid foundation in the clinical samples. The issue is linked to the discussion above about the tumour-related clinical factors. However, it is important enough to be stressed once again. The samples should, in most instances, be well attached to good and full TNM pathology data. There is also a need of quite large clinical materials especially when looking into treatment subgroups. The data can be affected by the local treatment traditions and the risk of a systematic bias in assessment and registration. A population-based material can add strength to a study by being more homogeneous in the assessments. A multicentre approach can suffer weaknesses through having more people involved and disparate registrations, although it can gain in strength by the possible numbers. In a geographically wide area there could also be differences not only in the health care systems, but also in the genetic settings and through cultural influences. A good example is when using the length of stay as a parameter. When discussing the translational research we should also acknowledge the complexity of each component. The laboratory tests have their demand on skill and experience. To get valid results the testing must be repeatable and consistent over time as set in the GLP standards. The corresponding clinical standard is GCP, which also provides guidelines for conducting clinical studies and the associated enrolment of patients. The quality of the laboratory standard in research is about as important as the quality of delivered care.

Future directions

As described, there are several difficulties and possible pitfalls in the area of biomarker research. We fully acknowledge the difficulty in finding good and solid markers. It could be a part of the explanation why so few markers reach the actual clinical use and why only a few ever gets validated in later studies. Two features that could be of benefit is a better consideration of the clinical data and a better adherence to the know factors like the TNM. The TNM system is functional but has several limitations. In my opinion, it will probably adapt with the increasing knowledge about the cancer disease. The tumour anatomy will

still be represented but might lose in impact and proportion. However, it is not unlikely that some factor of biological property and some of the genetics will find a role. Whilst this development requires a lot of scientific progress, an easier change could be to adapt the stage classification to be seen from a functional perspective.

Stage	Trait	Treatment	Sub-groups	Example vs. TNM
A	Early cancer	Local surgery	None	Tis, T1 (well diff T2)
B	No metastasis	Full resection	B1 Surgery/pathology ok B2 High risk (treat as C1)	T1-3, N0 T4, Rx, Nx (<12 nodes)
C	Lymphatic spread	Resection + adjuvant chemotherapy	C1 Low risk C2 High risk (treat as D1)	T1-3, N1-2, LNR<0.5 T4N1, TxN2, LNR>0.5
D	Metastatic disease	Multi disciplinary strategy	D1 Cure possible – surgery + chemotherapy D2 Cure unsure – chemotherapy + eventual surgery after re-evaluation and downstaging D3 Palliative – incurable spread	

Some small changes, as suggested below, could serve to adapt the staging to the clinical setting. Two important changes would be the clinical differentiation between stages I and II and the use of LNR to differentiate the risk in stage III. Added is also a suggestion of a “second line” of adjuvant chemotherapy so a high risk stage III is assessed in the same way as the best prognosis stage IV patients regarding choice of drugs and the handling of adverse effects.

The continued development of predictive factors and pharmaco-genetics will facilitate the choice of treatment and head toward individually tailored therapies. Another clinically important issue is to develop and research the possibilities of an improved preoperative staging. It can be facilitated by conducting research along the pathways of the established treatment algorithms. With increased knowledge, better preoperative assessments could be made and thus both tailor the surgical procedure and get a proper use of the neoadjuvant therapy. Another key factor in oncology is the metastasis potential of a tumour. An interesting field in the near future would be to find a factor that could predict advanced disease.

Conclusion

Colorectal cancer is one of the most common forms of neoplastic disease in the Western world. Factors that can affect the treatment and outcome are discussed in this thesis. A distinction is made between prognostic factors, which concerns the patients overall cancer outcome, regardless of therapy, and predictive factors that can give information on the effect of a therapeutic intervention. Discussed are also the basics of a factor, some means of analysis an evaluation as well as some possible pitfalls and difficulties in this aspect of translational research.

In paper I, the pathology assessment quality is shown to be of central importance for both clinical practise and associated research and thus should be monitored and improved. In paper II, the lymph node ratio was shown to be a prognostic indicator and could aid in prognostic differentiation within stage III disease. In paper III the role of the MTHFR polymorphism C677T in CRC was evaluated. It was shown to be of significant predictive value for the risk of side-effects from 5-FU treatment and thus also the outcome. There was no evidence that it would affect the carcinogenetic process or any specific pathology factor. In paper IV, cyclin E was found to be expressed as both full length and shorter isoforms in both tumour and adjacent mucosa. It correlated significantly to the risk of recurrence in stage I/II colon cancer.

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Appendix

Paper I-IV