Colorectal and appendiceal peritoneal metastases

From population studies to genetics

MALIN ENBLAD
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Abstract

Peritoneal dissemination of colorectal and appendiceal origin was previously considered the end-stage of malignant disease. Today, treatment with cytoreductive surgery (CRS) in combination with hyperthermic intraperitoneal chemotherapy (HIPEC) has prolonged survival and cured some patients with peritoneal metastases (PM). Unfortunately, a majority of patients still have fatal outcomes. In this thesis, colorectal and appendiceal PM were studied from a wide population-based perspective down to the detailed perspectives of histopathology and genetics, with the aim of further contributing to prolonged survival.

In Paper I, the heterogeneous histopathology of PM was investigated and a substantial proportion of patients undergoing CRS and HIPEC were found to have surgical specimens lacking neoplastic epithelium. These patients had a favourable prognosis and the results illustrate the importance of thorough analysing and reporting of histopathology for understanding differences in survival outcomes and for improving patient selection. In Paper II, the role of inflammation in colorectal and appendiceal carcinogenesis was investigated at a population-based level. Patients with non-surgical treatment of appendicitis had an increased incidence of cancer (especially of appendiceal and right-sided colon cancer) compared to the general population. This should be taken into consideration in the discussion of optimal management of patients with appendicitis. In Paper III, risk factors for PM were studied with the aim of aiding in the detection of PM at earlier stages. Appendiceal and right-sided colon cancer, advanced tumour and node stages, mucinous histopathology and vascular invasion were identified as high risk features for developing PM, and should increase awareness of potential PM. In Paper IV, genome-wide chromosomal copy number alterations of PM were explored and associated with prognosis after CRS and HIPEC. Colorectal PM exhibited a wide range of alterations of which copy number gain on parts of chromosome 1p and 15q were significantly associated with poor prognosis and have the potential to be used as prognostic molecular markers in the future.

In conclusion, this thesis provides new insights into the field of colorectal and appendiceal cancer and PM to be used for improved patient selection, early detection and prevention, ultimately contributing to improved survival.

Keywords: Peritoneal metastases; Peritoneal carcinomatosis; Pseudomyxoma peritonei; Colorectal cancer; Appendiceal cancer; Cytoreductive surgery; HIPEC; Appendicitis

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To Viktor
“I have never tried that before, so I think I should definitely be able to do that”
- Pippi Longstocking
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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<td>CCS</td>
<td>Completeness of cytoreduction score</td>
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<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CIN</td>
<td>Chromosomal instability</td>
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<tr>
<td>CMS</td>
<td>Consensus molecular subtypes</td>
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<tr>
<td>CN</td>
<td>Copy number</td>
</tr>
<tr>
<td>CNA</td>
<td>Copy number alterations</td>
</tr>
<tr>
<td>CRS</td>
<td>Cytoreductive surgery</td>
</tr>
<tr>
<td>DMPM</td>
<td>Diffuse malignant peritoneal mesothelioma</td>
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<td>DPAM</td>
<td>Disseminated peritoneal adenomucinosis</td>
</tr>
<tr>
<td>EMT</td>
<td>Epithelial mesenchymal transition</td>
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<tr>
<td>HR</td>
<td>Hazard ratio</td>
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<tr>
<td>HIPEC</td>
<td>Hyperthermic intraperitoneal chemotherapy</td>
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<td>ICD</td>
<td>International Classification of Disease</td>
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<tr>
<td>LAMN</td>
<td>Low-grade appendiceal mucinous neoplasm</td>
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<td>LS</td>
<td>Lesion size score</td>
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<td>MIP</td>
<td>Molecular inversion probe</td>
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<td>MSI</td>
<td>Microsatellite instability</td>
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<tr>
<td>NEA</td>
<td>Neoplastic epithelium absent</td>
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<td>NEP</td>
<td>Neoplastic epithelium present</td>
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<td>OR</td>
<td>Odds ratio</td>
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<td>PM</td>
<td>Peritoneal metastases</td>
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<tr>
<td>PCI</td>
<td>Peritoneal cancer index</td>
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<td>PMP</td>
<td>Pseudomyxoma peritonei</td>
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<td>PMCA</td>
<td>Peritoneal mucinous carcinomatosis</td>
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<tr>
<td>PMCA-I</td>
<td>Peritoneal mucinous carcinomatosis−intermediate</td>
</tr>
<tr>
<td>PMCA-S</td>
<td>Peritoneal mucinous carcinomatosis−signet ring</td>
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<tr>
<td>PSOGI</td>
<td>Peritoneal Surface Oncology Group International</td>
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<tr>
<td>SIR</td>
<td>Standardised incidence ratio</td>
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Introduction

The presence of peritoneal metastases (PM) was previously considered a manifestation of generalised and terminal malignant disease. As it was recognised that a group of patients with PM rarely had haematogenous metastases, the interest of peritoneum and peritoneal surface malignancies increased. Today, peritoneal dissemination is considered a separate entity of dissemination, often referred to as loco-regional and the peritoneum has been upgraded from a simple surface structure to an organ with unique properties. One of these unique properties is the “peritoneal-plasma-barrier”, which effectively prevents intravenously administered chemotherapy from reaching sufficient cytotoxic levels in the peritoneal cavity. Reversely, it prevents large intraperitoneal molecules from reaching the systemic circulation. This enables intraperitoneal administration of chemotherapy with high concentration in the PM without reaching toxic levels in the circulation. Intraperitoneal chemotherapy in combination with surgical removal of all disease-affected peritoneum and organs has proven to be a successful option for patients with PM.

Currently, cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) is standard treatment for patients with PM originating from colorectal cancer, appendiceal tumours and diffuse malignant peritoneal mesothelioma (DMPM). Despite advances in the treatment of PM, there are still many challenges ahead. In colorectal and appendiceal PM, a large proportion of patients are diagnosed at a late stage with extensive disease and with limited benefit from CRS and HIPEC, and the need for identifying risk factors for PM is essential. In appendiceal PM, this is further complicated by the low incidence of appendiceal tumours and the heterogeneous histopathology. In contrast to extensive PM, a limited tumour burden in combination with blunt selection criteria leads to potential over-treatment with CRS and HIPEC and resulting unnecessary morbidity. In addition, some patients with seemingly treatable tumour burden experience rapid disease recurrence and in retrospect without benefit of CRS and HIPEC. This illustrates the need for studies in the field of genetics and PM for identifying prognostic molecular biomarkers to aid in patient selection.

In all, even though there has been rapid progression in the field of PM and the condition is no longer incurable, much remains before knowledge
in PM corresponds to what we already know of haematogenous metastases. This applies to everything from population-based epidemiology to genetics, and this thesis is a start.
Background

Peritoneal surface malignancies

Peritoneum
The peritoneum consists of a single layer of mesothelial cells superficial to a basement membrane and a layer of loose connective tissue\(^8,\)\(^9\). The parietal peritoneum lines the inside of the abdominal wall and is continuous with the visceral peritoneum covering a majority of the intra-abdominal organs. The parietal and visceral peritoneum creates the peritoneal cavity and has an estimated surface corresponding to the surface of the skin. The peritoneum was initially thought of only as a smooth surface facilitating frictionless movement of the intra-abdominal organs. However, the peritoneum is a complex structure involved in the transport of fluid, solutes and particles between the peritoneal cavity and blood and lymphatics. It also acts as a “peritoneal-plasma-barrier”, preventing the movement of oxygen and nutrients from the capillaries to the peritoneal cavity\(^10\). The peritoneum is also an immunologically active structure that plays an important role in the innate and the adaptive immune defence and several “stomata”, lymphatic openings, are present in the peritoneum\(^9,\)\(^10\). In metastatic cancer, the peritoneum acts as a protecting physical and immunologic barrier against neoplastic cells trying to invade through the peritoneum to reach the underlying structures\(^11\).

Primary peritoneal neoplasms
Primary peritoneal neoplasms are defined as neoplasms with a manifestation in the abdominal cavity and without visceral primary origin. Tumours derived from mesothelium include benign mesothelioma, well-differentiated papillary mesothelioma and DMPM\(^12\). Most common is primary peritoneal adenocarcinoma, which mainly occurs in women and histopathologically and clinically resembles surface epithelial ovarian tumours\(^13\). In addition, the benign condition diffuse peritoneal leiomyomatosis, with multiple benign nodules of well-differentiated smooth muscle cells, is macroscopically easily mistaken for a secondary peritoneal neoplasm\(^12\). Finally, desmoplastic small
round cell tumour is an extremely rare and malignant condition of unknown origin that primarily occurs in boys and young men\textsuperscript{14}.

Secondary peritoneal neoplasms
Secondary peritoneal neoplasms, or PM, often referred to as peritoneal carcinomatosis, constitute the majority of peritoneal neoplasms and are the main focus of this thesis. PM most often originate from a primary gastrointestinal or gynaecological tumour and the dissemination is a multistep process\textsuperscript{11, 12} (Figure 1). First, cancer cells detach from the primary tumour and freely spread within the peritoneal cavity along with the peritoneal fluid\textsuperscript{15, 16}. The detachment occurs spontaneously by cell-shedding from a tumour penetrating the overlaying peritoneum or from a preoperatively damaged tumour. Second, the cancer cells attach to the peritoneal surface and third, invade either “transmesothelially”, “translymphatically” or through peritoneal defects. Transmesothelial invasion is prevented by the poor nutrient environment created by the “peritoneal-plasma-barrier” and the physical barrier created by the mesothelial cells. However, some cancer cells can induce a contraction of mesothelial cells and reach the underlying basement membrane through the created space between the mesothelial cells\textsuperscript{11}. Matrix proteinases complete the invasion by degrading the submesothelial tissue for the cancer cell to gain access to the capillary bed\textsuperscript{17}. Translymphatic invasion occurs via lymphatic “stomata” followed by proliferation in the submesothelial lymphatic tissue\textsuperscript{9, 11}. The phenotypic changes involving avoiding apoptosis when detaching and the ability to invade surrounding tissue is called epithelial mesenchymal transition (EMT) and is a key step in metastasis\textsuperscript{17}. \section{Materials and Methods}
Figure 1. The multistep process of peritoneal dissemination. A tumour cell detaches from the primary tumour and floats freely in the peritoneal cavity (A). The cell adheres to the mesothelium (B) or to endothelial cells of lymphatic stomata (C) and invades transmesothelially or translymphatically to the submesothelial tissue (D).

Colorectal cancer and peritoneal metastases

Epidemiology

Colorectal cancer is the third most common cancer with an incidence of 32 per 100,000 a year in men and 25 per 100,000 a year in women (age adjusted to the world standard population) in Sweden 2011–2015. The five-year relative survival is approximately 64% in women and 67% in men (age adjusted to the world standard population)\(^1\), but decreases rapidly if distant metastases are present.

Peritoneum is the third most common site of distant metastases after liver and lungs\(^2\), and is often referred to as peritoneal carcinomatosis when originating from colorectal cancer. The currently accepted term is PM and will here be used also for colorectal peritoneal dissemination. The prevalence of synchronous PM is reported between 4%–7%\(^20\text{–}23\) and metachronous PM between 2%–19%\(^22\text{–}25\). Overall survival rates in early studies are poor, with a median survival of six months from the time of diagnosis of PM\(^26\text{,}27\). When treated with systemic chemotherapy, individuals with peritoneum as the sole site of distant metastasis have significantly shorter overall survival compared to other single-site distant metastases\(^28\). Treatment with CRS and HIPEC gained acceptance as superior to systemic chemotherapy for treating PM after Verwaal et al. published a randomised
trial in 2003\textsuperscript{29}. A meta-analysis by Huang et al. summarised survival data of fifteen controlled studies comparing CRS and HIPEC with systemic chemotherapy: median overall survival was 35 months (range 13–62) vs. 17 months (range 9–34); five-year survival rate was 38\% (range 26–58) vs. 18\% (range 0–44)\textsuperscript{30}.

**Anatomy**

Based on the location of the primary tumour, colorectal cancer can be divided into right-sided colon cancer (including cecum to proximal 2/3 of transverse colon), left-sided colon cancer (including distal 1/3 of transverse to sigmoid colon) and rectal cancer. The right colon is derived from the embryological midgut and the left colon and rectum are derived from the hindgut. This anatomical/embryological division is relevant because there are important biological and clinical differences, of which one is PM being more common in right-sided colon cancer\textsuperscript{31-34}. The peritoneum completely surrounds the more mobile cecum, transverse and sigmoid colon, while the more fixed ascending and descending colon and rectum are only partly covered by the peritoneum.

**Aetiology and risk factors**

An inherited predisposition to colorectal cancer is present in approximately 5–6\% of colorectal cancers, most often as Familial adenomatous polyposis and Hereditary non polyposis colorectal cancer\textsuperscript{35}. Another non-environmental risk factor is inflammatory bowel disease\textsuperscript{36}, where a chronically inflamed mucosa with inflammatory cells releasing reactive oxygen species leads to oxidative stress, DNA damage and dysplasia\textsuperscript{37}. Evidence supports that inflammation plays an important role not only in inflammatory bowel disease-associated colorectal carcinogenesis. One example is COX-2, a potent trigger of inflammation, normally not expressed in colonic mucosa. Individuals with regular intake of acetylsalicylic acid, a COX-2-inhibitor, have decreased incidence of colorectal cancer\textsuperscript{38}. In addition, environmental factors corresponding to a western lifestyle are responsible for a large proportion of sporadic colorectal cancer cases\textsuperscript{39}.

The benefit of treating PM with CRS and HIPEC is dependent on early detection and low tumour burden. It is therefore important to recognise an individual with high risk features of already established PM (synchronous PM) and patients with a risk of peritoneal recurrence (metachronous PM). Synchronous PM have been associated with right-sided colon cancer, T4 tumour stage, N1/N2 node stages, poorly/undifferentiated tumours, mucinous tumours and emergency surgery\textsuperscript{21-23}. The latter is often a result of complications from a T4 tumour or PM itself. Metachronous PM have been
associated with right-sided colon cancer, advanced tumour and node stages, mucinous tumours, non-radical surgery, and emergency surgery\textsuperscript{22, 23, 40}. Significant results and studied variables differ between studies for both synchronous and metachronous PM.

### Histopathology

A colorectal adenocarcinoma is most often non-mucinous but is classified as mucinous if it contains >50\% of extracellular mucin. If >50\% of the tumour contains signet ring cells, the tumour is classified as a signet ring cell carcinoma\textsuperscript{12}.

Histopathological characteristics of colorectal PM resemble that of the primary tumour. The classic colorectal PM, not containing mucin, are categorised as non-mucinous peritoneal carcinoma, and are associated with a poor prognosis\textsuperscript{41}. Mucinous PM are primarily associated with appendiceal tumours but can also originate from a mucinous colorectal cancer. The PM are then classified according to the Ronnett’s histopathological classification of pseudomyxoma peritonei (PMP)\textsuperscript{42}, which is thoroughly described below. Signet ring-containing metastases should be classified separately due to the very poor prognosis\textsuperscript{43}.

### Genetic alterations in colorectal cancer

The stepwise progression of genetic alterations transforming normal colorectal mucosa to a colorectal cancer was initially described by Fearon and Vogelstein in 1990\textsuperscript{44}. Since then, the “adenoma-carcinoma sequence” has served as the model for colorectal carcinogenesis and has been widened to at least three main pathways of tumour transformation: the chromosomal instability pathway (CIN), the microsatellite instability pathway (MSI), and the serrated pathway\textsuperscript{45}.

The CIN pathway accounts for approximately 85\% of colorectal cancers and is associated with an accelerated rate of gains or losses of whole or large portions of chromosomes which results in an imbalance of chromosomes (aneuploidy, other than diploid copy number, CN), and sub-chromosomal copy number alterations (CNA). CIN tumours are characterised by mutations of tumour suppressor genes and proto-oncogenes. Inherited mutations in the \textit{APC} gene lead to Familial adenomatous polyposis. The exact mechanism leading to instability is unknown and it is not clear whether the instability is an initiating event or a consequence of the malignant transformation\textsuperscript{45-47}. The MSI pathway is a result of defects in the mismatch repair system, which corrects errors during DNA replication. Microsatellites are repeated small sequences of the genome, susceptible to replication errors, and mutations in mismatch repair genes result in MSI. A germline mutation of mismatch
repair genes leads to hereditary non polyposis colorectal cancer and constitutes the MSI pathway. Sporadic MSI tumours are due to irreversible silencing of a mismatch repair promoter region by hypermethylation. This mechanism is sometimes referred to as the serrated pathway because of its association with serrated polyps and has been associated with poor prognosis. In all, approximately 15% of colorectal cancers have MSI.

The Colorectal Cancer Subtyping Consortium took on the task of analysing large-scale data to create the consensus molecular subtypes (CMS) 1–4 of colorectal cancer. CMS1 includes the majority of MSI tumours and is thereby also characterised by hypermethylation. CMS2–3 all have CIN but CMS3 tumours have less CNA and sometimes also MSI and hypermethylation. Somatic mutations are not limited to but enriched in the different subtypes. For example, BRAF mutation is common in CMS1 and KRAS mutation in CMS2. Other characteristics include immune infiltration in CMS1 and upregulation of genes involved in EMT in CMS4. CMS1 tumours cluster in the right colon and the others in the left colon and rectum. CMS4 tumours have the worst and CMS2 the best overall survival. CMS1 tumours have a very poor prognosis if relapsed.

The search for genetic alterations associated with recurrence and metastasis have not yet revealed the mutations responsible. While the “adenoma-carcinoma sequence” has been estimated to take seventeen years, the time from carcinoma to distant metastasis has been estimated at less than two years. It is suggested that few additional genetic alterations in a subset of cancer cells are required for dissemination but there is also evidence suggesting that many metastatic-specific mutations occur after dissemination. Several mechanisms behind peritoneal dissemination, such as genes involved in EMT, are suggested but studies have mostly been based on gynaecologic PM. Genetic alterations in colorectal PM and its association with prognosis after CRS and HIPEC can be considered largely unknown.

Appendiceal tumours and pseudomyxoma peritonei

Epidemiology

Appendiceal tumours are rare, with an incidence of 0.7 per 100,000/year in men and 1.0 per 100,000/year in women (age adjusted to the world standard population) in Sweden in 2014. Every year approximately 130 individuals are diagnosed with an appendiceal tumour in Sweden, but only 40% of these tumours are invasive adenocarcinomas. Survival data is limited and varies depending on histopathological subtype. However, appendiceal
Adenocarcinoma has a worse prognosis compared to colorectal cancer with a five-year overall survival of approximately 55%\textsuperscript{59}.

Appendiceal tumours have a tendency to metastasise to the peritoneum and mucinous appendiceal tumours are responsible for the majority of PMP cases. The incidence of PMP worldwide is approximately 0.2 per 100,000/year (not adjusted to the world standard population\textsuperscript{60}). If left untreated, PMP is a condition eventually leading to death, but patients treated with CRS and HIPEC have a median survival of 16 years and a five-year survival of 74\textsuperscript{6}. 

**Anatomy**

The vermiform appendix is an elongated tubular structure of approximately 8 cm that opens into the cecum below the ileocecal valve. It is derived from the embryological midgut and is sometimes considered part of the right colon\textsuperscript{61}. The appendix is an intraperitoneal organ, most often located in the right lower quadrant behind or below the cecum but with great variation due to its mobility during and after its development\textsuperscript{62}.

**Aetiology and risk factors**

The aetiology of appendiceal neoplasms is assumed to have similarities to that of colon cancer but is not specifically studied. However, the organ’s unique composition and anatomy and the histopathological and clinical characteristics of appendiceal tumours suggest that distinct pathways of neoplastic transformation might exist. In 1964, McVay et al. reported a higher incidence of colon cancer in individuals who were appendectomised\textsuperscript{63}. Since then, the potential protective role of the appendix has been in focus, hypothesising that the removal of appendiceal lymphoid tissue could play an important role in local carcinogenesis. However, evidence is insufficient since studies have reported inconsistent associations with cancer\textsuperscript{64-69}. To the contrary, an inflamed appendix left in the abdomen could theoretically contribute to carcinogenesis in the appendix and surrounding tissue. This association does not seem farfetched considering the strong association between inflammation and colorectal cancer\textsuperscript{37, 38}. However, the risk of later cancer development after non-surgical treatment of appendicitis is unknown.

**Histopathology**

Appendiceal tumours range from benign adenomas and polyps to low-grade appendiceal mucinous neoplasms (LAMN) and high-grade non-mucinous and mucinous adenocarcinomas (Table 1). In addition, the appendix is the most common site of intestinal neuroendocrine tumours but these are not the
focus of this thesis. Adenomas are limited to the mucosa and mucin dissection and extra-appendiceal mucin is not present. Adenomas are sometimes associated with mucin accumulation and the formation of a mucocele, which makes it hard to distinguish from LAMN. However, adenomas are per definition not associated with disseminated disease. LAMN is characterised by “pushing border invasion” of the appendiceal wall but infiltrative invasion is absent. Rupture of the wall is associated with peritoneal extra-appendiceal mucin, with or without neoplastic epithelial cells (Figure 2). If atypia is high-grade but all other features are of LAMN, the recently chosen term is high-grade appendiceal mucinous neoplasm. Non-mucinous adenocarcinomas histopathologically and clinically resemble colorectal adenocarcinomas (Figure 3). If >50% extracellular mucin is present, the tumour is designated mucinous and is associated with pools of mucin infiltrating the appendiceal wall, which often ruptures and leads to peritoneal dissemination. Tumours with poor differentiation and presence of signet ring cells are associated with poor prognosis and widespread peritoneal dissemination12, 43, 70, 71

<table>
<thead>
<tr>
<th>Terminology</th>
<th>Histopathology</th>
<th>Peritoneal dissemination</th>
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<tbody>
<tr>
<td>Adenoma</td>
<td>Usual tubular, tubulovillous or villous adenoma, with intact muscularis mucosae</td>
<td>No peritoneal dissemination</td>
</tr>
<tr>
<td>Serrated polyps</td>
<td>Serrated features and intact muscularis mucosae</td>
<td>No peritoneal dissemination</td>
</tr>
<tr>
<td>Low-grade appendiceal mucinous neoplasm</td>
<td>Pushing border invasion with loss of muscularis mucosae, dissecting acellular mucin, rupture of appendix with extra-appendiceal mucin or cells and low-grade atypia.</td>
<td>Risk of mucinous peritoneal dissemination</td>
</tr>
<tr>
<td>High-grade appendiceal mucinous neoplasm</td>
<td>High-grade atypia and LAMN-features.</td>
<td>Risk of mucinous peritoneal dissemination</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>Usual colorectal adenocarcinoma</td>
<td>Risk of non-mucinous peritoneal dissemination</td>
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<tr>
<td>Mucinous adenocarcinoma</td>
<td>Colorectal adenocarcinoma with &gt;50% mucin</td>
<td>Risk of mucinous peritoneal dissemination</td>
</tr>
<tr>
<td>Signet ring adenocarcinoma</td>
<td>Poorly undifferentiated with signet ring cells and &gt;50% mucin</td>
<td>Risk of mucinous signet ring peritoneal dissemination</td>
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</table>
Figure 2. Low-grade appendiceal mucinous neoplasm (LAMN). The mucinous tumour has a highly differentiated mucinous epithelium and the nuclei are placed at the bottom of the cell and show only a minor degree of dysplasia. The tumour extends to the depth of the appendiceal wall with pushing border and it is no longer possible to identify the submucosa. With kind permission from Dr A Wanders.

Figure 3. Invasive non-mucinous colorectal adenocarcinoma. The glands infiltrate the colon wall by dissecting the stroma. The epithelium exhibits a moderate degree of dysplasia. The surrounding stroma is infiltrated by inflammatory cells.
Appendiceal peritoneal dissemination most often originates from a mucinous neoplasm. The PM produce mucinous ascites and can cause the clinical syndrome PMP\textsuperscript{43}. Historically, the origin of PMP was unknown but was suspected to arise from appendiceal or ovarian tumours. The tendency to metastasise to ovarian peritoneum is today considered the probable explanation for the suspected ovarian origin\textsuperscript{60}. However, ovarian teratomas can contain mucinous tumours and cause PMP\textsuperscript{72}. PMP classically arises from an underlying LAMN or mucinous adenocarcinoma with rupture or growth through the peritoneum, which releases neoplastic epithelial cells into the peritoneal cavity. This is followed by a “redistribution phenomenon” as described by Sugarbaker in 1994\textsuperscript{15}. First, the PM float freely in peritoneal fluid and circulate towards sites of absorption, the great omentum and below the right hemi-diaphragm. Second, gravitation leads to accumulation within the pelvis, right retrohepatic space, left abdominal gutter and at the ligamentum of Treitz. Peristaltic movement of the small intestine prevents adhesion but is finally seen in patients with late-stage PMP. PMP left untreated will lead to massive mucin accumulation which eventually causes intestinal and bile obstruction and death.

Histopathological classification of PMP has been problematic due to the uncertainty of its origin and the existence of both benign and malignant histopathological features. A modified version of the classification according to Ronnett\textsuperscript{42} has been used at our institution. Ronnett divides PMP into three subgroups: disseminated peritoneal adenomucinosis (DPAM), which is associated with scant presence of non-invasive neoplastic epithelium, minimal mitotic activity and a favourable prognosis (Figure 4A); peritoneal mucinous carcinomatosis (PMCA), which is associated with abundant neoplastic epithelium with pronounced atypia, mitotic activity and poor prognosis and in between, PMCA-intermediate (PMCA-I), which describes a condition with both DPAM and PMCA features. In addition, the modification includes peritoneal acellular mucin and signet ring cell containing metastases (PMCA-S), the latter associated with very poor prognosis. In 2016, the Peritoneal Surface Oncology Group International (PSOGI) published a consensus on PMP classification and recommend the use of low-grade and high-grade mucinous carcinoma peritonei, but the Ronnett- equivalent is an alternative and accepted classification\textsuperscript{42} (Table 2). Finally, non-mucinous appendiceal adenocarcinoma results in PM corresponding to usual non-mucinous colorectal PM (Figure 4B)\textsuperscript{41}.
Table 2. The histopathological classification of pseudomyxoma peritonei according to Ronnet\textsuperscript{42} and the Peritoneal Surface Oncology Group International (PSOGI)\textsuperscript{43}

<table>
<thead>
<tr>
<th>Modified Ronnett Classification</th>
<th>PSOGI classification</th>
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<tr>
<td>Acellular mucin</td>
<td>Acellular mucin</td>
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<tr>
<td>Disseminated peritoneal adenomucinosis (DPAM)</td>
<td>Low-grade mucinous carcinoma peritonei</td>
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<tr>
<td>Peritoneal mucinous carcinomatosis intermediate (PMCA-I)</td>
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<td>Peritoneal mucinous carcinomatosis (PMCA)</td>
<td>High-grade mucinous carcinoma peritonei</td>
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<tr>
<td>Peritoneal mucinous carcinomatosis with signet ring cells (PMCA-S)</td>
<td>High-grade mucinous carcinoma peritonei with signet ring cells</td>
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</table>

Figure 4. Disseminated peritoneal adenomucinosis (DPAM). The peritoneum is lined by a single layer of mucinous epithelium with a minor degree of dysplasia. Occasionally some small papillary formations of the epithelium can be seen (A). Non-mucinous colorectal peritoneal metastasis. The metastasis grows into the peritoneal wall in a cribiform manner. The nuclei are pleomorphic and hyperchromatic and with ongoing mitosis (B). With kind permission from Dr A Wanders.

Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy

History
Systemic chemotherapy alone has a limited effect on PM, since the “peritoneal-plasma-barrier” effectively prevents intravenously administered chemotherapy from reaching sufficient cytotoxic levels in the peritoneal cavity. This is especially true in patients with large tumour burden\textsuperscript{1}. The evolution of a combined treatment with aggressive tumour-reducing surgery and locally administered chemotherapy began in the 1930s when extensive
debunking surgery was proposed for treating ovarian PM. In the 1960s and 1970s, the extent of residual disease was found to be an important prognostic factor in advanced ovarian cancer\textsuperscript{73-75} and improved survival was reported in patients with PMP who underwent repeated surgery\textsuperscript{76}. At the same time, Shingleton et al. studied regional perfusion of chemotherapy in the abdomen. By occluding the aorta, inferior vena cava and azygos vein, the concentration of the chemotherapeutic agent was increased in the abdominal circulation\textsuperscript{77}. In addition, promising results of total body hyperthermia was reported in the late 1970s. Hyperthermia was created by wrapping the patient in a hot-water blanket and 50\% of the patients were reported to have disease regression\textsuperscript{78}. A combination of hyperthermia and a regional intraperitoneal perfusion technique was then introduced by Spratt et al. in the 1980s\textsuperscript{79}. This was soon followed by the first treatment of a patient with PMP\textsuperscript{80}. After that, the successful combination of intraperitoneal administration and small residual tumour burden after resection was confirmed for ovarian PM\textsuperscript{81, 82}, followed by gastrointestinal PM\textsuperscript{83}. To standardise and optimise CRS, Sugarbaker described the stepwise technique of the peritonectomies and visceral resections in 1995\textsuperscript{84}. During the past decades, the modality of intraperitoneal chemotherapy administration has varied from normothermic to hyperthermic, closed to open technique and postoperative repeated administration to peroperative administration only\textsuperscript{85}. Today, the open “Coliseum technique” described below is the most widely used\textsuperscript{86}.

Cytoreductive surgery

CRS aims to remove all disease-affected peritoneum and disease-affected organs to optimise the effect of HIPEC. The procedure consists of several peritonectomies and visceral resections, as previously described\textsuperscript{84}. Briefly, the operation begins with evaluation of resectability and estimation of peritoneal cancer index (PCI, see below). If the patient is resectable and a decision to proceed is taken, exposure is obtained by elevating and attaching the abdominal wall of the incision to a retractor ring for optimal visualisation and dissection. A diathermy is used for stripping the peritoneum from the abdominal wall, pelvic walls, and the diaphragm. Depending on the extent of macroscopic tumour growth, this is followed by visceral resections and resections of other tumour-affected tissues such as perihepatic ligaments. Commonly affected structures are the undersurface of the right hemi-diaphragm, right retrohepatic space, the ligament of Treitz, left abdominal gutter, pelvis, and greater omentum according to the “redistribution phenomenon” of PM. The peristaltic movements of the bowel prevent cell adhesion in the earlier stages\textsuperscript{15}. If present, small superficial nodular bowel and mesenteric metastases can be removed by diathermy and invasive metastases by segmental resections. However, diffuse small bowel
involvement is the most frequent cause of inoperable PM and the decision not to perform CRS and administer HIPEC.

Hyperthermic intraperitoneal chemotherapy
Microscopic tumours left in the peritoneal cavity after CRS are treated with HIPEC, which is performed immediately after cytoreduction, according to “the Coliseum” technique. “The Coliseum” is created by the elevated skin edges attached to the retractor ring and covered with plastic film. One inflow catheter is placed centrally in the abdomen and four closed suction drains are inserted through the lateral abdominal wall, allowing for intraperitoneal administration and circulation of hyperthermic chemotherapy. Prior to start of perfusion, the patients are cooled to 35°C. The inflow and outflow catheters are connected to a perfusion pump allowing intraperitoneal circulation of the chemotherapeutic agent for 30 to 90 minutes depending on the primary tumour and agent used. Meanwhile, the surgeon manually distributes the chemotherapeutic agent in the abdominal cavity and the intra-abdominal temperature is kept at 42°C. Desirable properties of the chemotherapeutic are sufficient tumour penetration and large molecular weight which limits systemic absorption. Several regimens of chemotherapy have been used but Oxaliplatin is frequently used for colorectal PM and Mitomycin C for PMP.

Surgical scores
The most important aspect of CRS is to achieve complete cytoreduction. Besides controlling resectability at common failure sites such as extensive small bowel involvement, the surgical score PCI can aid in the evaluation of overall resectability and potential benefit from proceeding with CRS and HIPEC. The PCI (range 1–39) is used to quantify tumour load in the abdominal cavity at the beginning of CRS. The PCI score is calculated by summing lesion size scores (LS, range 0–3) in 13 different abdominal regions: LS-0 = no visible tumour, LS-1 = tumour up to 0.5 cm, LS-2 = tumour up to 5 cm and LS-3 = tumour larger than 5 cm. Figure 5 shows an example of a peritoneal nodule assessed with the PCI. The PCI is a strong prognostic indicator and PCI scores higher than 20, 17, 15 and 10 have been suggested as cut-offs for proceeding with CRS and HIPEC in colorectal PM due to poor prognostic association. No consensus on cut-off has been reached since it is difficult to base the decision solely on PCI. However, patients with colorectal PM and high PCI scores are less likely to benefit from CRS and HIPEC compared to patients with PMP who can have a high PCI score and still benefit from surgery. Another important aspect of PCI is the difficulty to judge if there really is presence of neoplastic cells in the
macroscopically suspected metastasis or pool of mucin, especially when the “tumour burden” is small\textsuperscript{91}. Recent evidence suggests that proceeding with CRS and HIPEC could be unnecessary in some cases with limited extra-appendiceal mucin, even if neoplastic cells are present\textsuperscript{92}.

*Figure 5. Characteristic non-mucinous colorectal peritoneal metastases on the small bowel mesentery. With the patients informed consent.*

The completeness of cytoreduction is quantified by the completeness of cytoreduction score (CCS) (range 0–3)\textsuperscript{89}. The CCS describes the size of remaining tumours: CC-0 = no macroscopic residual disease, CC-1 = nodules less than 2.5 mm, CC-2 = 2.5 mm–2.5 cm and CC-3 = nodules larger than 2.5 cm\textsuperscript{89, 93}. The goal is to achieve CC-0 when all macroscopic tumour growth is resected and only microscopic tumour growth remains. For patients with PMP, CC-1 is considered acceptable and the prognosis corresponds to the prognosis of patients with complete cytoreduction\textsuperscript{6}. For patients with colorectal PM, scores higher than CC-0 are considered unsuccessful and palliative\textsuperscript{29, 94}, but HIPEC is sometimes administered. CC-2/3 corresponds to debulking surgery in both PMP and colorectal PM and HIPEC is usually not administered\textsuperscript{94}.
Aims of the thesis

The overall aim of this thesis was to study colorectal and appendiceal tumours with a focus on PM from a wide population-based perspective down to the detailed perspectives of histopathology and genetics.

The specific aims were:

I To study the prevalence and prognostic importance of absence of neoplastic epithelium in specimens from CRS, and to investigate the association with clinical and histopathological characteristics.

II To study the risk of colorectal and appendiceal cancer after non-surgical treatment of appendicitis.

III To identify risk factors associated with synchronous and metachronous PM in patients treated with an abdominal resection of appendiceal, colon or rectal adenocarcinoma.

IV To explore genome-wide CNA in colorectal PM, and identify alterations associated with prognosis in patients treated with CRS and HIPEC.
Materials and Methods

Patients and follow-up

Paper I
All patients with peritoneal surface malignancy treated with CRS and HIPEC at Uppsala University Hospital between January 2004 and December 2012 were included. Patients with inoperable disease, patients undergoing debulking surgery, patients with a PCI score of zero, and patients who received sequential postoperative intraperitoneal chemotherapy instead of HIPEC were excluded. The proportion of patients classified as neoplastic epithelium absent (NEA) was analysed for all primary tumours and the prognostic importance of NEA was analysed in patients with appendiceal and colorectal PM only. Information on vital status was retrieved from the Swedish Population Registry and information about recurrence and last follow-up was retrieved from medical records and from follow-up forms sent to the referring hospitals.

Paper II
All patients with non-surgical treatment of appendicitis in Sweden between 1987 and 2013 were included. Patients with appendicitis were identified from the Swedish National Inpatient Register using the Swedish version of the International Classification of Disease (ICD) codes (Appendix A)\(^{95, 96}\). Patients were excluded if a surgical procedure code\(^{97, 98}\) implying appendectomy was registered at time of appendicitis. Patients who developed bowel cancer were identified by ICD codes in the Swedish Cancer Registry and information on emigration and vital status was retrieved from the Register of Total Population and the Cause of Death Register, respectively. Patients were considered at risk until cancer diagnosis, surgical removal of the organ, death, emigration or end of study period (31 December 2013).
Paper III
All patients diagnosed with appendiceal, colon or rectal adenocarcinoma treated with a bowel resection in Sweden between January 2007 and February 2015 were included. Patients were identified from the Swedish Colorectal Cancer Registry. Information on the presence of PM at time of primary tumour diagnosis (synchronous PM) is entered into the registry as written descriptions of PM when the patient is diagnosed with cancer. Information on peritoneal recurrence (metachronous PM) and last follow-up is added to the registry at time of recurrence or planned follow-up.

Paper IV
All patients with non-PMP colorectal PM treated with CRS and HIPEC at Uppsala University Hospital between January 2004 and December 2015 were included. Patients with inoperable disease, patients undergoing debulking surgery, patients with a PCI score of zero, patients who received sequential postoperative intraperitoneal chemotherapy instead of HIPEC, and patients with primary appendiceal tumours and PMP were excluded. Information on vital status was retrieved from the Swedish Population Registry and information about recurrence and last follow-up was retrieved from medical records and from follow-up forms sent to the referring hospitals.

Surgical methods

Papers I and IV
The included patients were treated with CRS and HIPEC and fulfilled the general criteria of the procedure: adequate renal, liver and hematopoietic function; a WHO performance status of ≤2; and no distant metastases. Exceptions in the presence of liver metastases may have occurred in recent years as combined resection of liver metastases and CRS and HIPEC is under evaluation.

The CRS procedure was performed according to the descriptions made by Sugarbaker. Briefly, a diathermy was used for stripping of the peritoneum from underlying structures. This was followed by visceral resections, resections of other non-visceral tissues and resections of small superficial nodular metastases using diathermy or sharp dissection. All resections were performed depending on the extent of macroscopic tumour.

HIPEC was performed according to “the Coliseum” technique: one inflow catheter and four closed suction drains were inserted through the
abdominal wall, allowing for intraperitoneal administration and circulation of HIPEC. The intra-abdominal temperature was kept at 42°C while HIPEC was administered for 30-90 minutes depending on primary tumour and chemotherapeutic agent used.

The extent of macroscopic tumour load before start of CRS was quantified using PCI, (range 1–39). The size of remaining tumour after CRS was quantified using CCS (range 0–3)89.

Paper II

Non-surgical treatment of appendicitis was defined as no registered surgical procedure code97, 98 corresponding to any kind of removal of appendix, at time of appendicitis (Appendix B). If a surgical procedure code indicating appendectomy was registered after appendicitis, the patient was censored from that date.

Paper III

The included patients were treated with any of the following registered bowel resections: appendectomy, ileocecal resection, right hemicolectomy, resection of transverse colon, left hemicolectomy, resection of sigmoid colon, total colectomy, anterior resection and abdominoperineal excision of rectum.

Histopathology

Papers I and IV

Surgical specimens from CRS were fixed in 4% buffered formaldehyde and embedded in paraffin, sliced into 3 to 4 µm sections and stained with haematoxylin and eosin.

In Paper I, the absence or presence of neoplastic epithelium in surgical specimens was based on the histopathology report from CRS. Patients lacking neoplastic epithelium, with or without mucin, were classified as NEA and patients with neoplastic epithelium were classified as neoplastic epithelium present (NEP). Primary tumours were classified according to the World Health Organization12. A specification of presence or absence of mucin was done for NEA and a peritoneal histopathology diagnosis was defined for NEP41. PMP was classified according to Ronnett42 after new assessment of the early cases. This was done due to changes in the classification during the study period.
In Paper IV, all specimens were reviewed and regions of PM with the maximum tumour cell content were identified and marked. 10μm sections of the formalin-fixed paraffin-embedded (FFPE) specimens corresponding to the marked area were sliced for DNA-preparation. Colorectal PM were included if criteria of PMP were not fulfilled.41

Molecular biology

Paper IV

DNA was extracted from the FFPE sections using QIAamp FFPE Tissue Kit (QIAGEN) according to the manufacturer’s recommendations. DNA was quantified using Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific). The MinElute Reaction Cleanup Kit (50) (QIAGEN) was used for concentrating for samples with a low concentration of DNA.

The DNA microarray analysis was performed according to standard protocols for Affymetrix OncoScan® Arrays (Affymetrix OncoScan® FFPE Assay Kit User Guide (P/N 703175 Rev. 2), Affymetrix Inc.)99-101 based on molecular inversion probe (MIP) technology102 (Figure 6). The MIP technology was developed to enable analysing large numbers of short DNA sequences in parallel without compromising sensitivity and specificity. Briefly, a MIP consists of two regions complementary to target DNA, two polymerase chain reaction primer sites, two cleavage sites and one Tag sequence for array detection of the MIP. First, 80 ng of genomic double-stranded DNA was incubated overnight for annealing of the MIPs, which circularised the MIP. Second, each sample was then divided into two channels, one for adenine and thymine nucleotides and one for guanine and cytosine nucleotides and the gaps formed after the annealing process were filled with the complementary nucleotides and closed by a ligase. Third, a cleavage enzyme linearised the probe followed by amplification by polymerase chain reaction. The samples were then prepared for hybridisation onto the OncoScan® Array after digestion with the HaeIII enzyme. Hybridised probes were bound to streptavidin-phycoerythrin conjugates using GeneChip® Fluidics Station 450 (Affymetrix Inc.) and arrays were scanned using GeneChip® Scanner 3000 7G (Affymetrix Inc.). The DNA microarray analyses generate absolute intensities in fluorescence, and the allele-specific signals, corresponding to the MIPs, are used to determine the CN.
Figure 6. The principles of molecular inversion probe technology. The probe consists of two sequences complementary to target DNA (H1, H2), two polymerase chain reaction primer sites (P1, P2), two cleavage sites (X1, X2) and one array detection sequence (Tag) (A). The probe anneals to target DNA (B) and the gap is filled with nucleotides and is ligated (C). Exonucleases cleave the probe at X1, primers bind to P1/P2 and the linearised probe is amplified with polymerase chain reaction (D). Endonucleases cleave the amplified probes at X2 and the Tag sequences are hybridised onto the array.

The DNA microarray data (probe intensities) were normalised in relation to a set of diploid reference samples using Affymetrix OncoScan console 1.3 and segmented using BioDiscovery Nexus Copy Number 8.0 with the TuScan algorithm with default settings. Analyses of allele-specific CN and average ploidy were performed using Tumor Aberration Prediction Suite103, suitable for tumour samples containing a mixture of genetically normal and abnormal cells. In short, to visualise the CNA in a sample, average log ratio of the CN is plotted against allelic imbalance ratio for all segments (Figure 7A). The log ratio is the logarithm of the probe intensity and a log-ratio of zero corresponds to the median CN of the sample. An increased CN compared to the median leads to a log ratio >0 and a decreased CN of the sample leads to a log ratio <0. The allelic imbalance ratio reflects the relationship between the alleles; values close to zero indicate a balanced CN and values close to one indicate unbalanced CN. In reality, the allelic imbalance ratio is affected by all cells in the sample and normal cells with diploid CN result in a ratio shift towards zero. In addition, if the proportion
of tumour cells is low, the ratio is too close to zero for detecting CNA, which is illustrated in Figure 8C.

Figure 7. Log ratio of copy number plotted against allelic imbalance ratio for all segments of the genome for three patient samples. Sample ID 20 contained clearly separated clusters of segments with different copy numbers (A). Poor technical quality of sample ID 62 affected the ability to separate segments with different copy numbers (B) and sample ID 44 had too low tumour cell content (C). ID=identification number, CN=copy number, LOH=loss of heterozygosity.

Statistical analysis

General statistics
Descriptive data were presented as median and inter-quartile range. The chi-square test or Fisher’s exact test were used to compare proportions and the Mann-Whitney U test was used to compare continuous data of two groups. Survival rates and cumulative incidence were calculated using the Kaplan-Meier method and the log-rank test was used for analysing differences
between two groups. Univariate and multivariate logistic regression were used to identify categorical variables associated with PM and presented as odds ratio (OR) with 95% confidence interval (CI). Univariate and multivariate Cox proportional hazard regression were used to identify categorical variables associated with time to PM and presented as hazard ratio (HR) with 95% CI. P-value <0.050 was considered statistically significant. Statistical analysis was performed with SPSS version 22 (IBM, Armonk, New York, USA) and R version 3.1.2 and 3.41 (R foundation for Statistical Computing, Vienna, Austria).

Paper II
The incidence of cancer among patients with non-surgical treatment of appendicitis was compared with the total population of Sweden using standardised incidence ratio (SIR). The year of diagnosis, age, sex, and site-specific incidence rates were extracted from the Swedish Cancer Registry and multiplied with the accumulated person-years at risk in each stratum. The ratio of observed to expected number of cases was expressed as SIR with 95% CI and CIs were calculated assuming that the observed number of cases followed a Poisson distribution, and by using Byar’s normal approximation\(^{104}\). The SIR analyses were stratified by cancer diagnosis, time to cancer, attained age and diagnosis of appendiceal abscess or no abscess.

The numbers needed to treat with appendectomy to avoid one appendiceal cancer was estimated by comparing the probability of cancer between six months and 25 years after non-surgical treatment of appendicitis with the probability of cancer after appendectomy (=zero). The numbers needed to treat were calculated as the inverse of the absolute risk reduction.

Paper III
The register data retrieved from the Swedish Colorectal Cancer Registry contained missing data. A sensitivity analysis of the missing data was performed by running a multiple imputation by chained equations procedure using the fully conditional specification method\(^{105}\). Instead of randomly inserting one of the potential values, multiple imputation uses the observed data to estimate the values of the missing data. Multiple imputation by chained equations was chosen due to missing values occurring in several variables. This procedure replaces the missing values for one variable at the time based on the other observed values using logistic regression models. This is repeated for several cycles, to create one dataset. Five complete datasets were created and pooled in one dataset for calculation of OR and HR. The results were then compared with the original complete case analyses.
Paper IV

Tumour Aberration Prediction Suite\textsuperscript{103} was used to calculate frequencies of CNA (gain to \textgreater{}2 copies per cell, loss to \textless{}2 copies per cell) in the population and for short-term (\textlessthan{}=2 years) and long-term (\textgreater{}=2 years) survivors, over the whole genome.

Correlation between CNA in different parts of the genome and survival probability were calculated using the log-rank test. The genome was divided in segments of 10 Mbp and for each segment, the average CN was calculated and the population was grouped according to the CNA status in this segment (e.g. gain vs. no gain). The log-rank test was then used to calculate the probability of difference in survival between the groups. To correct for multiple testing and difference in sensitivity of the log-rank test for different group sizes, permutation testing with 50,000 replicates was used to determine the distribution of the smallest p-value when randomly assigning the individuals of the study population to groups based on simulated genome segments. This distribution of extreme p-values was then used to calculate empirical p-values for actual genomic segments\textsuperscript{106, 107}.

Multivariate Cox proportional hazard regression was used to determine the relative contribution to the hazard model of: CN gain on chromosome 15 (between 40 Mbp and 103), CN gain on chromosome 1 (between 120 Mbp and 130 Mbp), mean CN \textgreater{}=2.5, PCI, CCS in different combinations.

Ethical considerations

All four studies were approved by the regional ethics committee of Uppsala County, Sweden (Dnr 2013/203, Dnr 2014/421, Dnr 2014/512, Dnr 2015/396).
results

Paper I

Of the 353 included patients, all with macroscopically suspected PM, 78 (22%) had no neoplastic epithelium in surgical specimens from CRS. Mucin was found in 28 of the 78 patients with NEA. The distribution of primary tumours in relation to NEA and NEP is shown in Figure 8.

![Figure 8](image-url)

Figure 8. Primary tumour diagnoses in patients with neoplastic epithelium absent without mucin (n=50), neoplastic epithelium absent with mucin (n=28) and neoplastic epithelium present (n=275). DMPM=diffuse malignant peritoneal mesothelioma, LAMN=low grade appendiceal mucinous neoplasm.

Patients with LAMN/mucinous adenoma (n=91) and appendiceal/colorectal adenocarcinoma were further analysed (n=171) for differences in baseline characteristics and survival between NEA and NEP. There were no differences in age, gender and follow-up time between NEA and NEP. Preoperative chemotherapy was more common in patients with LAMN/mucinous adenoma and NEA. The tumour marker carcinoembryonic antigen was lower in NEA, as were median PCI scores. CC-0 was more
often achieved in NEA. The five-year overall survival and recurrence-free survival rates were 100% in patients with LAMN/mucinous adenoma and NEA. The five-year overall survival rate was 84% and the recurrence-free survival rate 59% for patients with NEP (Figure 9). The five-year overall survival rate for appendiceal/colorectal adenocarcinoma was 61% for NEA and 38% for NEP and the recurrence-free survival was 60% for NEA and 14% for NEP (Figure 10).

Figure 9. Overall- and recurrence-free survival for patients with low-grade appendiceal mucinous neoplasm (LAMN)/mucinous adenoma and neoplastic epithelium absent (n=31) and present (n=60). Only patients with CC-0 were included for recurrence-free survival analysis. A p=0.023, B p=0.001.

Figure 10. Overall- and recurrence-free survival for patients with appendiceal/colorectal adenocarcinoma and neoplastic epithelium absent (n=32) and present (n=139). Only patients with CC-0 were included for recurrence-free survival analysis. A p=0.056, B p=0.001.
Paper II

Of the 13,595 patients with non-surgical treatment of appendicitis, 352 (2.6%) were later diagnosed with small bowel, appendiceal, or colorectal cancer. Thirteen patients had an additional bowel cancer. Patients who developed cancer had a median age of 66 (IQR 54−75) compared to 38 (IQR 21−61) in patients without cancer at time of appendicitis. There were 52% men in the cancer group compared to 50% men in the non-cancer group. The majority of the patients with cancer had a complicated appendicitis with appendiceal abscess (63%) compared to 36% in patients without cancer. The majority of tumours were located in the right colon (n=228), followed by appendix (n=50), left colon (n=30), rectum (n=26) and the small bowel (n=12). Adenocarcinoma was the most common histopathology (77%), but neuroendocrine tumours were common in the small bowel (n=58%) and appendix (n=16%). Time to cancer was less than three months in 44% of the patients.

Patients with non-surgical treatment of appendicitis had a greater incidence of bowel cancer than expected in the general population (SIR 4.1, 95% CI 3.7−4.6). The largest incidence increase was observed for cancer in the appendix (SIR 35, 95% CI 26−46) followed by right-sided colon (SIR 7.5, 95% CI 6.6−8.6) and small bowel (SIR 3.2, 95% CI 1.6−5.6). The risk of left-sided colon cancer and rectal cancer was not increased. The observed and expected events of cancer per 1000 person-years and SIR in relation to time since appendicitis are shown in Figure 11. The short-term incidence of cancer (within 6 months) was increased for all sites and then gradually decreased with time since appendicitis. Patients with right-sided colon cancer had an elevated risk of cancer up to five years after appendicitis (SIR 3.5, 95% CI 2.1−5.4).

The number needed to treat with appendectomy to prevent one appendiceal cancer was 375 (95% CI 237−894).
Of the 35,120 included patients, 327 patients had appendiceal cancer, 24,399 patients had colon cancer and 10,394 patients had rectal cancer. The 35,120 patients were included for analysis of factors associated with synchronous PM. A total of 894 (2.5%) patients had synchronous PM, which was most common in appendiceal cancer (n=77, 23.5%), followed by colon cancer.
The number of patients with appendiceal cancer was too small to perform multivariate risk analyses. Descriptively, the majority of appendiceal tumours were mucinous (n=204, 62.4%) and the most common tumour stage was T4 (n=142, 43%). The proportion of mucinous (70% vs. 60%), T4 tumours (79% vs. 32) and N2 node stage (20% vs. 6%) were larger in patients with synchronous PM. Radical surgery was more common in patients without synchronous PM (71% vs. 20%). For colon cancer, female gender, age <60, right-sided tumour, T3 and T4 tumour stages, N1, N2 and NX node stages, perineural invasion, vascular invasion, mucinous histopathology and emergency surgery were independent risk factors for synchronous PM (Table 3). For rectal cancer, proximal tumour (OR 2.37, 95% CI 1.15−4.90), T4 tumour stage (OR 19.12, 95% CI 5.52−66.24), NX node stage (OR 5.29, 95% CI 1.29−21.73) and mucinous tumour (2.89, 95% CI 1.55−5.36) were associated with synchronous PM. The five-year overall survival rate was 14.9% in patients with synchronous PM and 64.0% in patients without synchronous PM. Patients with synchronous PM who did not undergo bowel surgery (not included in risk analyses) had a five-year overall survival rate of 1.6% (n=576). Of these, the five-year overall survival was highest in patients with appendiceal PM (40.8%, n=14), while patients with colonic PM (0%, n=467) and rectal PM (1.1%, n=95) had poorer survival.

After excluding patients with <1 year since diagnosis, patients who were dead within 30 days after surgery and patients lacking date of metachronous PM, 27,255 patients were included for analysis of factors associated with metachronous PM. Of patients with appendiceal metachronous PM (n=10, 4.8%), six had T4 tumours and two had T3 tumours, one had N2 node stage and four had N1 node stage, and five had mucinous tumours. Colonic metachronous PM (n=263, 1.4%) was associated with right-sided tumour (HR 1.68, 95% CI 1.29−2.19), T4 tumour stage (HR 5.64, 95% CI 2.80−11.36), N1 (HR 2.07, 95% CI 1.49−2.88) and N2 (HR 3.48, 95% CI 2.51−4.83) node stages, vascular invasion (HR 1.74, 95% CI 1.28−2.37), mucinous histopathology (HR 1.54, 95% CI 1.16−2.04) and emergency surgery (HR 1.92, 95% CI 1.47−2.51). Rectal metachronous PM (n=64, 0.7%) was associated with T4 (HR 17.66, 95% CI 5.00−62.33) and T3 (HR 4.55, 95% CI 1.38−15.07) tumour stages and mucinous histopathology (2.69, 95% CI 1.53−4.73). Cumulative incidences of metachronous PM are shown in Figure 12.
Table 3. Univariate and multivariate logistic regression analysis of the risk of synchronous peritoneal metastases in patients with colon cancer (n=24,399).

<table>
<thead>
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<th>Univariate analysis</th>
<th>Multivariate analysis&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p-value</td>
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<tr>
<td><strong>Sex</strong></td>
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<td>1.00</td>
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<tr>
<td>Female</td>
<td>1.28 (1.10 – 1.48)</td>
<td>0.001</td>
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<tr>
<td><strong>Age</strong></td>
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<td>&gt;80</td>
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<td>70-79</td>
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<tr>
<td>60-69&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.00</td>
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<tr>
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<td>2.19 (1.66 – 2.90)</td>
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<tr>
<td>Right colon</td>
<td>1.28 (1.10 – 1.49)</td>
<td>0.001</td>
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<tr>
<td>Left colon&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.00</td>
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<tr>
<td>Colon NOS</td>
<td>8.06 (2.71 – 23.96)</td>
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<td><strong>Tumor stage</strong></td>
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<td>T1</td>
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<tr>
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<td>1.00</td>
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<td>T3</td>
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<td>56.26 (25.14 – 125.93)</td>
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<td>T0</td>
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<tr>
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<td>N2</td>
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<tr>
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<td>13.04 (7.50 – 22.68)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Differentiation grade</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well/moderately&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Poorly</td>
<td>2.69 (2.27 – 3.18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Not specified</td>
<td>2.29 (1.61 – 3.24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Perineural invasion</strong></td>
<td></td>
<td></td>
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<tr>
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<td>1.00</td>
<td>1.00</td>
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<tr>
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<td>4.22 (3.55 – 5.00)</td>
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<tr>
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<td>1.84 (1.53 – 2.20)</td>
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<tr>
<td><strong>Vascular invasion</strong></td>
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<td></td>
</tr>
<tr>
<td>No&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.00</td>
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<tr>
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<td>5.23 (4.42 – 6.19)</td>
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<td>3.33 (2.66 – 4.17)</td>
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<td></td>
</tr>
<tr>
<td>Non-mucinous&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.00</td>
</tr>
<tr>
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<td>2.05 (1.74 – 2.40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Not specified</td>
<td>1.17(0.90 – 1.51)</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Perforation (close to tumour)</strong></td>
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<td></td>
</tr>
<tr>
<td>No&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.00</td>
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<tr>
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<td>2.19 (1.38 – 3.46)</td>
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</tr>
<tr>
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<td>1.00</td>
</tr>
<tr>
<td>Emergency</td>
<td>2.83 (2.44 – 3.28)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Complete case analysis (missing=327), adjusted for all significant variables in the univariate analysis except differentiation grade. <sup>b</sup>Reference group. OR=odds ratio, CI=confidence interval, NOS=not otherwise specified.
Figure 12. Cumulative incidence of metachronous peritoneal metastases stratified by primary tumour localisation (A). Cumulative incidence of colon cancer PM stratified by: tumour T stage (B), node N stage (C) and the combination of risk factors T4 tumour stage, N2 node stage and mucinous histopathology (D).

Paper IV

Inclusion criteria were fulfilled for 114 patients after histopathological examination but CN analysis was unsuccessful in 61 of these patients due to inability to extract sufficient amounts of DNA, due to signals caused by interstitial components, and due to the influence from normal-cell DNA in the analyses. Individuals with unsuccessful analysis were more likely to have appendiceal cancer, lower PCI and lower carcinoembryonic antigen levels. There was no statistically significant difference in overall survival between individuals with successful (n=53) and unsuccessful CN analysis (n=61) (p=0.676). The only patient with appendiceal cancer and successful CN
analysis was excluded in the final analysis for homogenising the material, leaving 52 patients for final analysis.

The frequency of CN gain and loss at each position in the 52 patients with colorectal PM is shown in Figure 13. Overall, there was extensive CNA affecting large proportions of the genome.

Figure 13. Copy number frequency in patients with colorectal peritoneal metastases. Green indicates >2 copies per cell; orange indicates <2 copies per cell.

To detect potential correlations between CNA and prognosis, the population was divided into short-term survivors (≤2 years) and long-term survivors (>2 years) after CRS and HIPEC and compared with respect to frequency of gain/loss in CN. Short-term survivors had an overall higher frequency of gains, and gains on parts of chromosome 1p and a majority of chromosome 15q were associated with short-term survival (p≤0.005). There were no significant losses associated with survival.

In an alternative approach to identify CNA associated with prognosis, the population was separated by CN status in all 10 Mbp segments of the genome and analysed with the log-rank test. When adjusting for multiple testing and using empirical p-values, gain in parts of chromosome 1p (120–130 Mbp) and 18p (20–30 Mbp) and gain on major parts of chromosome 15q (40–103 Mbp) were found to be significantly associated with shorter survival (empirical p-value <0.05, Figure 14). Gain on chromosome 18p was found only in individuals who also had gain on 1p. In addition, a significant association between having gain on both 1p and 15q (p=0.002) and on both 1p and 18p (p-value= 0.0004) were found. The gain of chromosome 1p included the colorectal cancer-associated genes REG4 and NOTCH2 and gain on 15q included the genes MAP2K1, SMAD3, SMAD6 and IGF1R, among others.
Figure 14. Probability of association between gain within segments of 10 Mbp and difference in survival. Values are given as empirical p-values corrected for multiple testing (A). Survival probability in individuals with and without gain in any 10 Mbp segment of chromosome 1 (120-130 Mbp), chromosome 15 (40-103 Mbp) and chromosome 18 (20-30 Mbp) (B). Chr=chromosome

The genomic composition ranged from minimal CNA (diploid, CN 2) to almost total aneuploidy. Individuals with no gain in chromosome 1p or 15q had a mean CN of 2.24. Individuals with either gain on chromosome 1p or both 1p and 15q had a higher mean CN than individuals with no gain (Figure 15).

Figure 15. Percent of probes per copy number and mean copy number for the 52 individuals with colorectal peritoneal metastases, separated by gain or no gain in any segment of chromosome 1 (120-130 Mbp), chromosome 15 (40-103 Mbp), or both.

In a multivariate hazard model, gain on chromosome 15 (HR 7.42, 95% CI 2.28–24.2) and gain on both chromosome 1 and 15 (HR 16.39, 95% CI 5.66–47.5) were independently associated with short survival. A mean CN>2.5 was not an independent prognostic factor. When including PCI and
CCS in the hazard models, the association between poor prognosis and gain on chromosome 15 and gain on both chromosomes 1 and 15 remained. PCI and CCS were also associated with poor prognosis.
Discussion

Peritoneal dissemination is a heterogeneous condition ranging from the more common and aggressive colorectal PM to the rare appendiceal PM with mucin accumulation that slowly causes failure of normal gastrointestinal functions. Since CRS and HIPEC became an established treatment option, peritoneal dissemination is no longer an incurable condition. However, for the majority of patients, the outcome is still fatal, and some patients do not seem to experience any effect from the treatment. To prolong survival and avoid unnecessary morbidity, there is need for improving patient selection, optimising the treatment and increasing the overall knowledge of this heterogeneous condition.

In Paper I, the prevalence of no neoplastic epithelium in surgical specimens from CRS was analysed and found to be surprisingly high and associated with a favourable prognosis. All these patients were judged to have a PCI score >0 corresponding to macroscopic peritoneal tumour burden and the results illustrate the difficulty of assessing peritoneal lesions macroscopically during surgery. The main question is if this difficulty resulted in these patients undergoing unnecessary surgery? Since the absence of neoplastic epithelium can have several explanations, it is not possible or correct to say unnecessary surgery. First, complete response after preoperative chemotherapy has previously been described for colorectal and appendiceal PM and could explain some of the cases with adenocarcinoma and NEA\textsuperscript{108, 109}. Second, the histopathological characteristics of the primary tumour partly explain NEA. A common finding in patients with LAMN is extra-appendiceal mucin without neoplastic epithelium\textsuperscript{43}. In this study, patients with LAMN and NEA had 100% five-year overall and recurrence-free survival, indicating either that the treatment was very effective in these patients, or that there was no established peritoneal dissemination. More recently, definitions of PMP more clearly state that acellular mucin limited to the right-lower quadrant is not automatically PMP unless the clinical picture is characteristic\textsuperscript{43, 71}. Recent evidence also suggest that proceeding with CRS and HIPEC could be unnecessary in some cases of LAMN, even if extra-appendiceal neoplastic cells are present\textsuperscript{92}. Third, some of the NEA in this patient cohort might be a reflection of a shift towards altered patient selection and treating minimal disease or in some cases prophylactic surgery. Irrespective of reason for the absence of neoplastic epithelium, it is a
common finding in patients undergoing CRS and HIPEC and it is associated with a favourable prognosis. The results indicate that increased awareness and reporting of NEA is needed for interpretation and understanding of differences in survival between reported series and for further optimisation of patient selection.

In Paper II, the association between an inflamed appendix left in the abdomen and cancer incidence was investigated. The short-term incidence of cancer was increased for all sites (small bowel to rectum), especially close to the appendix. The long-term incidence of right-sided colon cancer was still elevated five years after non-surgical treatment of appendicitis. A reverse causation (malignancy causing signs of appendicitis) is the probable explanation for the increased incidence of cancer in the early time period after appendicitis. This illustrates the limitations of radiological methods when it comes to visualising different pathologies in the ileocecal region and is especially important in an era of increased interest in non-surgical treatment options for appendicitis. The large proportion of patients with appendiceal abscess in the cohort is a reflection of the initial treatment with drainage or antibiotics and the cohort is therefore pooled towards complicated appendicitis. However, the incidence of bowel cancer was increased both for appendicitis with and without abscess. Likewise, with increasing age and number of comorbidities, there is a shift towards non-surgical treatment, which results in an older population with increased incidence of cancer. However, the incidence of cancer was increased for all age groups. The increased long-term risk of right-sided colon cancer could be the result of an inflammatory environment and its effect on premalignant lesions corresponding to the role of inflammation in colorectal cancer carcinogenesis\textsuperscript{37, 38}. The decreased incidence of cancer with distance to the appendix is further in line with the hypothesis that a local immunological process could contribute to carcinogenesis in the area. Finally, the ideal comparison group would have been patients undergoing appendectomy. Unfortunately, the main organ of interest would then have been resected and the diagnosis codes would have been affected by surgical findings with subsequent risk of low tumour incidence in the cohort. The safest was therefore to do a standardised comparison with the general population. The results can be compared with results of Song et al., who compared the risk of cancer among appendectomised patients with the general population of Sweden and found only a modest increase of colon cancer 5–14 years after appendectomy\textsuperscript{69}. In all, even though the chain of causation is not established after this population-based study, patients with non-surgical treatment of appendicitis have an increased incidence of bowel cancer, which should be taken into consideration before choosing not to perform an appendectomy.

In Paper III, risk factors for synchronous and metachronous PM was identified with the aim of aiding in the detection of PM at earlier stages.
Colorectal cancer is often studied as a single entity even though there are distinct differences in histopathology and pattern of dissemination between appendiceal, colon and rectal cancer\textsuperscript{31, 34}. Therefore, risk factors for PM were studied separately for appendiceal, colon and rectal cancer unlike previously reported studies\textsuperscript{20, 22, 23, 40}. PM were most common in appendiceal cancer, followed by colon cancer, and least common in rectal cancer. Appendiceal cancer constituted less than 1% of the cohort, which restricted the ability to perform multivariate analyses. However mucinous T4 tumours were common in these patients. For colon cancer, a right-sided tumour, advanced tumour and node stage, vascular invasion, mucinous histopathology and emergency surgery were main risk factors for PM. T4 tumour stage is an intuitive risk factor for PM as it is thought to develop when tumour cells shed into the peritoneal cavity\textsuperscript{11}. T3 tumour stage and advanced nodal stage indicate that tumours with general high-malignancy characteristics may be capable of peritoneal dissemination and confirm that free cancer cells can invade both “transmesothelially” and “translymphatically”\textsuperscript{11}. Rectal cancer more seldom has mucinous histopathology and preferably metastasises to the liver and lungs\textsuperscript{31, 34}. Accordingly, PM were uncommon, but when present, PM were associated with T4 tumour stage, mucinous histopathology and a proximal tumour. The prevalence of synchronous PM was 2.5% compared to previously reported prevalence of 4%–7\%\textsuperscript{20-23}. This could partly be explained by the exclusion of patients with inoperable disease. However, the main explanation is underreporting of PM to the Swedish Colorectal Cancer Registry. The reporting was dependent on writing descriptions of PM and not easily registered with check boxes as for liver and lung metastases. Although the prevalence of PM was low, this is to the author’s knowledge the largest study on risk factors for PM and reliable risk factors were identified. These risk factors should increase awareness of potential peritoneal dissemination during primary surgery for high risk tumours as well as during follow-up and might aid in upcoming proactive treatment strategies.

In Paper IV, genome-wide CNA in colorectal PM were explored and CNA associated with prognosis were identified. Although CNA and other genetic alterations in primary colorectal tumours have been thoroughly described by the Cancer Genome Atlas Network\textsuperscript{47}, CNA in PM are more or less unexplored. In this study, the peritoneal CNA ranged from normal diploid to almost total aneuploidy. This probably reflects MSI tumours (few CNA) and CIN tumours (extensive CNA) in the studied population. The pattern of CNA resembled the pattern seen in primary tumours\textsuperscript{47}, but gains were more common and present for almost all chromosomes. The difference between primary tumours and PM suggest that metastases acquire additional genetic alterations. In addition, it is probably not enough to analyse the primary tumour when aiming to predict prognosis or response to therapy and
the crucial next step in the field of PM is to identify reliable predictive biomarkers. In this study, CNA in the form of gain on chromosome 1p and 15q were associated with very poor prognosis after CRS and HIPEC and were also found to be independently associated with poor prognosis when analysed together with the well-established prognostic factors PCI and CCS. These patients also exhibited the most pronounced CNA which could indicate whole genome duplication\textsuperscript{110}. The prognostic impact of a CN $>$2.5 was analysed to assess if the association of gain on 1p and 15q were mainly attributed to whole genome duplication but CN$>$2.5 was not an independent prognostic factor. Although gain on 1p and 15q were clearly associated with poor prognosis in patients with successful CN analysis, the CNA remains unknown for the other half of the included patients. The method was limited by signals caused by normal cells and interstitial components and gain on 1p and 15q has to be further explored for these patients. However, there was no difference in survival between patients with successful and unsuccessful analysis, which strengthens the likelihood that the results could play an important predictive role in the future and thereby prevent unnecessary surgery-related morbidity.
Conclusions

I A substantial proportion of patients undergoing CRS and HIPEC have surgical specimens lacking neoplastic epithelium. These patients have a favourable prognosis and decreased risk of recurrence. Increased awareness and reporting of NEA is needed for interpretation of differences in survival between reported series and for further optimisation of patient selection.

II Patients with non-surgical treatment of appendicitis have an overall increased incidence of bowel cancer, especially appendiceal and right-sided colon cancer. This should be taken into consideration in the overall discussion about optimal management and follow-up of appendicitis.

III Appendiceal cancer, right-sided colon cancer, advanced tumour and node stages, mucinous histopathology and vascular invasion are high-risk features for PM. These risk factors should increase awareness of potential peritoneal dissemination during primary surgery and follow-up and might aid in upcoming proactive treatment strategies.

IV Colorectal PM exhibited a wide range of CNA, which were more pronounced in PM compared to primary tumours. Gains on parts of chromosome 1p and 15q were significantly associated with poor prognosis after CRS and HIPEC and are potential prognostic molecular biomarkers for future patient selection.
To be able to prolong survival or even cure patients with metastatic growth affecting peritoneum and adjacent intra-abdominal organs is a great advancement for cancer research and health care. The present thesis provides new insights into the field of colorectal and appendiceal PM and highlight the fact that there are still questions on what to treat, who to treat, and when to treat with CRS and HIPEC.

“What to treat” refers to the heterogeneous histopathology of colorectal and appendiceal PM, sometimes even without neoplastic epithelium. Confirmed DPAM and PMCA or a clinical presentation of characteristic PMP are strong indications for CRS and HIPEC. But how strong are the indications for a ruptured LAMN with extra-appendiceal acellular mucin or widespread signet ring-containing PM? Patients with LAMN and extra-appendiceal acellular mucin had a five-year overall and recurrence-free survival of 100% and similar results have been reported by others. In contrast, patients with signet ring-containing PM have a very poor prognosis, often reported with a median survival of 7–14 months after CRS and HIPEC. In the case of LAMN, it is possible to perform a randomised study to come closer to the answer. In the case of signet ring, a feasible next step would be to sharpen patient selection criteria by performing larger prognostic studies. In any case, routine preoperative biopsy of PM before deciding to perform CRS and HIPEC would contribute to further understanding.

“Who to treat” more specifically refers to patients undergoing CRS and HIPEC but suffering from almost immediate relapse without obvious explanation. Beforehand, no histopathological or clinical factors predict the non-response to therapy. This illustrates one of the most important future tasks: To increase knowledge in molecular biology and PM and to identify predictive markers. The mutational landscape of PM is probably at least as heterogeneous as the histopathology and clearly plays an important role in the response to therapy. Although precision medicine and targeted therapy are not in the nearby future, there is where we are heading. To begin with, the importance of gain on chromosome 1p and 15q will be further investigated.

Finally, “when to treat” refers to upcoming proactive treatment strategies. One major difficulty is to diagnose colorectal PM at an early and treatable
stage. Risk factors for colorectal PM can aid but some advocate second-look surgery or prophylactic HIPEC. Second-look surgery after primary resection of high-risk colorectal tumours has been shown to identify patients with PM at a treatable stage and prolong survival\textsuperscript{113}. Prophylactic HIPEC would be administered at time of primary resection of high-risk tumour, prevent free cancer cells from adhering and thereby preventing PM. If ongoing randomised studies on second-look surgery and prophylactic HIPEC turn out in favour of proactive treatment, it is likely to once again drastically change the overall management of PM.
Svensk sammanfattning (Summary in Swedish)

Bakgrund

Syftet med denna avhandling var att fördjupa kunskapen inom tjock- och ändtarmscancer och blindtarmscancer med fokus på spridning till bukhinnan för att ytterligare bidra till förlängd överlevnad för dessa patienter. Detta har gjorts både utifrån ett brett befolkningsperspektiv och utifrån patologins och genetikens detaljerade perspektiv.

**Delarbete I**

I denna studie inkluderades patienter med spridning till bukhinnan och som genomgått behandling med CRS och HIPEC på Akademiska sjukhuset i Uppsala mellan januari 2004 och december 2012. Bortopererad vävnad från CRS granskas rutinmässigt av patolog för att få en mikroskopisk beskrivning av spridningen. Utifrån dessa beskrivningar analyserades hur vanligt förekommande avsaknad av cancerceller varit i den bortopererade vävnaden. Hos 78 av de 353 (22%) inkludera de patienterna kunde inga cancerceller på bukhinnan påvisas. Detta trots att patienterna för ögat hade tydlig tumörväxt eller slem, vilket är vanligt förekommande vid denna typ av spridning. Patienter med avsaknad av cancerceller hade bättre prognos. Ingen av patienterna med en särskild form av blindtarmstumör och avsaknad av cancerceller hade återfall av sjukdomen och alla levde efter fem år. Denna avsaknad av cancerceller kan delvis förklaras av att några patienter hade fått förbehandling med cellgifter och haft effekt men detta är ovanligt vid spridning till bukhinnan. Resterande fall förklaras istället av att blindtarmstumörer med misstänkt spridning till bukhinnan i form av slemansamlingar, antingen hade haft mycket god effekt av behandlingen eller aldrig hade någon etablerad spridning. Oavsett orsak är det viktigt att andelen patienter utan cancerceller på bukhinnan rapporteras och studeras ytterligare så att vi i framtiden kan våga avvakta behandling och därmed undvika onödig behandlingsrelaterad sjuklighet.

**Delarbete II**

I delarbete II var syftet att få ökad förståelse kring varför en del människor drabbas av den ovanliga cancerformen blindtarmscancer. Det är redan känt att tjock-och ändtarmscancer kan orsakas av långvarig inflammation i tarmen men det är okänt om en inflammerad blintarm kan bidra till uppkomst av cancer i blindtarmen eller närliggande tarm. Utifrån svenska patientregistrets diagnoskoder identifierades alla de individer i Sverige som mellan 1987 och 2013 diagnosstiserades med blindtarmsinflammation. De patienter som sedan saknade en operationskod motsvarande att blindtarmen opererats bort inkluderades. Förekomsten av tarmcancer i denna patientgrupp jämfördes...

Delarbete III

I denna studie inkluderades alla patienter som opererats för blindtarms-, tjock- och ändtarmscancer i Sverige mellan januari 2007 och februari 2015. Information om dessa patienter inhämtades från Svenska kolorektalcancerregistret, som är ett register för uppföljning av vårdens kvalitet och som också används för forskningsändamål. Information som inhämtades var allmän information såsom diagnosdatum, kön och ålder vid cancerdiagnos samt tumörrelaterad information såsom tumörens växtsätt, tumörens mikroskopiska karaktäristika, eventuell spridning till olika organ och information om eventuella återfall. Syftet med studien var att genomföra riskanalyser för att identifiera vilka av dessa allmänna och tumörrelaterade faktorer som var associerade med spridning till bukhinnan. Av de 35120 patienter som inkluderades hade 894 patienter spridning till bukhinnan vid diagnos. Det var vanligast hos patienter med blindtarmscancer (77 av 327, 23.5%), följt av tjocktarmscancer (759 av 24399, 3.1%) och minst vanligt vid ändtarmscancer (59 av 10395, 0.6%). Resultatet från riskanalyserna visade att tumörer som växer ut genom tarmsllemhinna och tumörer med slemproduktion var associerat till bukhinnespridning vid alla tre tumörformer. Övriga riskfaktorer sågs framförallt vid tjocktarmscancer och innefattade lymfkörtelspridning, tumör som växer in i kärl och nerver, kvinnligt kön och en ålder yngre än 60 år vid diagnos. Genom att känna till vanliga risker för spridning till bukhinnan kan operation av huvudtumören planeras bättre och spridningen upptäckas tidigare och därmed vara behandlingsbar med CRS och HIPEC.
Delarbete IV


Konklusion

Denna avhandling om tjock- och ändtarmscancer och blindtarmscancer med spridning till bukhinnan har bidragit med kunskap som kan användas i förebyggande syfte, i syfte att förbättra behandlingsstrategier hos de som redan är drabbade och i syfte att undvika att förvärra en redan allvarlig situation: genom att operera bort inflamerade blindtarmar kanske vi kan förhindra några cancerfall; genom att känna till vanliga risker för spridning till bukhinnan kan vi diagnosticera spridningen i tid; genom ökad kunskap om de olika typerna av bukhinnespridning kan vi hitta de patienter som verkligen behöver behandling med CRS och HIPEC. Slutligen kan genetiska analyser få en avgörande framtidiga roll när det gäller val av behandlingsmetod för den enskilde patienten och finnas som underlag för att avstå behandling när det inte väntas göra nytta.
Acknowledgements

I wish to express my sincere gratitude to all those who have contributed to make this thesis possible:

Associate Professor Helgi Birgisson, my main supervisor, for inspiring and enthusiastic guidance through all phases of my doctoral studies. I am impressed by your knowledge, optimism, and your attention to details, and I believe that one of the most important factors for successful doctoral studies is the main supervisor.

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All unnamed who in one way or another have contributed to the accomplishment of this work.

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Jan, Pia, Per, Maja and Mats, for all our joyful memories and traditions.

Lennart and Anita, and Lilian and Lars, my grandparents and greatest supporters in life, for having created many of my happiest moments.

My sister and brother-in-law, Anna Pia and Kalle, and my sister Lovisa, for your love and support and for sharing life with me.

My parents, Gunilla and Per, for your love and never-ending support. My research in cancer and surgery is the result of me being a combination of you. I admire you more than words can describe and thank you for everything.

Finally, my husband Viktor, for being the love of my life. Without your never-ending love, support, patience, intelligence and encouragement, this thesis would not have been. Thank you!
Appendix A

Diagnosis codes for appendicitis according to Swedish version of the International Classification of Disease codes\textsuperscript{95, 96} (Paper II)

<table>
<thead>
<tr>
<th>ICD-10</th>
<th>ICD-9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>K35 Acute appendicitis</strong></td>
<td><strong>540 Appendicitis</strong></td>
</tr>
<tr>
<td>K351 Appendiceal abscess</td>
<td><strong>540A Acute appendicitis with generalised peritonitis, perforation or rupture of appendix</strong></td>
</tr>
<tr>
<td>K352 Acute appendicitis with generalised peritonitis</td>
<td><strong>540B Acute appendicitis with peritoneal abscess</strong></td>
</tr>
<tr>
<td>K353 Acute appendicitis with localised peritonitis</td>
<td><strong>540X Acute appendicitis without information on peritonitis</strong></td>
</tr>
<tr>
<td>K358 Other not specified acute appendicitis</td>
<td><strong>541X Unspecified appendicitis</strong></td>
</tr>
<tr>
<td><strong>K36 Other appendicitis</strong></td>
<td><strong>542X Other appendicitis</strong></td>
</tr>
<tr>
<td><strong>K37 Unspecified appendicitis</strong></td>
<td><strong>542X Other appendicitis</strong></td>
</tr>
</tbody>
</table>
Appendix B

Surgical procedure codes for removal of appendix according to Swedish version of NOMESCO Classification of Surgical Procedures\textsuperscript{97,98} (Paper II).

<table>
<thead>
<tr>
<th>Current procedures</th>
<th>Historical procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>JEA00 Appendectomy</td>
<td>4510 Appendectomy</td>
</tr>
<tr>
<td>JEA01 Laparoscopic appendectomy</td>
<td>4511 Appendectomy with drainage</td>
</tr>
<tr>
<td>JEA10 Appendectomy with drainage</td>
<td>4520 Inversion of appendix</td>
</tr>
<tr>
<td>JEW96 Other operation on appendix</td>
<td>4599 Other operations on appendix</td>
</tr>
<tr>
<td>JEW97 Other laparoscopic operation on appendix</td>
<td></td>
</tr>
<tr>
<td>JFB20 Ileocecal resection</td>
<td>4642 Ileocecal resection</td>
</tr>
<tr>
<td>JFB21 Laparoscopic ileocecal resection</td>
<td>4641 Right hemicolecotomy</td>
</tr>
<tr>
<td>JFB30 Right hemicolecotomy</td>
<td>4650 Total colectomy and ileorectostomy</td>
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<td>JFH00 Total colectomy with ileorectal anastomosis</td>
<td>4652 Proctocolectomy and ileostomy</td>
</tr>
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<td>4654 Total colectomy with ileoanal anastomosis</td>
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<td>0059 Inversion of appendix “en passant”</td>
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<td>JFH96 Other total colectomy</td>
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References


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