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Kristen Brantley Date

Serum Lipid Levels and Colorectal Cancer Recurrence

By

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Master of Public Health

Epidemiology

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Serum Lipid Levels and Colorectal Cancer Recurrence

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B.S., The University of North Carolina at Chapel Hill, 2012

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Abstract

Serum Lipid Levels and Colorectal Cancer Recurrence
By Kristen Brantley

Background: Biologic and epidemiologic evidence suggests that tumor cells depend on reprogrammed lipid metabolic function for survival and growth. Mechanistically, cholesterol and triglycerides may support tumor recurrence by providing energy needed for future proliferation. Altered serum lipid profiles have been observed in cancer patients at diagnosis and throughout treatment, and studies have found associations of serum lipids with cancer incidence, mortality, and disease-free mortality. Lipids may be particularly relevant in colorectal cancer (CRC) progression, though studies have yet to evaluate the prognostic potential of serum lipids for CRC recurrence.
Methods: A prospective cohort design was used to study the effect of serum lipids, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG), on CRC recurrence-free survival. The study actively followed 342 Danish colorectal cancer patients who underwent surgical resection between 2003-2011 from date of surgery until December 31, 2012, or death. Sixty patients experienced a recurrence, with a median follow-up time of 4.1 years (interquartile range [IQR] 2.3, 6.8). Serum lipids were collected at scheduled intervals throughout follow-up, and recurrence rate was assessed using Cox proportional hazards modeling. Lipids were assigned as time-varying exposures evaluated in the year preceding recurrence and models were adjusted for clinically relevant covariates. A simplified analysis was performed by excluding influence of statin use on results via censorship of patients at first prescription. All-cause and CRC-cause mortality were also assessed as outcomes of interest.
Results: Among 342 CRC patients, increased HDL-C appeared to have a beneficial impact on recurrence-free survival (RFS) for CRC patients, though protection was only observed among statin users (hazard ratio [HR]=0.80; 95% confidence interval [CI]: 0.65, 0.98). Increased LDL-C and triglycerides both had null effects on RFS. Among the subset of non-statin users (n=266) who were censored at first statin prescription, increased lipids showed a near-null effect on CRC recurrence. Triglycerides were associated with slightly decreased CRC-specific mortality among non-statin users (HR=0.83; 95% CI: 0.67, 1.01).
Conclusion: Our results suggest potential utility of HDL-C as a prognostic marker of CRC recurrence. However, small sample size and exposure-covariate relationships that are subject to time-varying confounding limits interpretation of results.

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Chapter I. Literature Review

Colorectal cancer - Burden of disease

Trends in incidence and mortality

Colorectal cancer (CRC) is the third most common cancer worldwide and represents the second highest incidence rate among all cancers in Europe, with 447,136 new cases diagnosed in 2012 (1). There has been a consistent upward trend in the incidence of CRC throughout Europe over the last 25 years, while mortality trends show a steady decline due to advances in treatment and adoption of screening practices. Despite this decline, the five-year survival rate remains low; among European cancer patients diagnosed between 2000 and 2007, five-year survival was only 57% for colon cancer and 56% for rectal cancer (2).

In Denmark, approximately 4,000 new cases of CRC are diagnosed annually. Five-year survival is estimated to be only 50 percent (3), lower than neighboring Nordic countries (4) and countries with similar health systems (5). While most (80%) patients undergo resection with curative intent at disease diagnosis, some have disseminated disease (20%) and are given the option of palliative resection (6). CRC, like all cancers, is most treatable if diagnosed in pre-metastatic stages. A screening program initiated in 2014 in Denmark (7) has shown early success, with the proportion of cases detected in early stages jumping from 26 to 53 percent in early reports (8).

Despite the notable advances in treatment and screening over time in Europe, and specifically within Denmark, CRC remains a leading cause of morbidity and cancer-related mortality. Low long-term survival is largely due to high rates of recurrence. It is estimated that 40-45 percent of those who are surgically treated for CRC will experience a recurrence (6), highlighting the need to understand the causes of CRC recurrence, and to find targeted interventions to halt the process of recurrence.

Colorectal cancer recurrence

Most CRC recurrences occur in the first two years following surgery (9, 10) , and may occur locally, distally, or both. As many as 33% of recurrent cases are locoregional, seen in the pelvic nodes, at the anastomotic site or rectal stump, or in the presacral area (11). Distal recurrence most often appears in the liver or lungs (12), though less common sites for distant recurrence include the bone, brain, and ovaries. Five-year survival following recurrence is poor (13); recent estimates suggest five-year survival is cut in half in the case of local recurrence and even further in the case of distant recurrence (14).

Identifying and Treating Recurrence

Current methods to predict recurrence risk rely on standard clinical variables such as stage, size, and grade of tumor (15). Tumor stage is recognized as the most significant prognostic factor for local recurrence, with surgical skill also contributing to recurrence risk (10); however, these factors are overall poor indicators of prognosis following resection and have limited utility in identifying patients at risk for recurrence. Given the inability to identify a subpopulation at high risk for recurrence, consistent monitoring of all patients following surgery is optimal. Clinicians rely on surveillance to capture early recurrent disease as early identification provides a better prognosis. Surveillance commonly includes use of fluorodeoxyglucose positron emission tomography/ computed tomography (PET/CT), which has been shown to successfully find early recurrent disease (11).

A recent meta-analysis of clinical trials of follow-up procedures after curative resection for CRC revealed a reduction in all-cause mortality among those who underwent intensive follow up after resection (combined risk ratio [RR]=0.81; 95% confidence interval [CI]: 0.70, 0.94). The analysis of five trials and 1,342 patients also found an association of intensive follow up with earlier detection of recurrence and increased detection for isolated recurrence, attributing part of the survival benefit to this factor (16). It remains unknown what components of active follow-up are most essential for early detection, and there is a recognized need to uncover predictive and

prognostic markers for recurrence that may provide a clearer benefit for treatment of CRC patients (9).

Treatment options following recurrence include chemotherapy and radiation, or re-resection. Five-year survival after recurrent cancer diagnosis has remained low over the years, though it is improved for those who have a re-resection, compared with those who do not have further surgery (12, 17). A study on 1,417 Dutch CRC patients from the randomized Dutch TME trial examined recurrence trends in rectal cancer under two different therapeutic regimens: 1) preoperative radiotherapy and total mesorectal excision, and 2) total mesorectal excision alone. Among the first group, five-year local recurrence rate was less than five percent, while it was 11 percent in the second group, with varying prognosis based on the site of local recurrence (18). This finding aligns with early evidence demonstrating the benefit of combining re-resection with radiotherapy for recurrence treatment (12, 18).

Distant recurrences offer a distinct set of problems in treatment. Survival time is much lower for patients who have liver or lung metastases, even with metastatectomy (11). Pulmonary metastases are particularly difficult to diagnose and to treat, as they often present with no symptoms, and have multiple deposits, making re-resection inadvisable (12).

Based on the poor prognosis following recurrent disease, it is essential to find recurrences early to provide the best potential for improved survival. Current methods rely on clinical surveillance, which is not offered consistently for all CRC patients (9). Discovery of prognostic biomarkers for recurrence may enable earlier detection and improved risk-stratification of patients. In turn, differential monitoring plans could be implemented based on each patient's risk, improving outcomes and saving resources.

Improving outcomes in colorectal cancer

Based on continued low survival for CRC, research has focused on finding ways to improve outcomes following CRC diagnosis. Cyclooxygenase (COX) inhibitors, both non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin, have been associated with decreased

CRC incidence and improved survival. These drugs are thought to act by increasing apoptosis to suppress tumor growth (19). Evidence from randomized trials has shown that regular use of NSAIDs improve CRC outcomes (20), and this has also been reflected in observational studies. For example, evaluation of the Women's Health Initiative cohort recently found consistent NSAID use was associated with reductions in CRC mortality (hazard ratio [HR]=0.72; 95% CI: 0.54, 0.95) (21). Recurrence risk also appears lower among current NSAID users, compared to non-users (22). However, some results have shown inconsistent associations; for example, a large study using the General Practice Research Database in the UK found no association between non-aspirin NSAID use and CRC survival (23).

Aspirin has been consistently shown to produce benefits for CRC patients, with regular use associated with decreased all cause and cancer-specific mortality (21, 24-26). The UK study by Walker et al. mentioned above did find some beneficial effects of aspirin use, with regular use associating with decreases in mortality. A recently conducted pooled analysis of 14,000 patients from randomized cardiovascular disease prevention trials found daily aspirin use to be associated with over a 30% reduction in CRC mortality over 20 years (27). In addition, a randomized trial of non-metastatic colorectal cancer patients investigated the role of daily aspirin treatment on risk of recurrence, finding use to be associated with a 35% reduced risk of recurrent adenoma or carcinoma over three years (28). This finding has also been observed in other randomized trials (24).

Other modifiable risk factors that may influence CRC outcomes, including obesity and diet, have been studied. While obesity is associated with decreased survival time among CRC patients (29-33), it has not been consistently associated with recurrence (34, 35). Diet also does not appear to influence CRC recurrence, as an analysis of "Western" and "prudent" dietary patterns did not find associations with CRC recurrence or mortality (36). Without clear modifiable factors to reduce recurrent disease focus remains on treatment and early detection.

Mechanisms behind CRC development, progression, and recurrence

There are two well-recognized mechanisms responsible for CRC incidence, however, little is known about mechanisms of CRC recurrence. With respect to incidence, one mechanism of carcinogenesis, observed in familial adenomatous polyposis (FAP), describes carcinoma development as being led by a series of gene mutations, beginning with mutations in the tumor suppressor adenomatous polyposis coli (APC) gene. This gene encodes APC protein, which normally functions to control cell division frequency and attachment. Following APC mutation, subsequent gene mutations lead to hyper-proliferation and a cascade of increasingly severe adenoma development that transforms into invasive carcinoma (37, 38). The second mechanism for CRC incidence posits cancer to begin with microsatellite instability which causes mutations in DNA mismatch repair genes, driving hyperproliferation as replication errors pile up over time (38, 39).

Recurrence following surgery acts through a different pathway, the intricacies of which have not yet been defined, though several hypotheses have arisen to describe potential mechanisms. Early clinical studies suggested that local recurrence following resection occurs either by implantation of viable cells or by growth of residual tumor cells (12) that result from inadequate excision of primary tumor (10).

Current literature suggests that cancer stem cells, a small subpopulation of tumor cells, promote cancer propagation, including recurrence (40, 41). Cancer stem cells (CSCs), also referred to as metastatic initiating cells (MICs), tumor-initiating, or tumor-promoting cells, are thought to survive following cancer treatment and lay the seeds for future tumorigenesis. These undifferentiated cells histologically resemble the parent tumor, have the ability to self-renew, and can generate daughter cells that lack regenerative potential (42, 43). The presence of cancer stem cells may describe failed efforts to prevent recurrence by targeting remaining tumor cells with radiation and chemotherapy. In addition, patients whose cancers are resistant to therapies show markers of cancer stem cells in recurrent tumors (44). Cell surface protein CD133 has been

suggested as a marker to determine CRC stem cell fraction (45, 46). In a mouse model, CD133 positive cells showed exponential tumor growth of colorectal cancer cells that was not seen in CD133 negative cells (45). This evidence makes a case for increased investigation into CRC stem cells as potential progenitors of recurrent disease, though identification of stem cells remains difficult.

For a recurrence to occur following surgical treatment and subsequent chemotherapy or radiation treatment, tumor cells must find a way to survive under hypoxic conditions. It has long been known that cancer cells reprogram their metabolic function to overcome such harsh environments by converting energy production to rely on a high-rate of glycolysis, known as the Warburg effect (47, 48). Factors that support metabolic function of tumor cells are thus targeted as potential markers of recurrence. Lipids, including cholesterol and triglycerides, are integral to cellular metabolic function and are of interest in describing mechanisms of cancer recurrence.

Lipids in Cancer Progression and Recurrence

Tumor cells depend on lipids for growth

Lipids play a number of diverse roles in human biology, serving as cell membrane components and promoting cell growth and division. In turn, they contribute to a variety of aspects of tumor biology including growth, energy and redox homeostasis, and dissemination of cancer cells (49). Tumor cells are dependent on lipids to survive hypoxic conditions (50), and elevated lipid synthesis along with increased expression of lipogenic enzymes is a hallmark of cancer cells (51, 52). Breakdown of free fatty acids from lipid components to ATP can serve as a key energy source for cancer survival and development (53). Tumor cells use lipid components to build cell membranes and to proliferate (54, 55), and it has also been demonstrated that long chain fatty acid catabolism drives high proliferation rates (53). In addition to their role in cell membrane synthesis, cholesterol and lipid hormones can stimulate signaling pathways that lead to proliferation and invasion of tumors (49). In fact, cholesterol has been implicated as a

contributing factor of increased tumor aggressiveness (56), while inhibition of cholesterol esterification has been shown to suppress tumor growth in glioma (57), leukemia (58), pancreatic (59), and prostate cancers (60).

Evidence of lipid metabolic reprogramming in cancer

Tumors exhibit alterations in lipid and cholesterol-associated pathways that indicate reprogramming of lipid metabolism (61). Cancer cells avidly acquire cholesterol and lipids, either via de novo synthesis or through uptake of fatty acids from the blood. This uptake is primarily monitored through the hydrolysis of triglycerides and very low-density lipoprotein (VLDL) particles (50, 51). The enzyme responsible for this conversion (lipoprotein lipase, or LPL), is highly expressed in breast, liposarcoma, and prostate tumor samples (62), suggesting sequestering of lipids from the circulation. Lipid signatures of colorectal cancer patients have also revealed genes to be overexpressed in patients who are at a high risk of recurrence (61, 63), supporting lipid reprogramming as a mode for recurrent cancer development.

Tumor cells store excess lipids and cholesterol via lipid droplets (61), which are associated with cancer cell survival under hypoxic conditions. Investigators have found increased lipid droplets are increased in both breast cancer tumor cells (64) and colorectal cancer stem cells (65). Moreover, these lipid droplet-rich cells are resistant to chemotherapy (66), appearing like cancer stem cells, and further supporting the idea that lipids assist with disease progression and return.

Cholesterol and sphingolipids form lipid rafts, which control membrane dynamics as well as cell survival and apoptosis. Lipid rafts and cholesterol are enhanced in cancer cell membranes, including gastrointestinal (67), liver (68), and breast and prostate cancer (69).

Cancer cells also use surrounding adipocytes to acquire free fatty acids. The hydrolysis of triglycerides in adipocytes releases free fatty acids that tumor cells, if close by, can use as an energy source for growth (61). Acquisition of fatty acids from adipocytes has been observed in breast cancer (70).

Additional mechanisms of lipids in cancer

In addition to serving as a component of membrane synthesis and as an energy source for tumor development, cholesterol acts as the substrate for bile acid synthesis (71), a risk factor for CRC (72, 73). Based on lipid reprogramming, hormonal influence of increased triglycerides may also impact CRC outcomes. Increased triglycerides are associated with high insulin, a known growth factor, and as such, may exert their influence on cancer progression through the insulin-mediated pathway (74).

Taken as a whole, this biological evidence indicates potential for lipids to drive cancer progression and to influence survival.

Association of serum lipids with cancer in population based studies

Recognizing the biological plausibility for a role of lipids in cancer recurrence and progression, several epidemiologic studies have begun investigating serum lipid profiles to uncover associations between lipid components and various cancers and cancer-related outcomes. Investigators have examined influence of total cholesterol, LDL-C and HDL-C, VLDL, and triglyceride levels on cancer risk and prognosis. Evidence of whether lipids are increased, decreased, or unchanged throughout the course of cancer progression and treatment is largely inconclusive, though some general trends have been noted.

Total Cholesterol, low density lipoprotein (LDL-C) & Triglycerides

Increased LDL-C, total cholesterol (TC), and/or triglycerides, clinically defined as hyperlipidemia, has been indicated as a risk factor for CRC (75). Several case-control and cross-sectional studies evaluating adenoma patients support this theory, though whether the association appears with total cholesterol (76, 77), LDL-C (77), or triglycerides (76-79) differs between studies. A meta-analysis of 17 prospective studies found increased triglycerides to increase risk of CRC (pooled RR=1.18; 95% CI: 1.04-1.34), while total cholesterol also predicted increased risk (RR=1.11; 95% CI: 1.01-1.21) (80).

LDL-C, TC, and triglycerides have also been associated with advanced disease progression (81) and poor cancer-related outcomes. Levels of TC and TG in serum and the levels of TCH in cancerous tissue in patients with colorectal cancer were correlated with TNM stage (82), indicating potential for these factors to contribute to disease aggressiveness. With respect to outcomes, among patients with metastatic CRC, an elevated LDL-C/HDL-C ratio is predictive of poor prognosis (83). Additionally, an analysis of baseline lipid biomarkers and cancer mortality among a prospective cohort of 15,602 female health professionals enrolled in the Women's Health Study found increased mortality risk in CRC patients who had high triglycerides, measured by one standard deviation change (84).

Studies focused on recurrence as the outcome of interest are rare. One study of 35 ovarian cancer patients who relapsed following operation, compared with patients who did not relapse, found increased fatty acids among recurrent patients, suggesting a potential role for fatty acids as a biomarker for recurrent disease (85). A recent study on 843 prostate cancer patients who underwent radical prostatectomy observed increases of 10 mg/dl of triglycerides were associated with increased risk for recurrence among all patients, and enhancement of this risk occurred among patients with dyslipidemia (86). Another study using a veteran's cohort of 1,706 men, similarly found elevated LDL-C to be associated with recurrence following primary treatment for localized prostate cancer (HR=1.34; 95% CI: 1.03, 1.74) (87).

High Density Lipoprotein (HDL-C)

In contrast to trends seen for LDL-C and triglycerides, increased HDL-C, which acts as an efflux regulator (71), may decrease risk of cancer and improve outcomes among cancer patients. In the meta-analysis conducted by Yao et al., examining dyslipidemia and CRC risk,(80) authors found a potentially protective effect of increased HDL-C on risk (HR=0.84; 95% CI: 0.69, 1.02) (80). Increased HDL-C was also shown to decrease risk of CRC-mortality (HR=0.80; 95% CI: 0.60, 1.08) in the Women's Health Study cohort (84), while low HDL-C has been associated with development of pre-cancerous lesions (88).

HDL-C may be further protective for patients who have undergone surgery for cancer. Recovery of HDL-C levels following surgery have been associated with remission of disease in studies of ovarian cancer patients (85) as well as solid tumors in children (89). Elevations in HDL-C following treatment for non-metastatic CRC also found levels correlated with longer disease free and overall survival time (90).

Conclusion

The burden of colorectal cancer is largely driven by high rates of recurrent disease following surgical treatment and subsequent low survival among patients who experience a recurrence. Based on biologic plausibility of a role for lipids in driving colorectal cancer recurrence, along with epidemiologic findings suggestive of an association between altered lipids and cancer outcomes, it is of interest to investigate lipids further. From the review of current literature, it appears that increased levels of LDL-C and triglycerides may be associated with poor CRC prognosis, which may correlate with an increased risk of recurrence. In contrast, increased HDL-C may decrease recurrence risk among surgically treated patients, improving overall survival. Determining whether or not serum lipid profiling may be used as a prognostic tool in identifying CRC patients who are at high risk for recurrence will add to this area of research.

Chapter II. Manuscript

A. Abstract

Serum Lipid Levels and Colorectal Cancer Recurrence

By Kristen Brantley

Background: Biologic and epidemiologic evidence suggests that tumor cells depend on reprogrammed lipid metabolic function for survival and growth. Mechanistically, cholesterol and triglycerides may support tumor recurrence by providing energy needed for future proliferation. Altered serum lipid profiles have been observed in cancer patients at diagnosis and throughout treatment, and studies have found associations of serum lipids with cancer incidence, mortality, and disease-free mortality. Lipids may be particularly relevant in colorectal cancer (CRC) progression, though studies have yet to evaluate the prognostic potential of serum lipids for CRC recurrence.

Methods: A prospective cohort design was used to study the effect of serum lipids, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG), on CRC recurrence-free survival. The study actively followed 342 Danish colorectal cancer patients who underwent surgical resection between 2003-2011 from date of surgery until December 31, 2012, or death. Sixty patients experienced a recurrence, with a median follow-up time of 4.1 years (interquartile range [IQR] 2.3, 6.8). Serum lipids were collected at scheduled intervals throughout follow-up, and recurrence rate was assessed using Cox proportional hazards modeling. Lipids were assigned as time-varying exposures evaluated in the year preceding recurrence and models were adjusted for clinically relevant covariates. A simplified analysis was performed by excluding influence of statin use on results via censorship of patients at first prescription. All-cause and CRC-cause mortality were also assessed as outcomes of interest.

Results: Among 342 CRC patients, increased HDL-C appeared to have a beneficial impact on recurrence-free survival (RFS) for CRC patients, though protection was only observed among statin users (hazard ratio [HR]=0.80; 95% confidence interval [CI]: 0.65, 0.98). Increased LDL-C and triglycerides both had null effects on RFS. Among the subset of non-statin users (n=266) who were censored at first statin prescription, increased lipids showed a near-null effect on CRC recurrence. Triglycerides were associated with slightly decreased CRC-specific mortality among non-statin users (HR=0.83; 95% CI: 0.67, 1.01).

Conclusion: Our results suggest potential utility of HDL-C as a prognostic marker of CRC recurrence. However, small sample size and exposure-covariate relationships that are subject to time-varying confounding limits interpretation of results.

B. Introduction

Colorectal cancer (CRC) is the second leading cause of morbidity and cancer-related mortality in Europe and the United States (1, 91). Despite advances in both early detection and treatment, the five-year survival rate has not improved dramatically, and remains below 65 percent in Europe (2). In Denmark the rate is lower than that of its European counterparts (4) and countries with similar health systems (5), with an estimated 50 percent five-year survival rate (3). Screening programs have improved outcomes through diagnosis in pre-metastatic stages, which enables surgical intervention (8). However, nearly fifty percent of CRC patients will experience a recurrence following surgical resection (10), and most will die from this relapse (13).

Recurrence typically appears within the first two years following surgery (9, 10, 92), with the majority of patients experiencing distant recurrence, often in the liver or lung, and as many as 33 percent experiencing loco-regional recurrences (11). Treatment options for patients who experience a recurrence are limited; further surgery is often radical and not often beneficial, unless complete resection, free of margin, is possible (93). The burden of recurrent disease is particularly high, given the dramatic drop in five-year survival rates following local or distant recurrence (14). Determination of recurrence risk remains elusive, as it cannot be predicted using standard clinical features such as stage, size, and grade of tumor (15). Based on the high probability of recurrent CRC and lack of knowledge on how to prevent its return, identification of prognostic markers for recurrence in asymptomatic stages represents an area of great potential for public health impact (94).

Accumulating evidence points to lipids as key drivers of tumor biologic function and cancer progression, given the ability of lipids to enhance growth, alter energy and redox homeostasis, and promote the dissemination of cancer cells (49). It is well-recognized that cancer cells reprogram their metabolic function by increasing glucose uptake and fermentation to survive and grow under harsh conditions (48, 95). As a part of this survival and development process,

specific lipid metabolic reprogramming also occurs (61), in which tumor cells may sequester free lipids to fulfill cholesterol needs for membrane biosynthesis, while also undergoing de novo lipid synthesis (54, 71). This enhanced lipid uptake is additionally advantageous, as cholesterol and other lipid-related hormones can stimulate signaling pathways that lead to proliferation and invasion of tumor cells (49). Aside from serving as energy reserves for tumor cells, lipids may also promote tumor growth by influencing bile acid secretion, a known risk factor for CRC (72, 73), or through influence on circulating hormones, insulin and IGF-1, both established pro-tumor growth factors (74).

The potential for these changes in lipid metabolic function to promote development of recurrent disease has also been described. Storage of triglycerides benefits tumor cells in a hypoxic state and provides fuel for growth after re-oxygenation (50, 74), indicating a mechanism for residual tumor cells to endure through treatment regimens and eventually recur. Moreover, lipid-related gene expression profiling reveals four genes in CRC that are only overexpressed in stage two patients with elevated risk of relapse (63). Recently, investigators have observed that colorectal cancer stem cells, which are believed to be a factor behind therapeutic resistance and recurrence (40, 41), show increased lipid droplet accumulation (96).

In response to biologic studies, epidemiologic evidence has begun to uncover linkages between lipids and cancer outcomes. Increased low density lipoprotein cholesterol (LDL-C) has been suggested as a prognostic marker for disease progression in several cancer types, including breast, ovarian, colon, and gastric cancers (81). On the other hand, increased high-density lipoprotein cholesterol (HDL-C) may indicate decreased risk; for instance, a study of ovarian cancer patients found recovery of high density lipoprotein cholesterol (HDL-C) levels after surgery to be associated with cancer remission (85). With respect to CRC, studies have found an association between triglycerides and total cholesterol and disease aggressiveness, measured as TNM stage (82). A study of 453 CRC patients found that a high LDL-C: HDL-C ratio was associated with poor prognosis in CRC, indicating lower overall survival (83). However, the exact role of dyslipidemia

in CRC remains controversial, as studies have found abnormal cholesterol and triglyceride levels to both increase (97, 98), and decrease (99, 100), risk of mortality. Research evaluating serum lipids with respect to cancer recurrence is rare, though a recent study of 843 radical prostatectomy patients found an association between increased triglycerides and total cholesterol with increased prostate cancer recurrence risk (86).

There are few studies examining lipid profiles in surgically-treated cancer patients over time, and none that have observed profiles in CRC patients during the period from surgical resection to recurrence or remission. Moreover, current studies are limited by small sample sizes, incomplete covariate information, and restriction to patients who are not using cholesterol lowering medications, limiting generalizability of findings. This study of 342 Danish colorectal cancer patients who have undergone surgical resection aims to examine the effect of HDL-C, LDL-C and triglyceride levels on recurrence-free survival (RFS) time. It is hypothesized that serum lipid profiles measured in the year preceding recurrence will indicate risk for recurrence and may have utility as prognostic markers for recurrence.

C. Methods

Study Population

This prospective cohort study combines members of two cohorts of Danish colorectal cancer (CRC) patients who underwent treatment at Aalborg Hospital in Denmark. An observational cohort consisted of 210 CRC patients admitted between October 2003 and November 2005 for intended curative surgery. Patients were followed throughout course of treatment, and received regular physical examinations for recurrence and related outcomes. Exclusions included seven patients with non-malignant disease, ten who were not surgically treated, and 27 with residual CRC or metastases within three months of surgery, leaving a total of 166 eligible patients.

The second cohort consisted of 245 participants enrolled in a clinical trial (COLOFOL, clinicaltrials.gov reference #NCT00225641). The trial was a multi-center study comparing

different control regimens after resection among patients with Stage II and III CRC. Patients were enrolled from January 2006-January 2011 and were given CT-scans or MR scans of the liver, X-rays or CT-scans of the lungs, and control of carcinoembryonic antigen (CEA) either (1) at 12 and 36 months following resection, or (2) at 6, 12, 18, 24, and 36 months following resection. Among the 245 COLOFOL participants, six were excluded from this study by invalid CPR number and one participant was already enrolled in the observational cohort.

There were 404 eligible patients from the observational and COLOFOL cohorts combined. An additional 49 participants were excluded due to: no record in the DCCG database (8), metastatic disease at diagnosis (7), unknown stage at diagnosis (5), previous cancer other than CRC (15), CRC diagnosis date more than 60 days after surgery date (2), new primary cancer within 180 days from CRC diagnosis (4), death within 180 days from CRC diagnosis (6), or CRC recurrence detected within 180 days from CRC diagnosis (5). Some patients were excluded for more than one reason. After further excluding patients with no available blood samples (n=12), 342 participants remained (**Figure 1**). Follow up concluded December 31, 2012.

Definition of Analytic Variables

DCCG Variables

The Danish Colorectal Cancer Group (DCCG) manages a database that serves as a clinical registry of all CRC patients in Denmark. This registry includes information on date of diagnosis and surgery, stage at diagnosis, and receipt of neoadjuvant and adjuvant therapy.

Eligible CRC patients from the combined Aalborg cohorts were categorized into age groups at CRC diagnosis (<55, 55-64, 65-74, 75-84, or ≥85 years). Patients were also grouped into calendar period of diagnosis (2003-2004, 2005-2008 or 2009-2011). Designation of stage at CRC diagnosis was defined by guidelines of the American Joint Committee on Cancer (AJCC) as: IA, localized; IIB, localized; or 3C, regional. Charlson comorbidity score (101) was defined at diagnosis as 0, 1-2, or ≥3. Receipt of chemotherapy either pre- or post-operatively is included as a dichotomous variable.

Exposure to statins, aspirin, and non-steroidal anti-inflammatory drugs (NSAIDs) was classified dichotomously as use or non-use in the year preceding surgery for CRC and for up to ten years following surgery. Users were identified as those who had at least one prescription recorded in the Danish National Registry of Medicinal Products. Chemical codes used to identify exposure to statins, aspirin, and NSAIDs were: (1) codes beginning with "C10AA" (statins); (2) B01AC06, N02BA01, N02BA51 (aspirin); and (3) M01A (other NSAIDs).

Events

Recurrences were defined as (1) tumor growth at or near the site of the original tumor and in the same organ or (2) metastases to tissue adjacent to the original tumor site or to a distant organ. Cases of recurrence were identified by direct record from clinic follow-up.

Because patients were excluded if an event occurred within 180 days of diagnosis, follow-up began 180 days after the DCCG date of diagnosis. Delayed start of follow-up avoids potential for reverse causation due to preexisting cancers that may influence exposures, in this case, lipid levels. Time to recurrence was defined as time between first recorded recurrent event and time of start of follow-up. Vital status and date of death were obtained from the Danish Central Personal Registry, and death from CRC was identified using the Danish Register of Causes of Death. Cause-specific death was defined in a broad and specific form. First, death from CRC was considered if CRC was listed as any of the eight causes of death on the registry. Second, death from CRC was considered only when CRC was listed as the underlying cause of death.

Visit Schedule

Starting at date of surgical intake, relevant clinical information was collected for participants of the observational and COLOFOL cohorts according to different schedules. A total of 12 visits were possible for participants of the observational cohort, with the last visit occurring five years after intake. Patients enrolled in the COLOFOL clinical trial had up to six visit dates recorded, with the last visit occurring three years after intake. Visits schedules are outlined in **Figure 2**.

Serum Profile & Clinical Standards

Lipid measurements included triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL-C), and low density lipoprotein (LDL-C). Total and HDL cholesterol and triglycerides were measured in serum (mmol/L) at each follow-up visit. LDL-C was calculated based on the equation: LDL-C=TC - (HDL-C) - (0.45*TG). Where triglycerides were >4 mmol/L LDL-C calculations were not initially performed, due to potential instability of measurements. Clinical standards are defined by European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) guidelines (102). Patients are determined to be within clinical standards for total cholesterol if their measurement is less than 5 mmol/L. The accepted clinical standard for LDL-C is under 3 mmol/L, and recommended TG levels are below 1.7 mmol/L. HDL-C standards are sex-specific; among men, values of HDL-C ≥1 mmol/L are considered optimal, and among women this value is slightly higher, with a recommended HDL-C ≥1.2 mmol/L.

Statistical Analysis

Imputation of visit dates & exposure variables

Imputation was performed for both missing dates and missing lipid measurements for participants. Where missing dates were observed, imputation was based on appropriate participant schedules (see **Figure 2**). Missing HDL-C and triglycerides were recorded by carrying the measurement from the previous visit forward.

Missing LDL-C calculations were imputed in two ways. First, where LDL-C calculations were missing due to high triglycerides (>4 mmol/L), we calculated LDL-C using the standard equation described above, noting potential instability of the calculated measurements. Following this calculation all other missing LDL-C measurements were filled in by last observation carried forward. The resulting LDL-C measurements were then used to assign clinical standards. By calculating missing LDL-C measurements according to the equation above we resolved LDL-C measurements at baseline of 10 participants for whom values were initially missing. Among these 10 participants with initially missing values, three were classified as within clinical standards at

baseline, and seven were classified as outside of clinical standards for LDL-C at baseline. For patients with LDL-C initially missing for occasions after the first visit, 12 patients were classified as inside clinical standards, and only one was classified as outside of clinical standards.

For continuous analysis, a second LDL-C imputation method was tested to evaluate the effect of the new, potentially unstable, calculated LDL-C measurements. Using this conservative approach, where TG were greater than 4 mmol/L, missing LDL-C measurements were not recalculated. Any missing LDL-C measurements were assigned based solely on the previous visit measurement, and baseline LDL-C values that were missing (n=10) remained missing.

LDL-C measurements were similar between the two imputation methods. Upon recalculation, classification of individual LDL-C measurements as within or outside of clinical standards changed for a total of 22 measurements. This included 10 measurements that were reclassified as outside of clinical standards, and 12 that were reclassified as within clinical standards. Median and mean LDL-C measures remained consistent between the two imputation methods, and the maximum LDL-C only increased for one measurement occasion using the calculation-based method (6.79 to 7.24 mmol/L). Both versions of series LDL measurements were tested in proportional hazards regression models described below, and there were no noticeable changes in effect estimates when examining models using different imputation methods; thus, LDL measurements assigned based on the first method are reported here.

Descriptive Statistics

All statistical analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics including frequency and proportion of all considered covariates and mean lipid measurements were evaluated for the cohort. Univariate associations between lipid measurements and covariates were explored. Unadjusted hazard ratios for CRC recurrence were calculated for covariates and time to recurrence was examined using Kaplan-Meier plots and the log-rank test. Distribution of total cholesterol, LDL-C, and HDL-C measurements for all dates reported was approximately normal; while distribution of triglyceride measurements was

slightly skewed, logarithmic transformation was not performed based on near-normality and to facilitate interpretation.

Lipid Profile and Time to Recurrence

Proportional hazards regression was used to estimate hazard ratios associating time-varying HDL-C, LDL-C, and triglyceride values with the rate of recurrence. The latest available lipid measurement in the year preceding recurrence or censorship represented exposure for each participant. Fourteen patients who did not have a full year interval between baseline lipid measurement and event or censorship were assigned lipid levels based on the earliest available value. Lipids were assessed both continuously and as dichotomous values determined by clinical standards categorization. For continuous lipid measures, interpretations were based on a 0.1 mmol/L increase based on previous studies that have examined serum lipids as a marker for cancer risk or prognosis, though we also assessed results when considering larger incremental changes as a consensus on clinically relevant HDL-C, LDL-C, and TG changes has not been established for cancer outcomes.

Models included each lipid measurement with adjustment for covariates as defined above: age group, sex, year of diagnosis, chemotherapy treatment (neoadjuvant and adjuvant), stage, Charlson comorbidity score, NSAID use, aspirin use, and statin use. Covariates were selected for inclusion based on prior literature evidence and exploratory analysis. Prescription use of NSAIDs, aspirin, and statins were included as time-dependent covariates, with usage status based on the year preceding event or censorship. Noting the potential for statin use to modify the impact of lipid measurement on recurrence outcome, we tested for interaction between lipid measures and statin use in the model. We performed stratified analysis based on baseline hyperlipidemia designation (baseline LDL-C, TC, or TG above clinical standards) and baseline HDL-C level ($\geq$1 or 1.2 mmol/L vs. < 1.0 or 1.2 mmol/L).

Lipid Profile and Mortality Rate

To evaluate the effect of lipids on mortality rates, we used proportional hazard models considering time to death as the outcome variable of interest and re-assigned exposures and time varying covariates as values in the year preceding death or censorship. Both all-cause and CRC-specific death, listed as one of eight potential causes of death, were used in analysis. There were too few events (n=8) for CRC-death as the underlying cause to be evaluated as a separate outcome.

Statin User Exclusion

Considering the complicated relationship between statin use and lipid measurements over time, we performed a simplified analysis on a subset of participants who were not using a statin prior to surgery, and censoring participants at date of first statin prescription. This subset included 266 participants, among whom 43 experienced a recurrence during follow up time. Cox proportional hazards regression was used to estimate hazard ratios comparing rates of recurrence and mortality, adjusting for the same covariates mentioned above.

D. Results

Characteristics of the cohort

Among the 342 patients, median follow-up time was 4.1 years (interquartile range [IQR] 2.3, 6.8) A total of 60 patients experienced a recurrence within follow-up, three of whom were diagnosed with a second primary cancer prior to their recurrence. The majority of the cohort fell within the ages of 55-74 (73%), and patients were most often diagnosed in the years 2005-2008 (52%). Mean HDL-C and LDL-C measures fell within clinical standards categorization in the year preceding recurrence or censorship (mean HDL-C=1.28, standard deviation [SD]=0.41; mean TG=1.63, SD=0.85), though mean LDL-C was above clinical standards (mean LDL-C=3.19, SD=0.94). Sixty-eight percent of the actively followed cohort (n=233) reported no statin use during the time interval relevant to this study, from one year preceding surgery through up to 10-years of follow up.

Compared with patients who had optimal total cholesterol (<5 mmol/L) in the year before recurrence/censorship, those with high cholesterol (n=210, 62%) had fewer comorbidities, were less likely to be statin or aspirin users, and had higher mean LDL-C, HDL-C, and triglycerides. Groups did not differ by cancer type, therapy, lymph group, stage of tumor, sex, year of diagnosis or age at diagnosis. Mean BMI also did not differ between groups, though participants who had normal cholesterol in the year preceding recurrence were more likely to be classified as obese (27%) than those who had high cholesterol (17%). As expected, those who had cholesterol >5 mmol/L had higher mean HDL-C, LDL-C, and triglycerides, compared with those who had normal total cholesterol. Twenty-one (35%) recurrences occurred in the group with low cholesterol in the year preceding recurrence, and 39 (65%) of recurrences occurred in the high cholesterol group. Participant characteristics by covariate are displayed in **Table 1**.

Distribution of lipid measurements by statin use

We compared clinical standards designations for each lipid measurement by statin usage group (**Supplementary Table 1**) to examine data balance. Among users in the year preceding recurrence, only 28% had high LDL-C in the same year, compared with 67% of non-users. The majority of patients fell within recommended ranges for HDL-C and TG both at baseline and in the year preceding recurrence, and distribution of measurements was similar between statin usage groups.

Covariate associations with recurrence-free survival (RFS)

Univariate proportional hazards models revealed independent associations of tumor stage and age with RFS time. Hazard rates increased with increasing stage, (hazard ratio [HR] stage 2 v. 1=2.19; 95% confidence interval [CI]: 0.66, 7.27; and HR stage 3 v. 1=5.22; 95% CI: 1.60, 17.07). Those aged 55-64 and 65-74 both had a reduced rate of recurrence relative to those under 55 years (HR age 55-64=0.44; 95% CI: 0.22, 0.88; and HR 65-74=0.44; 95% CI: 0.22, 0.87). Among prescription drugs, aspirin use appeared to have the most benefit on RFS in independent assessment. Ever-users of aspirin had a reduced hazard rate of recurrence, compared to never-users

(HR=0.50; 95% CI: 0.26, 0.97); use in the year preceding recurrence showed less protection (HR=0.79; 95% CI: 0.40, 1.56). Ever use versus never use of other NSAIDs also appeared protective (HR=0.63; 95% CI: 0.37, 1.01), as did ever statin use (HR=0.59; 95% CI: 0.32, 1.10).

Associations of LDL-C and triglycerides with RFS and mortality

Increased LDL-C and TG do not affect RFS overall

Crude models showed null associations between LDL-C and TG and RFS time in continuous assessment **(Table 2)**. Evaluation at a 0.3 mmol/L increase did not change effect size. The fully adjusted model included statin, NSAID, and aspirin use in the year preceding recurrence, age group, sex, year of diagnosis, tumor stage, neoadjuvant and adjuvant chemotherapy, and comorbidities (**Table 2**). Interaction was observed between statin use and continuously measured lipids. Following multivariable adjustment, a 0.1 mmol/L increase in LDL-C had a near-null effect on hazard rates for statin users (HR=1.05; 95% CI: 1.00, 1.11) and non-users (HR=1.01; 95% CI: 0.98, 1.04). Similar null effects were seen for increased triglycerides.

While null effects were observed in continuous evaluation of LDL-C and TG, potential harmful associations were revealed when assessing impact of moving outside of clinical standards for either lipid measure (**Table 2**). Increased hazards apparent in crude assessment for high LDL-C (HR=1.43; 95% CI: 0.83, 2.45) and high TG (HR=1.31; 95% CI: 0.78, 2.31), remained consistent following adjustment (**Table 2**), though high LDL-C appeared to more negatively impact recurrence among statin users (HR=2.27; 95% CI: 0.66, 7.75) than non-users. High TG (≥1.7 mmol/L) resulted in a nearly 2-fold increased hazard rate of recurrence among statin users, compared with a 1.3-fold increased rate among non-users.

Increased LDL-C may negatively influence RFS among patients with baseline hyperlipidemia

Stratified analyses were performed to assess how baseline lipid level may influence the effect of pre-recurrence lipid measurements on RFS. Among the full cohort, there were 92 patients who were within clinical standards (CS) for total cholesterol, LDL-C and TG at baseline, 19 of whom experienced a recurrence within the study period; two were censored in analysis by prior

event. There were 250 patients whose TC, LDL-C, or TG did not fall within clinical standards at baseline, 41 of whom experienced a recurrence; one was censored in analysis. Among patients who were outside of CS at baseline for TC, LDL-C, or TG, increased LDL-C appeared harmful given statin use (HR=1.13; 95% CI: 1.03, 1.24) (**Table 3a**). Increased TG did not change hazards of recurrence for either group.

Patients were also stratified based on baseline HDL-C categorization alone (**Table 3b**). There were 128 patients who had low (non-ideal) HDL-C measurements at baseline. Twenty-five of these patients experienced a recurrence, two of whom were censored in analysis. Among the remaining 214 patients who had normal HDL-C at baseline, 35 experienced a recurrence, and one was censored. Null associations of LDL-C and TG on recurrence rate remained consistent in both baseline HDL-C groups.

LDL-C and TG levels do not affect RFS among non-statin users

Associations of lipid levels were evaluated in a subset of 266 patients who were not using a statin prior to surgical intervention, and by censoring patients at first statin prescription. Characteristics of this non-statin user subset are described in **Supplementary Table 2**. Overall, covariate distributions were similar to distributions within the entire cohort. A total of 48 recurrences were observed within the group, five of which were censored. Median time of follow up was 3.5 years (IQR 1.5, 5.4). A total of 32 (67%) of recurrences occurred among those with high total cholesterol (≥5 mmol/L) in the year preceding recurrence. A lower percentage had tumors classified as Stage II at diagnosis compared with the overall cohort (52% v. 60%), and those who did not use statins at baseline were also less likely to have used aspirin (23%) within the study period compared with the entire group (31%).

Crude models, mutually adjusted for other lipid components, showed a null effect of each 0.1 mmol/L increase of LDL-C and TG on RFS (**Table 4**), that remained null following covariate adjustment. Total cholesterol, assessed as a single exposure, also reflected this null effect on RFS among non-statin users (HR=1.00; 95% CI: 0.97, 1.03). Based on clinical standards categorization,

unadjusted and adjusted models showed a potentially harmful effect of increased LDL-C (aHR=1.56; 95% CI: 0.78, 3.11), as well as TG (HR=1.23; 95% CI: 0.62, 2.46), though wide confidence intervals limit interpretability of findings.

Hazard ratios did not indicate any differential associations of LDL-C and TG with CRC recurrence by baseline hyperlipidemia status in this subset (**Table 5**). Increases in both LDL-C and TG showed near-null associations in both groups. The same trends were seen when patients were stratified by baseline HDL-C levels (**Supplementary Table 2**).

Triglyceride levels are inversely associated with CRC-cause mortality rate

In the full cohort, after a median follow-up time of 4.5 years (IQR 3.1, 7.0), 27 patients died, and 14 deaths were attributable to CRC. Unadjusted models showed null effects for increased LDL-C and TG on both all-cause and CRC-specific mortality **(Table 6a)**. Similarly, each 0.1 mmol/L increase in total cholesterol in the year preceding death did not predict all-cause mortality (HR=0.97; 95% CI: 0.93, 1.00), or CRC-cause mortality (HR=0.99; 95% CI: 0.94, 1.04). Adjusted models showed a slight decrease in CRC-cause mortality with increased TG (HR=0.83; 95% CI: 0.67, 1.01) among non-statin users.

These results were consistent within the non-statin user subset (**Table 6b**), in which 16 patients died during median follow-up of 4.2 years (IQR 2.2, 5.5), and eight had CRC listed as one of eight underlying causes of death.

Associations of HDL-C with RFS and mortality

HDL-C provides a protective benefit on RFS for patients using a statin

For the entire cohort, crude models showed no influence of a 0.1 mmol/L increase in HDL-C on time to recurrence. Following adjustment, increased HDL-C had a protective association with recurrence free survival, though this was only notable among statin users (HR=0.80; 95% CI: 0.65, 0.98). This protection was enhanced with further increases in continuous HDL-C, though confidence intervals also widened (HR at 0.3 mmol/L=0.50; 95% CI: 0.27, 0.94). Corresponding with findings from continuous assessment, high HDL-C, classified as

≥1 or 1.2 mmol/L, had an overall protective effect on RFS for statin users, (HR=0.49; 95% CI: 0.15, 1.67). An overall harmful effect was observed among non-users (HR=1.92; 95% CI: 0.89, 4.16), however, these results should be interpreted cautiously due to uneven distribution of lipid measurements by statin usage groups (**Table S1**).

Protective benefits of HDL-C are enhanced in patients with baseline hyperlipidemia

The protective association of HDL-C was enhanced among those who were outside of clinical standards for lipid measurements at baseline. Given statin use, increased HDL-C in patients with hyperlipidemia at baseline resulted in an even longer RFS time than observed in the overall cohort (HR for 0.1 mmol/L increase=0.49; 95% CI: 0.28, 0.84) **(Table 3a)**. Patients who were inside clinical standards for LDL-C, TC, and TG at baseline experienced a minimal benefit with increased of HDL-C.

In addition, increased HDL-C appeared more protective for the group with low HDL-C to begin, compared with the group who already had high HDL-C at baseline **(Table 3b)**.

Increased HDL-C does not affect RFS in a non-statin user subset

In the non-statin user analytic group, associations of continuously assessed HDL-C with time to recurrence were null in both unadjusted and adjusted models **(Table 4)**. High HDL-C appeared potentially harmful when assessed dichotomously as high or low HDL-C, (HR=1.86; 95% CI: 0.84, 4.11), though dichotomous assessment was subject to a high degree of variability. Null associations of continuously measured HDL-C remained consistent when this subgroup was assessed by baseline hyperlipidemia status **(Table 5)** and by baseline HDL-C status **(Table S3).**

HDL-C has a null effect on all-cause and CRC-cause mortality

Increased HDL-C showed a near-null effect on all-cause mortality in the full cohort, though a slight protection was observed among statin users (HR=0.92; 95% CI: 0.78, 1.08) (**Table 6a**). Null associations of increased HDL-C with CRC-specific mortality were observed in both statin usage categories, and remained consistent in analysis of non-statin users alone (**Table 6b**).

E. Discussion

This study following 342 colorectal cancer patients treated with surgical resection found an inverse association between HDL-C and hazard rate of recurrence, dependent on statin use and baseline hyperlipidemia status. The prognostic impact of HDL-C appeared most relevant among statin users. Despite observed associations with time to recurrence, increased HDL-C did not have a noticeable impact on CRC-cause mortality rates. LDL-C showed a slightly harmful association with CRC recurrence among patients with baseline hyperlipidemia. Assessing recurrence rate by clinical standards categorization of lipid measures revealed heightened associations, indicating a need to further examine tipping points at which hazards for recurrence may be increased. The varying associations of individual lipid measures with recurrence rate indicate a unique role for each component of the lipid profile in CRC relapse.

Our results support an emphasis on HDL-C as a potential prognostic marker for colorectal cancer recurrence. This result aligns with current literature evidence, as HDL-C has been associated with both decreased risk of CRC and decreased risk of death following diagnosis (84, 90). Further supporting this finding, recovery of HDL-C from low levels at cancer diagnosis has been observed in cancer patients who experience remission (85, 89). Low HDL-C has also been observed as a factor in advanced cancer progression. A small study of gastrointestinal, colon, and rectal cancer patients undergoing surgical treatment in Italy found that patients with more invasive disease had lower serum HDL-C, suggesting that low levels were associated with increased cholesterol metabolism in proliferating tumors (67). A similar association between low HDL-C and advanced disease was observed among 83 breast cancer patients (103).

While it has been previously proposed that there is a link between bile acid circulation and VLDL triglycerides (104), which may affect recurrence risk for CRC, our results did not indicate associations between triglycerides, which primarily carry VLDL, and hazard rates of recurrence. If the mechanism through which lipids promote recurrence is fecal-acid driven, we would expect to see an increase hazard rate of recurrence with increased serum triglycerides.

Alternatively, if the mechanism by which lipid metabolic reprogramming affects cancer recurrence relies on derivation of energy from lipid sources, we might also expect triglyceride levels to be associated with increased hazard rates. Because we did not observe a deviation from a null association between triglycerides and time to recurrence, alternative theories may be needed to describe the link between lipid metabolism and recurrence risk.

The observed null associations between increased lipids and hazard rates of recurrence among a group of non-statin users, defined by censoring patients at first statin prescription, correspond with results reported by other groups. A study using data from the Shared Equal Access Regional Cancer Hospital (SEARCH) database that investigated the association between pre-operative cholesterol, LDL, HDL, and triglycerides and biochemical recurrence risk of prostate cancer in 843 patients found no associations between total cholesterol, LDL and HDL and recurrence risk (86). This same study did find an impact on recurrence risk among patients with hyperlipidemia, especially for HDL-C (HR=0.61; 95% CI: 0.41-0.91), mirroring the trend seen in this study of CRC patients.

Our near-null results for the association of serum lipids with overall survival and CRC-cause mortality agree with results from a previous study assessing overall survival among 266 CRC patients (105), although this study considered pre-operative serum lipids as opposed to values in the year preceding death. Discrepancies between recurrence analysis and CRC-cause mortality analysis in our study may be due to competing risks or misclassification of CRC-cause mortality. Interestingly, increased triglycerides appeared slightly protective against CRC-cause mortality, which does not correspond with prior evidence (84). A more precise analysis that includes CRC-death as the underlying cause alone is needed to further explore the relationship between lipid measurements and cancer-related mortality, though it was not possible here given the relatively small sample.

There has been increased focus on the role of statins in cancer therapy in recent years. Several biologic mechanisms have been proposed that explain how these drugs may act to inhibit

cellular proliferation or otherwise interrupt signaling of tumor cells, though whether or not statin use is truly beneficial for cancer patients remains controversial. Some epidemiologic studies have observed reductions in both cancer-specific mortality and recurrence for several cancers, including breast (106), prostate (107), lung (108), and colorectal cancer (109). This last study of 7,657 CRC patients with newly diagnosed stage I to III disease, identified from the National Cancer Data Repository in the UK from 1989 to 2009, found that post-diagnostic statin use was associated with reduced CRC-specific mortality (HR=0.71; 95% CI: 0.61 to 0.84). A second study by the same group using 8,391 patients from the Scottish Cancer Registry found statin use prior to diagnosis, but not after, was associated with improved survival (110).

Several additional studies have revealed no association between statin use and colorectal cancer outcomes. The meta-analysis conducted by Gray et. al. found only weak associations between pre-diagnostic statin use with improved survival and no clear associations of post-diagnostic use with survival (110). An American study of 842 CRC patients with stage III colon cancer, which examined recurrence as the outcome of interest, did not detect any association between patient-reported statin use after diagnosis and RFS (111). Recently, our group showed a null effect of statin use on recurrence rate (adjusted HR=1.01; 95% CI: 0.93, 1.09) within a large prospective Danish study including 21,152 patients and 5,036 recurrences (112). It has also been argued that the driver for any improved cancer-related outcomes based on statin use may simply be a result of the healthy user effect (113).

While we found differences between statin usage groups for effects of lipids on CRC recurrence, results may be subject to bias based on uneven distribution of lipid measures between statin users and non-users and lack of accounting for time varying-confounding in our analysis. Future analyses will consider a marginal structural modeling approach examining statin use as a time-varying confounder and applying lipid measures throughout the study as contributing exposures.

This study's strengths include its prospective design and active follow-up of CRC patients. Complete and reliable data from the Danish Colorectal Cancer Group allowed for adjustment of all covariates of interest within the study. Based on active follow-up, time to recurrence detection should not vary by participant health status, and potential for lead time bias is eliminated. We defined lipid measurements as time-varying exposures, assessing a time of exposure (post-surgery) that is clinically actionable. Prescription drug use was assessed using a lag time of one year. This negates the influence of increased contact with medical professionals directly proceeding death as a factor for prescription use (114). Excluding prescription drug use in the year that recurrence occurs is also beneficial to reduce the possibility of reverse causation biasing results, as statin prescriptions are often discontinued in the months directly preceding death (115). Though NSAIDs and aspirins are available over the counter as well as by prescription in Denmark, it is unlikely that prescription drug use is subject to a large degree of misclassification here, as NSAID and aspirin prescriptions are reimbursed by the Danish healthcare system, encouraging regular users to obtain these drugs through the system.

This main limitation of this study is a relatively small cohort (n=342) and limited number of recurrences (n=60), which drives the precision of effect estimates. The uneven distribution of lipid measures among covariate groups, particularly between current statin users versus non-users, also leads to an issue in positivity assumptions. Combined, these two factors result in a high degree of variability in stratified analyses.

We did not adjust for BMI based on uncertainty in timing of available measurement (recorded in DCCG). However, lack of substantial evidence for a specific effect of BMI on CRC recurrence may allow for its exclusion. While obesity is well-recognized as a strong risk factor for CRC incidence and has been shown by some groups to have a harmful effect on overall survival (29, 116), there is no consensus on associations between pre-surgical BMI and CRC-specific, progression-free (116) or disease-free survival, suggesting that obesity may affect survival through a pathway that does not involve recurrence. In fact, several studies evaluating

pre-diagnosis BMI either (a) did not see an association with CRC- specific mortality (29), or (b) did not see an association of BMI with either overall survival or CRC-specific survival (33, 117). A pooled study using the Adjuvant Colon Cancer Endpoints (ACCENT) Group database evaluated the association with BMI measured at enrollment with time to recurrence (TTR), DFS, and OS, and found obesity as a risk factor for DFS and OS in univariate and multivariate analysis, but did not find it to be a risk factor for TTR (34). Instead, underweight BMI was associated with worse TTR, showing that post-diagnosis BMI may act as a proxy for health status, i.e., a more aggressive cancer, which in turn predicts recurrence.

In addition, evidence for the impact of obesity on CRC-specific survival has been contradictory. A recent pooled prospective study found an increased risk of developing second cancers among obese CRC survivors; however, because the risk of second cancer was similar to the risk of primary cancer development, the findings suggested that increased risk was due to higher prevalence of obesity among CRC survivors compared with the overall cohort, as opposed to actual increased susceptibility (118). In contrast, an assessment of BMI at baseline in the European Prospective Investigation into Cancer and Nutrition (EPIC) found increased hazard among obese patients (HR=1.28; 95% CI: 1.0-1.63) for CRC-mortality, and a similar effect of all-cause mortality (HR=1.32; 95% CI: 1.12-1.56) (32). The Iowa women's study on 1,096 females found increased CRC-specific mortality for those with BMI above 30 (HR=1.35, 95% CI 1.00-1.82) that was not also seen for overall survival (119).

If BMI is associated with recurrence risk, it is suggested that it affects cancer return through insulin resistance and the IGF pathway mediators (35). Because this pathway is one of two pathways also suggested as potential lipid-oriented mechanisms for promotion of cancer recurrence (74), adjustment for BMI is unlikely to produce substantially different effect estimates than already observed. Despite these factors, the current analysis may benefit from incorporation of pre-diagnostic BMI within a marginal structural model that includes baseline lipid measurements.

Given the potential for lipid metabolic reprogramming to influence survival of CRC stem cells (96) and the observed altered lipid profiles observed in cancer patients from diagnosis to treatment and beyond, we hypothesized that serum lipids following surgical resection may have utility as a prognostic tool for recurrence. Our finding that levels of LDL-C, and triglycerides do not affect RFS are consistent with associations found in studies of other cancer types. High HDL-C may be associated with increased RFS time, with an enhanced association among statin users and patients with hyperlipidemia. However, we recognize that data imbalances and sparse numbers of patients in these analyses limits interpretation of results.

Further examination of the role of serum lipids in CRC recurrence using a larger cohort may help to resolve the issues our analysis, and are warranted based on biologic plausibility and our findings of a potential role for HDL-C in recurrence. In addition, longitudinal analyses evaluating patient's lipid profiles may reveal more information regarding the role of lipids in CRC recurrence. Collection of lipid profiles among small patient groups have revealed unique alterations of total cholesterol, LDL-C, and HDL-C over time. For instance, a study of 144 radical gastrectomy patients found a decrease in total cholesterol that remained consistent 12 months after surgery, an initial but recoverable decrease in LDL-C, and an increase in HDL-C at 12 months (120). With measurements collected over a time course, this study is well suited for longitudinal assessment of lipid profiles throughout the course of treatment, up until recurrence, remission, or death. Understanding serum changes over time, and linking this to tumor-based changes can help to elucidate mechanisms of lipids in cancer recurrence.

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G. Tables

Table 1. Characteristics of 342 actively followed Danish colorectal cancer patients, by cholesterol measurement assessed in the year preceding recurrence or censorship

Characteristic	All participants (N=342)		Total Cholesterol <5 mmol/L (N=132)		Total Cholesterol ≥5 mmol/L (N=210)	
	N or mean	% or (SD)	N or mean	% or (SD)	N or mean	% or (SD)
Age group at CRC surgery						
<55	46	13	15	11	31	15
55-64	123	36	42	32	81	39
65-74	129	38	55	42	74	35
75-84	37	11	16	12	21	10
>=85	7	2.0	4	3.0	3	1.4
Sex						
Female	198	58	81	61.4	117	56
Male	144	42	51	38.6	93	44
Mean BMI (kg/m^2)	26.3	(4.3)	26.7	(4.9)	26.1	(4.0)
BMI category						
≥ 30	63	18	30	23	33	16
< 30	248	73	87	66	161	76
Missing	31	9	14	11	17	8.1
Date of cancer debut						
2001-2004	92	27	40	30	52	25
2005-2008	177	52	67	51	110	52
2009-2011	73	21	25	19	48	23
Charlson comorbidity score at CRC diagnosis						
0	254	74	90	68	164	78
1 or 2	78	23	35	27	43	21
>=3	10	2.9	7	5.3	3	1.4
No. of positive lymph nodes						
0	228	67	93	70	135	64
1	75	22	26	20	49	23
2	22	6.4	6	5	16	7.8
3	17	5.0	7	5	10	4.8
Stage at CRC diagnosis						
I	42	12	21	15.9	21	10
II	186	60	72	54.5	114	54
III	114	33	39	29.5	75	36
Family history of CRC						
No	74	22	28	18	46	25
Yes	211	62	84	54	127	69
Missing	57	17	45	29	12	6.5
Surgical urgency						
Non-acute	337	99	132	100	205	98
Acute	5	1.5	0	0	5	2.4
Receipt of neoadjuvant therapy						
No	266	78	98	74	168	80
Yes	76	22	34	26	42	20

Receipt of adjuvant therapy						
No	266	78	107	81	159	76
Yes	76	22	25	19	51	24
Aspirin, any use						
No	235	69	79	60	156	743
Yes	107	31	53	40	54	26
Aspirin use, one year lag						
No	260	76	92	70	168	80
Yes	82	24	40	30	42	20
NSAID, any use						
No	150	44	51	39	99	47
Yes	192	56	81	61	111	53
NSAID use, one year lag						
No	263	77	95	72	168	80
Yes	79	23	37	28	42	20
Statin use, ever						
No	233	68	70	53	163	78
Yes	83	24	62	47	47	22
Statin use, one year lag						
No	245	72	72	55	173	82
Yes	97	28	60	45	37	18
Pre-surgery statin use						
No	275	80	86	65	189	90
Yes	67	20	46	35	21	10
Cancer type						
Colon	180	53	73	55	107	51
Rectum	162	47	59	44	103	49
Mean lipids (mmol/L)						
HDL	1.28	(0.41)	1.35	(0.42)	1.45	(0.46)
LDL	3.17	(0.93)	2.31	(0.56)	3.81	(0.78)
LDL (recalculated)[1]	3.19	(0.94)	2.30	(0.57)	3.82	(0.80)
Triglyceride	1.63	(0.85)	1.43	(0.82)	1.80	(1.03)

[1]LDL calculated regardless of high TG

Table 2. Hazard ratios for recurrence by lipid profiles in year preceding recurrence in actively followed CRC cohort (n=342)

	Unadjusted Model		Multivariable Model[1]			
			Statin=1		*Statin=0*	
Lipid	HR	95% CI	HR	95% CI	HR	95% CI
Continuous[2]						
LDL-C	1.01	(0.99, 1.04)	1.05	(1.00, 1.11)	1.01	(0.97, 1.04)
HDL-C	0.99	(0.93, 1.06)	0.80	(0.65, 0.98)	1.02	(0.94, 1.10)
Triglycerides	0.99	(0.96, 1.03)	0.96	(0.90, 1.03)	0.99	(0.96, 1.03)
Clinical Standards						
LDL-C	1.43	(0.83, 2.45)	2.27	(0.66, 7.75)	1.36	(0.70, 2.64)
HDL-C[3]	1.16	(0.65, 2.08)	0.49	(0.15, 1.67)	1.92	(0.89, 4.16)
Triglycerides	1.34	(0.78, 2.31)	2.18	(0.59, 8.05)	1.33	(0.69, 2.57)

[1]Adjusted for diagnosis year, age at diagnosis, tumor stage, sex, chemotherapy (neoadjuvant and adjuvant), Charlson comorbidity score, aspirin, NSAID, and statin use in year preceding event
[2] Hazard ratios for 0.1 mmol/L increase
[3] HDL-C HR represents high HDL-C (≥1 or ≥1.2 mmol/L), considered optimal

Table 3a. Multivariable association between lipid profiles in year preceding recurrence and time to recurrence, by baseline hyperlipidemia status

		Statins=1		*Statins=0*	
Lipid Measurement	No. / Events[1]	HR[2,3]	95% CI	HR[2,3]	95% CI
Outside CS for TG, TC, or LDL-C	250 / 39				
LDL-C		1.13	(1.03, 1.24)	1.00	(0.96, 1.05)
HDL-C		0.49	(0.28, 0.84)	1.03	(0.83, 1.44)
Triglycerides		0.95	(0.85, 1.06)	1.00	(0.95, 1.04)
Inside CS for TG, TC and LDL-C	92 / 17				
LDL-C		1.09	(0.93, 1.28)	0.95	(0.85, 1.07)
HDL-C		0.89	(0.67, 1.17)	1.13	(0.89, 1.42)
Triglycerides		1.00	(0.41, 2.40)	1.09	(0.94, 1.28)

[1]Censored at time of new primary diagnosis
[2]Adjusted for: diagnosis year, age at diagnosis, tumor stage, sex, chemotherapy (neoadjuvant and adjuvant), Charlson comorbidity score, aspirin, NSAID, and statin use in year preceding event
[3]HR for 0.1 mmol/L increase in lipid

Table 3b. Multivariable association between lipid profiles in year preceding recurrence and time to recurrence, by baseline HDL-C measurement

		Statins=1		*Statins=0*	
Lipid Measurement	No. / Events	HR[1,2]	95% CI	HR[1,2]	95% CI
Outside CS, HDL	128 / 23				
LDL-C		1.08	(0.99, 1.17)	0.97	(0.91, 1.04)
HDL-C		0.60	(0.34, 1.05)	1.09	(0.89, 1.32)
Triglycerides		0.98	(0.88, 1.08)	0.99	(0.93, 1.06)
Inside CS, HDL	214 / 34				
LDL		1.05	(0.97, 1.15)	0.99	(0.94, 1.03)
HDL-C		0.85	(0.61, 1.17)	1.00	(0.90, 1.11)
Triglycerides		0.95	(0.78, 1.16)	1.01	(0.94, 1.08)

[1]Adjusted for: diagnosis year, age at diagnosis, tumor stage, sex, chemotherapy (neoadjuvant and adjuvant), Charlson comorbidity score, aspirin, NSAID, and statin use in year preceding event
[2]HR for 0.1 mmol/L increase in lipid

Table 4. Hazard ratios for recurrence by increased lipid measurements in year preceding recurrence, among never statin users (n=266, events=43)

	Unadjusted Model		Multivariable Model[1]	
Lipid	HR	95% CI	HR	95% CI
Continuous				
LDL-C	1.00	(0.97, 1.04)	1.00	(0.96, 1.03)
HDL-C	1.02	(0.95, 1.10)	1.02	(0.94, 1.10)
Triglycerides	0.99	(0.96, 1.03)	0.99	(0.87, 1.12)
Clinical Standards				
LDL-C	1.36	(0.71, 2.61)	1.56	(0.78, 3.11)
HDL-C[2]	1.42	(0.70, 2.88)	1.86	(0.84, 4.11)
Triglycerides	1.08	(0.57, 2.07)	1.23	(0.62, 2.46)

[1]Adjusted for diagnosis year, age at diagnosis, tumor stage, sex, chemotherapy (neoadjuvant and adjuvant), Charlson comorbidity score, aspirin and NSAID use in year preceding surgery.
[2]HR for HDL-C is for ≥1 or 1.2 mmol/L

Table 5. Hazard ratios for recurrence by increased lipid measurements in year preceding recurrence, by baseline hyperlipidemia status, among never statin users

		Unadjusted Model		Multivariable Model[1]	
Continuous lipid measurement	No. / Events	HR[2]	95% CI	HR[2]	95% CI
Outside CS for TC, TG, LDL-C	209 / 32				
LDL-C		1.01	(0.97, 1.05)	1.02	(0.98, 1.06)
HDL-C		1.00	(0.91, 1.09)	1.00	(0.91, 1.11)
Triglycerides		0.99	(0.95, 1.03)	0.99	(0.95, 1.03)
Inside CS for TC, TG, LDL-C	57 / 11				
LDL-C		0.98	(0.89, 1.08)	0.96	(0.94, 1.27)
HDL-C		1.09	(0.96, 1.24)	1.08	(0.87, 1.33)
Triglycerides		1.06	(0.93, 1.20)	1.09	(0.94, 1.27)

[1]Adjusted for: diagnosis year, age at diagnosis, tumor stage, sex, chemotherapy (neoadjuvant and adjuvant), Charls comorbidity score, aspirin, and NSAID use in year preceding event
[2]HR for 0.1 mmol/L increase in lipid

Table 6a. Association of continuous lipid profile with all-cause mortality and CRC-specific mortality, n=342

	Unadjusted Model		Multivariable Model[1]			
			Statin=1		Statin=0	
	HR	95% CI	HR[2]	95% CI	HR[2]	95% CI
All-cause mortality (events=27)						
LDL-C	0.97	(0.93, 1.01)	1.02	(0.95, 1.09)	0.99	(0.93, 1.04)
HDL-C	1.00	(0.91, 1.09)	0.92	(0.78, 1.08)	1.03	(0.89, 1.19)
Triglycerides	0.97	(0.92, 1.02)	0.94	(0.83, 1.06)	0.93	(0.81, 1.12)
CRC-cause mortality (events=14)						
LDL-C	0.99	(0.94, 1.05)	1.02	(0.96, 1.10)	1.01	(0.93, 1.10)
HDL-C	1.03	(0.91, 1.16)	1.10	(0.87, 1.38)	0.99	(0.82, 1.21)
Triglycerides	0.96	(0.88, 1.05)	1.01	(0.89, 1.14)	0.83	(0.67, 1.01)

[1]Adjusted for: diagnosis year, age at diagnosis, tumor stage, sex, chemotherapy (neoadjuvant and adjuvant), Charlson comorbidity score, aspirin, NSAID, and statin use in year preceding event

[2]HR for 0.1 mmol/L increase in lipid

Table 6b. Association of continuous lipid profile with all-cause mortality and CRC-specific mortality, for never-statin users, n=266

	Unadjusted Model		Multivariable Model[1]	
	HR[2]	95% CI	HR[2]	95% CI
All-cause mortality (events=16)				
LDL-C	0.97	(0.92, 1.03)	0.98	(0.92, 1.03)
HDL-C	1.00	(0.88, 1.12)	1.01	(0.87, 1.18)
Triglycerides	0.96	(0.89, 1.03)	0.93	(0.85, 1.02)
CRC-cause mortality (events=8)				
LDL-C	0.98	(0.91, 1.06)	1.10	(0.93, 1.10)
HDL-C	0.94	(0.79, 1.12)	1.04	(0.84, 1.29)
Triglycerides	0.83	(0.68, 1.00)	0.82	(0.67, 1.02)

[1]Adjusted for: diagnosis year, age at diagnosis, tumor stage, sex, chemotherapy (neoadjuvant and adjuvant), Charlson comorbidity score, aspirin, and NSAID use in year preceding event

[2]HR for 0.1 mmol/L increase in lipid

H. Figures/Figure Legends

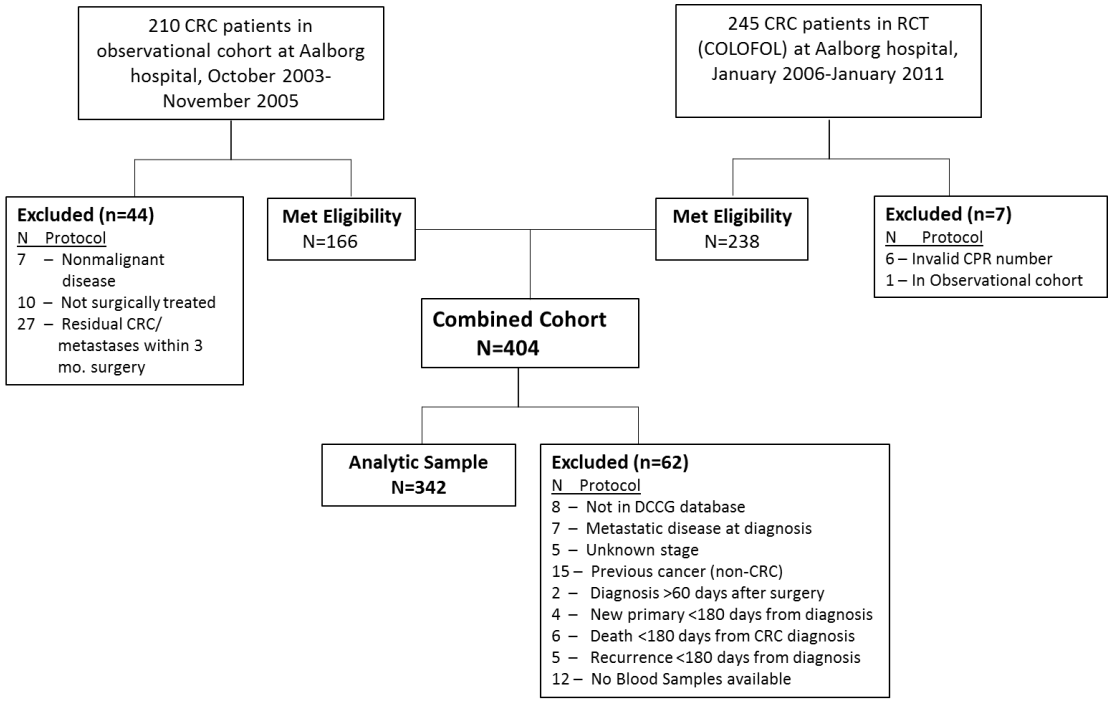

Figure 1. Flow chart of participants included in final analytic cohort.

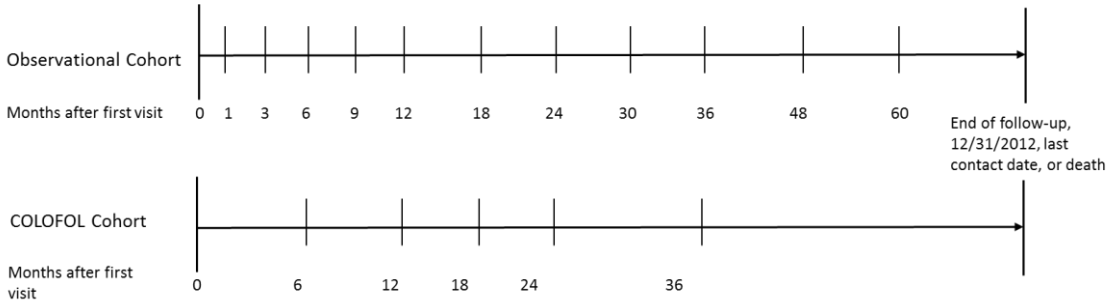

Figure 2. Patient visit schedules by original cohort.

Chapter III: Public Health Implications and Future Directions

Colorectal cancer recurs in approximately 40-50% of patients who are surgically treated (6, 10), and survival following recurrence is very low (13, 14). If identified prior to metastases, re-resection provides the best prognosis for recurrent CRC patients. While it is essential to understand underlying mechanisms behind relapse in order to work toward prevention of recurrent disease, biological mechanisms are not yet described. Given the inability to target development of recurrence specifically, current public health action should focus on finding ways to recognize patients who may be at high risk for recurrence and to ensure early diagnosis of recurrent disease. Current methods to determine high-risk patients are poor, and uncovering biomarkers that describe recurrence risk is a priority for CRC research.

Prognostic and predictive biomarkers have been identified for several cancer types, including breast, prostate, colorectal, and bladder cancers, among others (88). These markers have proven utility as risk-stratification tools that can provide more directive therapies for patients. Some of the greatest clinical successes have occurred in breast cancer, including the use of estrogen receptor (ER) profiling to identify patients who may benefit from hormone-based interventions including tamoxifen (121, 122). Large mRNA profiles have also been used more recently to determine patient prognosis in both breast (123-125) and colorectal (126-128) cancers. Despite the successful application of prognostic biomarkers in cancer medicine, many discovered markers are not clinically actionable. Even among markers that can be used in the clinical setting, requirement of intensive assays, immunohistochemistry of tumor sections, and extensive genetic profiling, may limit use. Serum-based biomarkers allow for a less invasive and less costly way to assess patient prognosis. For instance, in colorectal cancer, serum carcinoembryonic antigen (CEA) can indicate prognosis (129, 130). However, while CEA profiling has been recommended to monitor recurrence by the European Group on Tumor Markers (131), it is not prognostic for patients with normal levels of CEA pre-surgery (132), and offers an incomplete way to assess recurrence.

In this study, we investigated the association of serum lipid profiles with time to recurrence. Our findings suggest that increased HDL-C may increase time to recurrence in particular subsets of patients, including statin users and patients with hyperlipidemia, and as such, HDL-C may have prognostic potential in identifying patients at risk for recurrence. However, several limitations of our study, including small sample size and potential time-varying confounding by statin use, preclude public health action based on this finding.

While the evidence from our study does not clearly reveal the utility of serum lipid profiling to identify patients at risk for recurrence, our findings do warrant further study into serum lipids as biomarkers for CRC prognosis. If HDL-C is found to be associated with recurrence free survival in larger prospective studies, testing of serum lipids could be recommended for CRC patients at regular intervals following surgical resection. Given low cost and limited invasiveness of the serum test, this could be applied to all CRC patients, providing an equal standard of care for CRC follow-up, irrespective of factors such as socioeconomic status.

Building off of this work, other studies may consider the unique trajectory of lipid measurements over the course of recurrence progression, following patients from diagnosis to recurrence or censorship. Finding a specific time period following surgery where lipid measurements are most relevant to CRC prognosis will help to identify best-practice methods for surveillance of recurrent patients. Extensive follow up for CRC patients should continue based on current recommendations, covering the period over which patients are at elevated risk for recurrence, from surgery until five years following surgery. Future studies should examine potential non-invasive biomarkers to reveal patient groups at risk for recurrent disease.

Appendix

Supplementary Tables

Table S1. Frequency of participants within and outside of clinical standards for lipid components in actively followed cohort (n=342), at baseline and year preceding recurrence

	Baseline					*Year preceding recurrence/ censorship*				
	Statin Users		Non-users			Statin users		Non-users		
Lipid Measurement	N	%	N	%	X^2 p[1]	N	%	N	%	X^2 p[1]
LDL-C[2]										
Outside CS	16	24	177	64	<.0001	27	28	163	66	<.0001
Inside CS	50	76	98	35		70	72	82	33	
HDL-C[3]										
Outside CS	28	42	100	36	0.36	33	34	66	27	0.19
Inside CS	38	58	175	64		64	66	179	73	
Triglycerides[4]										
Outside CS	28	42	92	33	0.17	36	37	88	36	0.84
Inside CS	38	58	183	66		61	63	157	64	
Total Cholesterol & LDL[5]										
Outside CS	20	30	196	71	<.0001	40	41	183	75	<.0001
Inside CS	46	70	79	29		57	59	62	25	

[1]Two-sided p-value for chi-squared test, α=0.05
[2]LDL-C outside CS ≥3 mmol/L
[3]HDL-C outside CS < 1 mmol/L or <1.2 mmol/L
[4]TG outside CS ≥1.7 mmol/L
[5]TC outside CS ≥5 mmol/L

Table S2. Characteristics of 266 non-statin user subset, by cholesterol measurement assessed in the year preceding recurrence or censorship

Characteristic	All participants (N=266)		Total Cholesterol <5 mmol/L (N=72)		Total Cholesterol ≥5 mmol/L (N=194)	
	N or mean	% or (SD)	N or mean	% or (SD)	N or mean	% or (SD)
Age group at CRC surgery						
<55	41	15.4	12	16.7	29	14.9
55-64	100	37.6	22	30.6	78	40.2
65-74	89	33.5	23	31.9	66	34.0
75-84	29	10.9	11	15.3	18	9.3
>=85	7	2.6	4	5.6	3	1.5
Sex						
Female	155	58.3	45	62.5	110	56.7
Male	111	41.7	27	37.5	84	43.3
Mean BMI (kg/m^2)	25.9	(4.2)	25.8	(4.8)	26.0	(4.0)
BMI category						
≥ 30	42	15.8	13	18.1	29	14.9
< 30	205	77.1	51	70.8	154	79.4
Missing	19	7.1	8	11.1	11	5.7
Date of cancer debut						
2001-2004	80	30.1	24	33.3	56	28.9
2005-2008	131	49.2	34	47.2	97	50.0
2009-2011	55	20.7	14	19.4	41	21.1
Charlson comorbidity score at CRC diagnosis						
0	217	81.6	60	83.3	157	80.9
1 or 2	45	16.9	11	15.3	34	17.5
>=3	x	1.5	x	x	3	1.5
No. of positive lymph nodes						
0	175	65.8	51	70.8	124	63.9
1	58	21.8	11	15.3	47	24.2
2	19	7.1	5	6.9	14	7.2
3	14	5.3	5	6.9	9	64.3
Stage at CRC diagnosis						
I	36	13.5	13	18.1	23	11.9
II	139	52.3	38	52.8	101	52.1
III	91	34.2	21	29.2	70	36.1
Family history of CRC						
No	61	22.9	17	23.6	44	22.7
Yes	163	61.3	45	62.6	118	60.8
Missing	42	15.8	10	13.9	32	16.5
Surgical urgency						
Non-acute	261	98.1	72	100.0	189	97.4
Acute	5	1.9	0	0.0	5	1.9
Receipt of neoadjuvant therapy						
No	198	77.3	48	66.7	150	77.3
Yes	68	25.6	24	33.3	44	22.7
Receipt of adjuvant therapy						
No	208	78.2	60	83.3	148	76.3
Yes	58	21.8	12	16.7	46	23.7

Aspirin, any use						
No	204	76.7	56	77.8	148	76.3
Yes	62	23.1	16	22.2	46	23.7
Aspirin use, one year lag						
No	228	85.7	59	81.9	169	87.1
Yes	38	14.3	13	18.1	25	12.9
NSAID, any use						
No	125	47.0	33	45.8	92	47.4
Yes	141	53.0	39	54.2	102	52.6
NSAID use, one year lag						
No	212	79.7	57	79.2	155	79.0
Yes	54	20.3	15	20.8	39	20.1
Statin use, ever						
No	233	68	70	53	163	78
Yes	83	24	62	47	47	22
Cancer type						
Colon	131	49.3	39	54.2	92	47.4
Rectum	135	50.7	33	45.8	102	52.6
Mean lipids (mmol/L)						
HDL	1.42	(0.43)	1.33	(0.41)	1.46	(0.43)
LDL	3.49	(0.95)	2.46	(0.55)	3.87	(0.78)
LDL (recalculated)[1]	3.50	(0.98)	2.45	(0.57)	3.89	(0.80)
Triglyceride	1.63	(0.85)	1.43	(0.82)	1.80	(1.03)
Cholesterol	1.61	(0.82)	1.45	(0.90)	1.67	(0.78)

[1]LDL calculated regardless of high TG

Table S3. Hazard ratios for recurrence among non-statin users for 0.1 mmol/L increase in lipid measurements, by baseline HDL-C value

		Unadjusted Model		Multivariable Model[1]	
Continuous lipid measurement	No. / Events	HR	95% CI	HR	95% CI
HDL-C (<1 or 1.2 mmol/L)	97 / 16				
LDL-C		1.03	(0.98, 1.08)	1.04	(0.98, 1.10)
HDL-C		1.08	(0.92, 1.27)	1.02	(0.82, 1.27)
Triglycerides		0.99	(0.94, 1.05)	0.97	(0.915, 1.04)
HDL-C (≥1 or 1.2 mmol/L)	169 / 27				
LDL-C		0.98	(0.94, 1.03)	0.98	(0.93, 1.03)
HDL-C		1.02	(0.92, 1.13)	1.01	(0.90, 1.12)
Triglycerides		0.99	(0.93, 1.05)	0.98	(0.91, 1.05)

[1]Adjusted for: diagnosis year, age at diagnosis, tumor stage, sex, chemotherapy (neoadjuvant and adjuvant), Charlson comorbidity score, aspirin, and NSAID use in year preceding event