Universidade do Minho
Escola de Medicina

André Bettencourt Goulart

Influence of metabolic syndrome and inflammatory markers in the outcomes of colorectal cancer surgery

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Tese de Doutoramento
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Trabalho efetuado sob a orientação do
Professor Doutor Pedro Alexandre Leão Araújo Gonçalves Teixeira
Professor Doutor Nuno Jorge Carvalho de Sousa

dezembro de 2021

Licença concedida aos utilizadores deste trabalho

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Statement of Integrity

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

Resumo

Influência do síndrome metabólico e de mediadores inflamatórios nos resultados da cirurgia do cancro colorretal

Objetivo: Avaliar a relação entre síndrome metabólico (SM) e gordura visceral nos resultados cirúrgicos do cancro colorretal (CCR) e avaliar a influência de biomarcadores em prever as complicações cirúrgicas.

Métodos: Foram desenvolvidos dois estudos: a) retrospetivo - doentes operados por CCR no Hospital de Braga entre janeiro de 2007 e dezembro de 2009, quantificação da gordura visceral e recolha de dados para análise de sobrevida; b) prospetivo - doentes operados por CCR entre agosto de 2015 e agosto de 2016, com avaliação clínica e analítica pré-operatória e pós-operatória seriada até dois anos de seguimento e recolha de amostra da lesão tumoral para estudo imunohistoquímico.

Resultados: No estudo retrospetivo (n=199), a taxa de sobrevida global aos 5 anos foi de 60%; não foram encontradas diferenças estatisticamente significativas na sobrevida de doentes com diferentes quantidades de gordura visceral. No estudo prospetivo (n=134), 26.9% dos doentes desenvolveram complicações (15.7% *minor* vs 11.2% *major*) e 1.5% faleceram nos primeiros 30 dias após a cirurgia, não tendo sido encontrada nenhuma associação estatisticamente significativa entre SM e os resultados cirúrgicos. Verificou-se um aumento significativo da concentração de PCR no D1 e D3 pós-operatório e aumento do rácio PCR/albumina no D3 pós-operatório nos doentes com infeção do local cirúrgico (AUC 0.639, 0.729 e 0.736, respetivamente). A análise de regressão logística multivariável mostrou que estes biomarcadores são preditores independentes da infeção do local cirúrgico (OR 7.355, 7.605 e 8.337, respetivamente). Foi encontrada uma associação significativa entre os valores de VEGF sérico e a expressão tumoral do VEGF-R3 ($p < 0.001$), com um tamanho do efeito estimado alto ($\eta = 0.35$).

Conclusão: A gordura visceral pode influenciar as complicações pós-operatórias, a deiscência da anastomose e o risco de re-operação nos doentes operados por CCR. O SM não parece influenciar os resultados cirúrgicos. O valor da PCR no D1 e D3 pós-operatório e o rácio PCR/albumina no D3 identificam doentes com baixo risco de infeção do local cirúrgico, o que poderá permitir o uso destes marcadores inflamatórios como uma ferramenta de prognóstico e de alta precoce. A correlação encontrada entre o VEGF sérico e o VEGF-R3 tumoral abre novos horizontes na investigação acerca do potencial uso deste biomarcador na seleção do tratamento e prognóstico dos doentes com CCR.

Palavras chave: cancro colorretal, cirurgia, obesidade, síndrome metabólico

Abstract

Influence of metabolic syndrome and inflammatory markers in the outcomes of colorectal cancer surgery

Aim: Evaluate the relationship between metabolic syndrome (MetS) and visceral fat (VF) on colorectal cancer surgery (CRC) outcomes and the influence of biomarkers in predicting surgical complications.

Methods: Two studies were developed: a) retrospective - patients submitted to curative CRC surgery at Hospital de Braga between January 2007 and December 2009, VF quantification and data collection for survival analysis; b) prospective - patients submitted to CRC surgery at Hospital de Braga between August 2015 and August 2016, with clinical and analytical evaluation before and after until reached two years of follow-up and selection of an histological specimen for immunohistochemistry.

Results: In the retrospective study (n=199), the 5-year overall survival rate was 60%; no significant differences of survival between patients with different amounts of VF were found. In the prospective study (n=134), 26.9% of patients developed complications (15.7% minor vs 11.2% major) and 1.5% died at the first 30 days after surgery. Statistical analysis didn't reveal any association between MetS and surgical outcomes. Higher CRP concentrations on POD1 and POD3 and CRP to albumin ratio on POD3 were found in patients with surgical site infections (AUC 0.639, 0.729 and 0.736, respectively). Multivariable logistic regression analysis showed that those biomarkers were independent predictors of surgical site infections (OR 7.355, 7.605 and 8.337, respectively). Regarding VEGF, results showed significant association of serum values of VEGF with VEGF-R3 expression ($p < 0.001$), with a high estimated effect size ($\eta = 0.35$).

Conclusion: VF may influence postoperative complications, anastomotic leakage and re-operation on colorectal cancer patients. MetS doesn't appear to influence surgical outcomes. The value of CRP on POD 1 and 3 and CRP to albumin ratio on postoperative day 3 can positively identify patients at low risk of surgical site infection, which may allow those inflammatory markers to be used as a prognostic tool for early discharge criteria. The correlation between serum VEGF and tumoral VEGF-R3 open up new horizons in terms of investigating its role as a potential biomarker for the selection of CRC treatment and for prognostic information.

Key-words: colorectal cancer, metabolic syndrome, obesity, surgery

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Abbreviations

AHA	American Heart Association
ALT	alanine transaminase
AST	aspartate transaminase
BMI	body mass index
CAR	C-reactive protein to albumin ratio
CI	confidence interval
CRC	colorectal cancer
CRP	C-reactive protein
CT	computed tomography
DFS	disease-free survival
ELISA	enzyme-linked immunosorbent assay
GPS	Glasgow Prognostic Score
HDL	high-density lipoprotein
HR	hazard ratio
IDF	International Diabetes Federation
IGF	insulin-like growth factor
IL-1β	interleukin 1 beta
IL-6	interleukin 6
LDL	low-density lipoprotein
MetS	metabolic syndrome
MRI	magnetic resonance imaging

NCEP-ATPIII National Cholesterol Education Program – Third Adult Treatment Panel

NHLBI National Heart, Lung, and Blood Institute

NPV negative predictive value

OS overall survival

PCR polymerase chain reaction

PCT procalcitonin

POD post-operative day

PPV positive predictive value

ROC receiver operating characteristic

RR relative risk

SIRS systemic inflammatory response syndrome

TGFβ transforming growth factor beta

TNFα tumor necrosis factor alpha

VEGF vascular endothelial growth factor

VEGFR vascular endothelial growth factor receptor

VF visceral fat

WBC white blood cells

WHO World Health Organization

Chapter 1: Introduction

Colorectal cancer (CRC) is a common disease that surgeons have to deal on a daily basis. When a patient is newly diagnosed with CRC, that first question he/she usually ask is whether or not they will be "OK", and that is, of course, very difficult to answer.

Prognosis is currently based on preoperative staging of the disease and pathology of surgical specimens but these are used for groups and do not accurately predict individual survival and relapse rates. There other factors contributing to outcomes of CRC patients that are yet unexplored.

On an empiric basis, there is an established idea that obese and diabetic patients have worst outcomes than thinner, healthier patients, especially when it comes to post-surgical morbidity and mortality: when an obese patient undergoes surgery a complication is immediately anticipated; but when a more fit and healthy patients' surgery complicates, we ask ourselves why this happened. However, when we look at data from studies, neither obesity nor metabolic syndrome are clearly defined as risk factors for complications or worst outcomes after CRC surgery.

A common discussion point among surgeons is the prognostic value of c-reactive protein (CRP) and leucocytes on the first days following surgery: when CRP or leucocytes have high values sometimes it is interpreted as "normal", attributing the rise to the surgical insult, but on other circumstances the patient is thought to have an infectious complication or anastomotic leak. The ability to use inflammatory biomarkers to predict complications would be highly useful but at the time this work was developed the results that had been published were not concordant.

Due to an evolving need for individual outcome prediction, and the other aforementioned reasons, we developed this research work with the purpose of determining if metabolic syndrome and inflammatory markers have the ability to predict outcomes after CRC surgery.

1.1. Epidemiology

1.1.1. Colorectal cancer

In 2018, CRC was the third most frequently diagnosed cancer in the world, with 1,849,518 new cases diagnosed (10.2% of all cancer cases diagnosed), standing just behind lung and breast cancer (with 11.6%

each).(1) Of notice, however, is the fact that projections suggest that annual incidence of CRC will increase by 72%, reaching 3,093,241 new cases in 2040.(1)

In Portugal, data from 2010 showed that CRC is the second most diagnosed of all cancers both in men and women (17.2% and 14.5% respectively), following prostate and breast cancer, respectively.(2)

1.1.2. Obesity

Obesity is currently defined as an elevated body mass index (BMI), which typically occurs as a consequence of excess of adipose tissue. According to the World Health Organization (WHO), in 2016, 39% of adults aged more than 18 years in the world had excess of weight (BMI ≥25kg/m^2) and 13% were obese (BMI ≥30kg/m^2).(3) Similarly to other developed countries, obesity in Portugal affects a considerable part of the population and data from 2016 shows that 63.8% of the population at adult age has excess of weight, of which 26.2% are obese.(4)

1.1.3. Metabolic syndrome

Kylin, in 1920, was the first to define metabolic syndrome (MetS), in order to demonstrate the association of hypertension, hyperglycemia, and gout.(5) Meanwhile, the syndrome was several times renamed as "syndrome X", "the deadly quartet" and "insulin resistance syndrome".(6) Currently, many different definitions have been proposed by several institutions, but they all converge on the same basic components: hypertension, dyslipidemia, insulin resistance and central obesity.

In 1998, WHO published their definition of MetS.(7) In 2001, the National Cholesterol Education Program – Third Adult Treatment Panel (NCEP-ATPIII) published new criteria for the diagnosis of MetS that included the waist circumference instead of BMI, as an indicator of obesity.(8) In 2005, the American Heart Association (AHA) and the National Heart, Lung, and Blood Institute (NHLBI) updated the NCEP-ATPIII criteria, in order to include the current use of medication for hypertension, triglycerides and high-density lipoprotein (HDL)-cholesterol as positive criteria.(9) In the same year, the International Diabetes Federation (IDF) published another definition for MetS that includes central obesity as a mandatory criteria associated with other two of four criteria (raised triglycerides or specific treatment for this lipid abnormality, reduced HDL-cholesterol or specific treatment for this lipid abnormality, raised blood

pressure or treatment of previously diagnosed hypertension, raised fasting plasma glucose or previously diagnosed type 2 diabetes).(10)

With the aim of standardizing the MetS definition, five groups (IDF, AHA, NHLBI, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity) released a joint statement in 2009 regarding the harmonization of the criteria. One of the main improvements resulting from that effort is that the criteria used for abdominal obesity (waist circumference) required refinement with regard to country-specific and population-specific definitions.(11)

The reported prevalence of MetS varies depending on the definition used, age, sex, socioeconomic status, and the ethnic background of study cohorts.(12) In Portugal, the PORMETS study, that included non-institutionalized Portuguese adults selected from primary health care centers' lists from February 2007 to July 2009, estimated that the prevalence of MetS was 36.5% using the NCEP-ATPIII criteria and 49.6% using the IDF criteria.(13) In Europe, a recent study that included 10 European countries quantified the prevalence of MetS according to NCEP-ATPIII criteria in 24.3%.(14) In the United States of America, data from the National Health and Nutrition Examination Survey stated that the prevalence of MetS in 2007-2012 according to NCEP-ATPIII criteria raised from 25.3% to 34.2% in the past two decades.(15)

1.2. Obesity and Cancer

Since 2003, obesity has been firmly established as a risk factor for different types of cancer, such as esophageal, pancreatic, colorectal, breast (postmenopausal), endometrium and kidney cancer, leading to an increased mortality rate.(16-19) The American Society of Clinical Oncology reported that obesity is quickly overtaking tobacco as the leading preventable risk factor. Cancer patients with obesity have higher probabilities of cancer mortality, worse prognosis after cancer diagnosis and higher risk of second malignancies and comorbidities.(20)

Adipose tissue deposits in two compartments in the body: subcutaneous and visceral. Visceral fat (VF) is more active metabolically than subcutaneous fat, has multiple endocrine, metabolic and immunological functions and has been shown to be central to the pathogenesis of the MetS.(21) The pathways by which visceral obesity promotes cancer development are: inflammation and adipokines, insulin resistance and insulin-like growth factor (IGF) axis and obesity related hypoxia.(22)

1.2.1. Obesity and cancer development

1.2.1.1. Inflammation and adipokines

The relationship between inflammation and cancer is an accepted paradigm.(22) Chronic inflammation influences the proliferation of tumor cells, angiogenesis, the risk of metastases and the response to cancer therapy.(23) Obesity is recognized as a cause of chronic subclinical inflammation that can promote cancer development. However, the influence of obesity in cancer incidence differs accordingly to the distribution of fat into visceral and subcutaneous compartments; in fact, evidence suggests that obesity and cancer are mediated by visceral rather than generalized body fat.(24)

The adipose tissue is now recognized as a complex and dynamic endocrine organ with an intricate role in whole body homeostasis rather than an inert tissue for energy storage.(24) The adipocyte secretes adipokines like leptin and adiponectin. In an obese patient, the production on adipokines is dysregulated with increased levels of leptin and decreased levels of adiponectin.(25) Leptin seems to be capable of promoting tumor growth by endorsing angiogenesis through vascular endothelial growth factor (VEGF) signaling and hypoxia inducible factor 1 alpha stabilization.(26) VEGF production is higher in omental fat than in fat located at any other site in the body.(27) In contrast, adiponectin presents anti-inflammatory proprieties and acts as a negative regulator of angiogenesis directly into tumor cells by reducing cellular proliferation and inducing endothelial apoptosis.(28)

The adipose tissue, particularly the VF tissue, also produces inflammatory cytokines such as tumor necrosis factor alpha (TNFα), transforming growth factor beta (TGFβ), interleukin 6 (IL-6) and interleukin 1 beta (IL-1β) (all known as proinflammatory cytokines).(29-31) The release of these cytokines attracts inflammatory cells (including monocytes and macrophages) which, in turn, secrete inflammatory cytokines themselves and contribute, amongst others, to the development of insulin resistance.(25)

Insulin resistance and activation of the IGF axis is thought to be an important link between visceral adiposity and carcinogenesis, principally by the pro-tumorigenic properties of insulin and IGF.(22) A chronic caloric excess desensitizes tissues to the effects of insulin determining insulin resistance. In turn, Insulin resistance leads to hyperglycemia and a compensatory release of insulin by pancreas, in order to restore glucose level (hyperinsulinemia).(32) Insulin is a potent growth factor of both normal and tumor cells directly through insulin receptor and indirectly through increase in hepatic production of IGF.(32)

1.2.2. Obesity and Colorectal cancer

The relation between obesity and CRC incidence has been intensely studied. However, the results of this relation depend of the criteria used to defined obesity. With respect to BMI, the increase of this index was associated with an increased incidence of CRC. A 5kg/m^2 BMI increase was related to an increased risk of CRC development (relative risk (RR) range from 1.12 to 1.24).(19, 33) In studies where waist circumference was used, the RR of CRC ranged from 1.39 to 2.56. However, the cut-off value for which waist circumference increases the risk of CRC is yet to be defined.(22) Several studies analyzed the differences of the impact of waist circumference and BMI as predictors of CRC and concluded that waist circumference is a stronger predictor of CRC risk than BMI.(34-36) One review of the literature concluded that higher adiposity was associated with an increase of colon cancer-related mortality and a decrease of disease free survival (DFS) of colon cancer in women and rectal cancer in men. This study emphasizes that the percentage of fat tissue or waist circumference are better indicators of adiposity than the BMI.(37) This may suggests that the VF, rather than general adiposity, that is involved in the carcinogenesis of CRC.(22)

Visceral obesity was associated with higher risk of colorectal carcinoma.(38, 39) Besides the risk of CRC, VF has also been associated, in some studies, with a significant increase of surgical complications when compared to VF-free patients (40-42) and as a better complication predictor than BMI.(43, 44) However, a recent paper suggested that VF has no influence on surgical complications in patients with rectal and sigmoid cancer.(45) VF significantly predicted DFS in patients with resectable CRC (46) and increased the likelihood of a poor prognosis in CRC patients receiving adjuvant chemotherapy.(47) Of notice is the fact that visceral obesity has a controversial influence in clinical response to anti-VEGF therapy in CRC.(48, 49)

1.3. Metabolic Syndrome and Colorectal Cancer

There is strong evidence of CRC risk related to MetS. A meta-analysis from 2013 that included 11,462 cancer patients, showed that MetS was associated with an increased risk of CRC in both men and women.(50) An epidemiologic study from South Korea that included a total of 22,809,722 individuals concluded that the hazard ratio (HR) for the development of CRC in individuals with MetS (IDF criteria) was 1.10, after multivariate analysis adjusted for age, sex, smoking status, alcohol consumption and

regular physical exercise. The risk for the development of CRC also increased with the number of MetS components involved (high fasting glucose, central obesity, high blood pressure, high triglycerides levels and low HDL cholesterol levels). A subgroup analysis by gender shows that the risk is significantly higher in men than in women (HR 1.41, 95% confidence interval (CI) 1.37–1.44 and HR 1.23, 95% CI 1.20–1.27, respectively).(51) The physiopathology behind the relationship between MetS and risk of CRC is not yet fully understood. It has been suggested that the mechanism that connects MetS and CRC is related with abdominal obesity and insulin resistance, as discussed before.(19, 33, 52-54)

Unlike obesity, the MetS has been less studied as risk factor for CRC patients' outcomes. With respect to 30-days overall morbidity, a recent study shows that in a multivariate analysis MetS remained significantly associated with renal complications, wound dehiscence and infection and unplanned readmissions, but not with overall morbidity, cardiac and septic complications, nor prolonged length of stay for laparoscopic procedures.(55) Another recent study concluded that MetS does not increase the risk of postoperative outcomes following laparoscopic colectomy.(56) MetS has been, however, associated with a significant increase of tumor recurrence.(57, 58) In what concerns mortality rates, there are several studies(53, 57, 59, 60) and a meta-analysis (50) showing that the presence of MetS has been associated with an increase in mortality rate of CRC patients. However, other studies did not find the same association between MetS and CRC survival.(61-63) One of the latter studies concluded that MetS had no apparent effect on cancer outcomes (overall survival (OS) and recurrence-free rates), probably because of the combined adverse effects of elevated glucose/diabetes mellitus and hypertension and the protective effect of dyslipidemia in patients with nonmetastatic disease.(61)

There are multiple theories that try to explain the negative impact that MetS has on prognosis of CRC patients. First of all, patients with MetS could have overt tissue inflammation and excessive systemic inflammatory response. The insulin-resistance state characteristic of MetS alters the metabolism of adipose tissue leading to an increase in serum levels of adipokines (including IL-6 and TNF-α). These, in turn, aggravate tissue inflammation, and reduce levels of protective adipokine (adiponectin). In these patients with MetS, there are also higher levels of other proinflammatory cytokines (CRP, fibrinogen, and plasminogen activator inhibitor-1).(9, 64) Secondly, MetS is correlated with an impaired microvascular circulation which could cause poor tissue healing and increase the risk of wound complication and anastomotic leakage.(64) Finally, patients with MetS could have a malfunction of polymorphonuclear neutrophils, in particular at the aggregation, adhesion and degranulation levels. This anomaly could be

related to low level of leukotriene B4 in these patients, because this leukotriene has a potent chemotactic and chemokinetic activity for polymorphonuclear neutrophils.(64, 65)

1.4. Prognostic Biomarkers in Colorectal Cancer

1.4.1. Biomarkers

There is an increasing interest in the research of prognostic biomarkers in CRC because they may help improve clinical or therapeutic management of CRC.(66)

1.4.1.1. VEGF

The lymphatic system is involved in the transportation of extravasated protein-rich fluid and cells back into blood circulation.(67, 68) The formation of new lymphatic vessels (lymphangiogenesis) occurs in both normal developing tissues as well as in pathological processes such as inflammation, wound healing, lymphedema and cancer.(68) Lymphangiogenesis may be involved in the earlier stage of CRC development.(66)

VEGF is the most widely studied pro-angiogenic factor.(69) There are three vascular endothelial growth factor receptors (VEGFR) identified, each one having a different participation in angiogenesis and lymphangiogenesis: VEGFR-1 and VEGFR-2 mediate angiogenesis, whereas VEGFR-3 is involved mainly in lymphangiogenesis.(68) The number and diameter of lymphatic vessels is increased in peritumoral tissues, providing a larger contact area and facilitating tumor cell metastasis.(66) The principal ligands of VEGFR-3 and, therefore, the principal inducers of new lymphatic vessels, are VEGF-C and VEGF-D.(66, 68) Tumoral lymphangiogenesis, measured by lymphatic microvessel density, is significantly associated with tumoral lymphatic vessel invasion, lymph node metastasis and adverse outcomes of CRC.(66, 70, 71)

The fast growth of the tumor demands nutrients and oxygen that, in turn, trigger tumor cells to produce VEGF, which consequently leads to the formation of new blood vessels and may facilitate the metastatic spread of tumor cells.(72-74) In addition, it appears that VEGF also has autocrine functions, acting as a survival factor for tumor cells, protecting them from stresses, such as hypoxia, chemotherapy and

radiotherapy.(72) Of relevance in the context of this Thesis, is the fact that circulating levels of VEGF are increased in obese patients, namely in visceral obese patients.(73, 75, 76)

The best way to determinate VEGF level remains unknown. This biomarker can be measured in tumor tissue by immunohistochemistry, reverse transcription with polymerase chain reaction (rtPCR) or in the plasma by enzyme-linked immunosorbent assay (ELISA).(77) In tumoral tissue it can be evaluated through the expression of different forms of VEGF (VEGF-A, VEGF-B, VEGF-C and VEGF-D) and VEGF receptor (VEGFR-1, VEGFR-2 and VEGFR-3).(78) In serum, VEGF and the soluble form of VEGFR-2 (sVEGFR-2) can also be measured.(77) The relationship between the circulating and tissue concentration of VEGF remains unclear.(69)

1.4.1.2. C-reactive protein

The production of CRP belongs to a nonspecific acute phase response to most forms of infection, tissue damage, inflammation and cancer.(79) During the acute phase response, cytokines, predominantly IL-6, are released from pathological site and trigger the liver to produce CRP.(79, 80) CRP has an affinity to phosphocholine, which is inaccessible in normal human cells. However, in damage human cells, bacteria, fungi and parasites, CRP, as part of the innate host defense, recognizes phosphocholine and activates the classical complement pathway.(80) This activation enhances phagocytosis by macrophages, thus acting as an early defense against infection.(81) CRP is a reliable, but non-specific, marker of acute inflammation and has been investigated as an early indicator of infectious complications following abdominal surgery.(82)

1.4.1.3. Procalcitonin

Procalcitonin (PCT) is an inactive pro-peptide that serves as the precursor for calcitonin in C-cells of the thyroid.(83) In addition to its endocrine role, patients with sepsis activate an alternative pathway in response to proinflammatory mediators (IL-1β, TNF-α, and IL-6). This increases the concentration of PCT by multiple non-thyroidal tissue like white blood cells, spleen, kidney, pancreas, colon, adipocytes and the brain.(83-85)

The presence of bacterial endotoxins stimulates synthesis of PCT that is rapidly released into the circulation after 3-4 hours and peaks after 8-24 hours.(86) Following surgery, PCT concentrations are

commonly elevated by transient bacterial contamination or bacterial translocation during the operation or preparation of intestinal anastomoses.(79) It has also been observed that patients with an abnormal postoperative course more frequently have increased PCT levels than patients with a normal postoperative course. PCT seems to be a more specific marker of septic complications than CRP.(79)

1.4.1.4. Albumin

Human serum albumin, the most abundant protein in plasma, is a macromolecule that acts as the main determinant of plasma oncotic pressure and controls fluid distribution between body compartments.(87) Tumors have the ability to trap the larger plasma proteins and use their degradation products for proliferation. The accumulation of albumin in tumors is not only explained by the enhanced permeability of the vascular system, but also by the absence of a lymphatic system in the tumor that impairs lymphatic drainage.(88) Thus, albumin levels might be of relevance in the context of CRC.

1.5. Outcomes after CRC surgery

1.5.1. 30-days morbimortality

Infection control measures and the use of preoperative antibiotic prophylaxis immediately before the surgery contributed to the reduction of postoperative complications in surgery. However, a quarter of the patients submitted to a CRC surgery may actually develop postoperative complications.(89, 90) Infectious complications remain a major clinical problem in CRC surgery, contributing to a significant postoperative morbimortality, prolonged hospital stay and additional costs.(91) Despite the importance of early diagnosis of infectious complications in order to initiate treatment as soon as possible, their diagnosis is usually misleading, delaying its resolution. Therefore, there is an increasing necessity for early sensitive and specific markers for postoperative infections.(91, 92)

Several biomarkers of infection (e.g. white blood cells (WBC) count, CRP and PCT) have proven to be useful in the diagnosis of infection in different clinical settings, as well as in the assessment of the response to antibiotic therapy.(91) In the setting of early diagnose of postoperative infectious complications, there is no consensus regarding the diagnostic accuracy of each one.(84, 93) After surgery, those markers are elevated in all patients and this process is mostly influenced by the extent of

surgical trauma (surgical procedure and approach), as well as individual variability.(94) The determination of an universal cut-off point is impractical because it would imply estimation of a threshold for each postoperative day, surgical procedure, and surgical approach.(94) Therefore, a novel CRP measurement is being validated to identify postoperative infectious complications in patients undergoing colorectal surgery. It consists of comparing the value of the inflammatory marker on the day on which a complication was suspected with the value recorded on the second postoperative day.(95)

Recently, and especially since 2014 when this work begun, interest in the negative predictive values (NPV) of these inflammatory biomarkers has been increasing.(92, 93, 96, 97) This statistic value allows the identification of patients with very low probability of postoperative complications, facilitating early discharge after colorectal surgery.

1.5.2. Relapse and survival

Individually, preoperative hemoglobin, CRP and albumin can predict outcomes following the diagnosis of CRC.(98, 99) The combination of several inflammatory markers like the *Glasgow Prognostic Score (GPS)*, *modified GPS* and *neutrophil-to-lymphocyte ratio* have also been suggested to predict survival.(100) *Lymphocyte-CRP ratio* is a new score that has showed to be an independent prognostic factor for both DFS and OS.(101)

It has also been established that overexpression of VEGF and VEGFR in CRC tissue indicates poor prognosis,(78, 102-104) predicts early relapse (105) and increases the risk of distant metastastization.(104) Following surgery, VEGF levels tend to decrease, but if VEGF levels after surgery remain elevated, this may indicate significant residual disease, even without macroscopic evidence.(106)

1.6 Aims

Considering the incidence of obesity and MetS and the increasing incidence of CRC in the population, we have decided to evaluate the consequences of the presence of MetS and the VF of those patients in the outcomes of the patients submitted to CRC surgery. We also intend to evaluate the ability of inflammatory markers in predicting disease outcomes after CRC surgery.

The research project herein presented intends to:

1. Evaluate the impact of VF on 30-days morbidity, tumor features and 5-year survival on patients undergoing CRC surgery with curative intent (retrospective work).
2. Explore the relationship between the concentration of serum VEGF and tumor VEGF-R expression in patients with CRC (prospective work).
3. Determine the influence of MetS in the outcomes of CRC 30-days after surgery and in DFS and OS (prospective work).
4. Estimate the relationship between different inflammatory biomarkers and early infectious complications of colorectal surgery (prospective work).

Chapter 2: Research Project and Technical Considerations

2.1. Retrospective work

2.1.1. Inclusion and exclusion criteria

All patients submitted to a curative CRC surgery at Hospital de Braga during 3 consecutive years (between January 2007 and December 2009) were included.

The exclusion criteria were: emergency surgery, evidence of metastatic disease at presentation (Dukes D) and CT scans unavailable for analysis.

2.1.2. Data collection

Detailed information was obtained from the clinical records, which included demographic information, length of hospital stay, complications at 30-days (Clavien-Dindo morbimortality classification), reoperation or readmission at 30-days, anastomotic leak and pathological reports. Anastomotic leak was defined as an abscess or air near the anastomotic site that was diagnosed based on endoscopic and radiologic findings together with clinical symptoms and signs, such as a change in drainage color or signs of peritonitis that required reoperation or antibiotic treatment (e.g., in patients with colorectal anastomotic leak submitted to anterior resection with protective ileostomy and no sign of sepsis).

Patient follow-up was analyzed during 5 years or until death or the last contact date. Tumor recurrence, place of recurrence and date of recurrence were recorded.

2.1.3. Fat quantification at CT-scan

A single cross-sectional scan at the level of the umbilicus was selected for fat quantification. A scientific image-analysis program, *ImageJ,* was used for subcutaneous and VF area measure (http://imagej.nih.gov/ij). Subcutaneous fat was defined as fat that is superficial to the abdominal wall musculature, whereas VF is deep in the muscular wall and includes the mesenteric, subperitoneal and retroperitoneal parts.

2.2. Prospective work

2.2.1. Inclusion and exclusion criteria

All patients submitted to elective CRC surgery at Hospital de Braga between August of 2015 and August of 2016 were included.

Exclusion criteria were: evidence of metastasis before or during surgery, removal of other organs due to tumor invasion identified during surgery, synchronous tumors or history of other malignant tumors within 5 years of diagnosis, history of familial adenomatous polyposis or hereditary non-polyposis colorectal carcinoma.

2.1.2. Data collection

The patients included were evaluated in eight different moments: pre-operative consult, day before surgery, surgery, hospitalization period, 30 days after surgery, six months after surgery, one year after surgery and two years after surgery.

For every moment of evaluation, the data collected were:

- Pre-operative consult: confirmation of inclusion and exclusion criteria, registration of patient's age, gender, history of arterial hypertension, diabetes mellitus, dyslipidemia and usual medication (with special concern for hypertension, diabetes, high triglycerides and low HDL cholesterol specific treatments).
- Day before surgery: registration of anthropometric data (height, weight, bioimpedance, waist circumference, hip circumference) and collection of blood samples (WBC, total proteins, albumin, aspartate transaminase (AST), alanine transaminase (ALT), HDL, low-density lipoprotein (LDL), triglycerides, glucose, PCT). A second blood sample tube was retrieved for subsequent VEGF analysis.
- At surgery: registration of the surgery performed and surgery complications. After surgery, a Pathologist collected a tumor sample for immunohistochemistry analysis of VEGF-R.
- Hospitalization period: registration of information related in-hospital complications and mortality and collection of blood samples on postoperative days 1 (for WBC count, CRP and PCT) and 3 (for WBC count, CRP, PCT and albumin).

- 30 days after surgery: registration of anthropometric data (weight, bioimpedance, waist circumference, hip circumference), collection of information related to the pathology report and 30-days complications and mortality.
- Six months, one year and two years after surgery: registration of anthropometric data (weight, bioimpedance, waist circumference, hip circumference) and collection of information related to adjuvant treatments, tumor recurrence and mortality.

2.1.3. Metabolic syndrome definition

In our work we used three definitions of MetS: ATPIII, AHA and IDF. In 2009, the joint definition of MetS by the IDF, AHA, NHLBI, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity was published. It is similar to the previous AHA definition except for waist circumference that is now categorized using population- and country-specific definitions. For the European population included in our study, those criteria remained the same.

2.1.4. Tumor VEGF determination

2.1.4.1 Immunohistochemistry

One representative histological specimen of each case was selected, at the deepest invaded area of the CRC lesion, by the same Pathologist for immunohistochemistry. Immunohistochemical staining was performed on the samples with a thickness of 2.5μm, which were cut using the Thermo – MicroM HM355S with a simultaneous water bath at 56℃ for flattening out and drying tissue sections (Medite TFB45). After the water bath, the cut samples were placed on specific slides for a period of 20 minutes at 60°C in the Memmert Model 100–800. For the removal of paraffin, BondTM Dewax Solution (Catalog number AR9222, Leica Biosystems, Wetzlar, Germany) was used followed by VEGF-R protocol for Mouse Monoclonal Antibody VEGFR-3 (dilution 1:50; clone KLT9; Product code NCL-L-VEGFR-3, Leica Biosystems). The antibody was diluted with Novocastra TM IHC Diluent (Product Code RE7133, Leica Biosystems). All sections were incubated with primary antibody incubation for 60 minutes at 25℃. Staining was performed using the BOND - MAX Automated from Leica following the manufacturer's procedures. It was used with the following products: BondTM Wash Solution 10X Concentrate (Catolog number AR9590, Leica Biosystems), BondTM Epitope Retrival solution 1 (Catolog number AR 9961, Leica

Biosystems) and BondTM Polymer Refine Detection (Catolog number DS9800, Leica Biosystems). Then, the slides were washed in distilled water. Afterwards, the slides were dehydrated in an ascending series of alcohols (70%, 96%, and 100%) and made diaphanous with xylene, and finally mounted with Entellan glue.

2.1.4.2 Microscopic assessment of VEGF-R3 expression

VEGF-R3 staining was graded according to the intensity and extent of staining of the endothelium of the vessels as previously published.(107) The scale included four grades: 0 = absent, 1 = weak/very limited moderate staining, 2 = moderate widespread/strong localized staining and 3 = strong widespread. Grading was assessed under ×100 magnification for all of the sections taken.

2.1.5. Serum VEGF determination

For the determination of serum VEGF levels, blood samples were collected from the day before surgery. Serum samples were obtained by centrifugation at 3,000 revolutions per minute for 10 minutes and were stored at -80°C until use. Serum levels of VEGF were determined using a commercially available sandwich enzyme immunoassay kit (Human VEGF ELISA kit; Catalog number KHG0111, KHG0112, Life Technologies, Carlsbad, CA, USA). Samples were prepared and tested in duplicate according to the manufacturer's instructions. The reported detection limit is <5 pg/mL.

Chapter 3: Experimental Work

3.1. Influence of Visceral Fat in the Outcomes of Colorectal Cancer. Goulart A et al. Dig Surg. (2019)

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Influence of Visceral Fat in the Outcomes of Colorectal Cancer

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Keywords
Visceral fat · Obesity · Colorectal cancer · Morbidity · Survival

Abstract
Aim: To determine the relationship of visceral fat (VF) with the surgical outcome of the patients with colorectal cancer (CRC) submitted to curative surgery. ***Methods:*** Retrospective analysis of all patients submitted to CRC surgery during 3 years with a minimum of 5 years of follow-up. We assessed the length of hospital stay, complications, pathologic reports, surgical re-interventions and hospital re-admissions, relapses, survival time and disease-free time. VF was calculated based on patients' pre-operative CT-scan. The patients were divided into quartiles according to the VF area. Linear regression models and logistic regression models were used to establish a relationship between VF and all data collected. ***Results:*** The study included 199 patients (129 with colon cancer [CC] and 70 with rectal cancer). The average area of VF was 115.7 cm^2. Patients with CRC revealed a direct relationship between VF and postoperative complications ($p = 0.043$), anastomotic leakage ($p = 0.009$) and re-operation ($p = 0.005$). The subgroup of patients with CC had an inverse association between VF and lymph nodes harvested ($p = 0.027$). Survival analyses did not reveal significant differences. ***Conclusion:*** VF has an influence on postoperative complications, anastomotic leakage and re-operation. A negative influence of VF on lymph nodes harvested was observed on CC patients.

Introduction

Colorectal cancer (CRC) is the third most common cancer in men (746,000 cases, 10.0% of the total) and the second in women (614,000 cases, 9.2% of the total) worldwide [1]. Obesity is a global health growing problem. According to the World Health Organization, 39% of adults aged 18 years and over were overweight in 2014, and 13% were obese [2]. In Portugal, like most developed countries, the prevalence of overweight is more than 50% [3, 4].

Although body mass index has been used as one of the most reliable anthropometric indices of obesity because of its simplicity and objectivity, it does not reflect body adipose tissue accumulation, especially intra-abdominal or visceral fat (VF) tissue, and is not always consistent with VF area [5].

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Waist circumference has been suggested to be a better marker for central obesity and is easier to obtain from patients. However, in some studies, it was proven to be insufficient to distinguish between subcutaneous and visceral fat [6]. A single slice area is sufficient to measure the abdominal fat [7]. Different levels are described in literature, but VF and subcutaneous fat quantification can be easily measured by single slice CT-scan going through the umbilicus [8].

Gastrointestinal surgeons know that the amount of fat can greatly influence the technical difficulty during abdominal surgery [9]. The increase of VF has been associated with a significant increase of surgical wound infection, anastomotic leak, re-interventions and an increase in post-operative hospital stay when compared to VF free patients [10–13]. However, other studies did not agree with this influence of VF [14]. The influence of VF on survival also remains unclear.

The main propose of this study is to evaluate the impact of VF on 30-days morbidity, tumor features and 5-year survival on patients undergoing CRC surgery with curative intent.

Material and Methods

Study Design and Selection of Patients

A retrospective study that included all patients submitted to a curative CRC surgery at Hospital de Braga during 3 consecutive years (between January 2007 and December 2009) was conducted. Of the 355 patients reviewed, 45 patients were excluded because they were operated in an emergency setting and 27 patients were excluded because they had a metastatic disease at presentation (Dukes D). Of the remaining 283 patients, 84 were excluded because they had CT scans from outside hospitals and images were not available for analysis (Fig. 1).

Data Collection

Detailed information was obtained from the clinical records, which included demographic information, length of hospital stay, complications at 30-days (Clavien-Dindo Classification), re-operation or readmission at 30-days, anastomotic leakage and pathological reports. Anastomotic leakage was defined as an abscess or air near the anastomotic site that was diagnosed based on endoscopic and radiologic findings together with clinical symptoms and signs such as a change in drainage color or signs of peritonitis that required re-operation or antibiotic treatment (e.g., in patients with colorectal anastomotic leak submitted to anterior resection with protective ileostomy and no sign of sepsis).

Patient follow-up was conducted during 5 years or until death or the last contact date. Tumor recurrence, place of recurrence and date of recurrence were recorded.

A single cross-sectional scan at the level of the umbilicus was selected for fat quantification. A scientific image-analysis programme, ImageJ, was used for subcutaneous and VF area measure (http://imagej.nih.gov/ij). Subcutaneous fat was defined as fat that is superficial to the abdominal wall musculature, whereas VF is deep in the muscular wall and includes the mesenteric, subperitoneal and retroperitoneal parts (Fig. 2). For statistical analysis, VF area was divided into 4 quartiles.

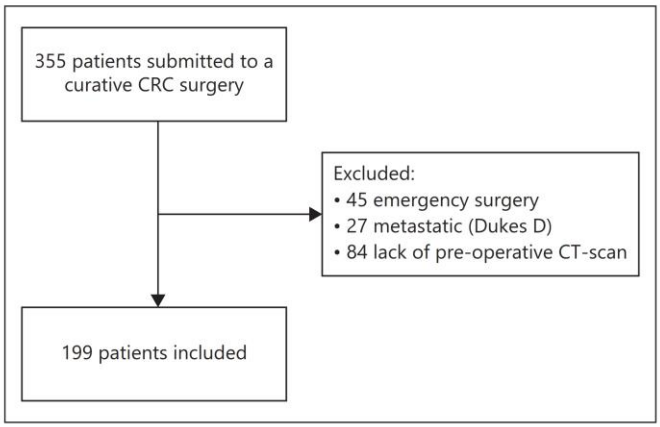

Fig. 1. Flowchart of the patients included in the study.

Data Analysis

Descriptive statistics are presented as absolute (n) and relative (%) frequencies for categorical variables, and mean (M) and SD for quantitative variables. The chi-square test was used to identify associations between dichotomous outcomes, and oneway analysis of variance was used to compare continuous variables. The Kaplan-Meier model with log-rank test was used to assess the impact of different characteristics on survival. A value of $p < 0.05$ was considered significant. All statistical analysis was performed with the IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA).

Ethical Issues and Informed Consent

This retrospective study is based on recorded data study. Informed consent was not able to be obtained from every patient, but this study design and use of data for the purpose intended was approved by the Ethic Committee of Hospital de Braga. The authors made sure to safeguard the anonymity and confidentiality of all the participants.

Results

The study included 199 patients with CRC with an average age of 68 years, of whom 124 (62.3%) were male. There were 129 patients with colon cancer (CC) and 70 with rectal cancer (RC). The clinicopathological characteristics of the patients are presented in Table 1.

Statistical analysis was performed for CRC patients group ($n = 199$) and for sub-groups of CC patients ($n = 129$) and RC patients ($n = 70$; Table 2).

The CCR group of patients presents significant differences in terms of 30-days morbidity ($p = 0.043$),

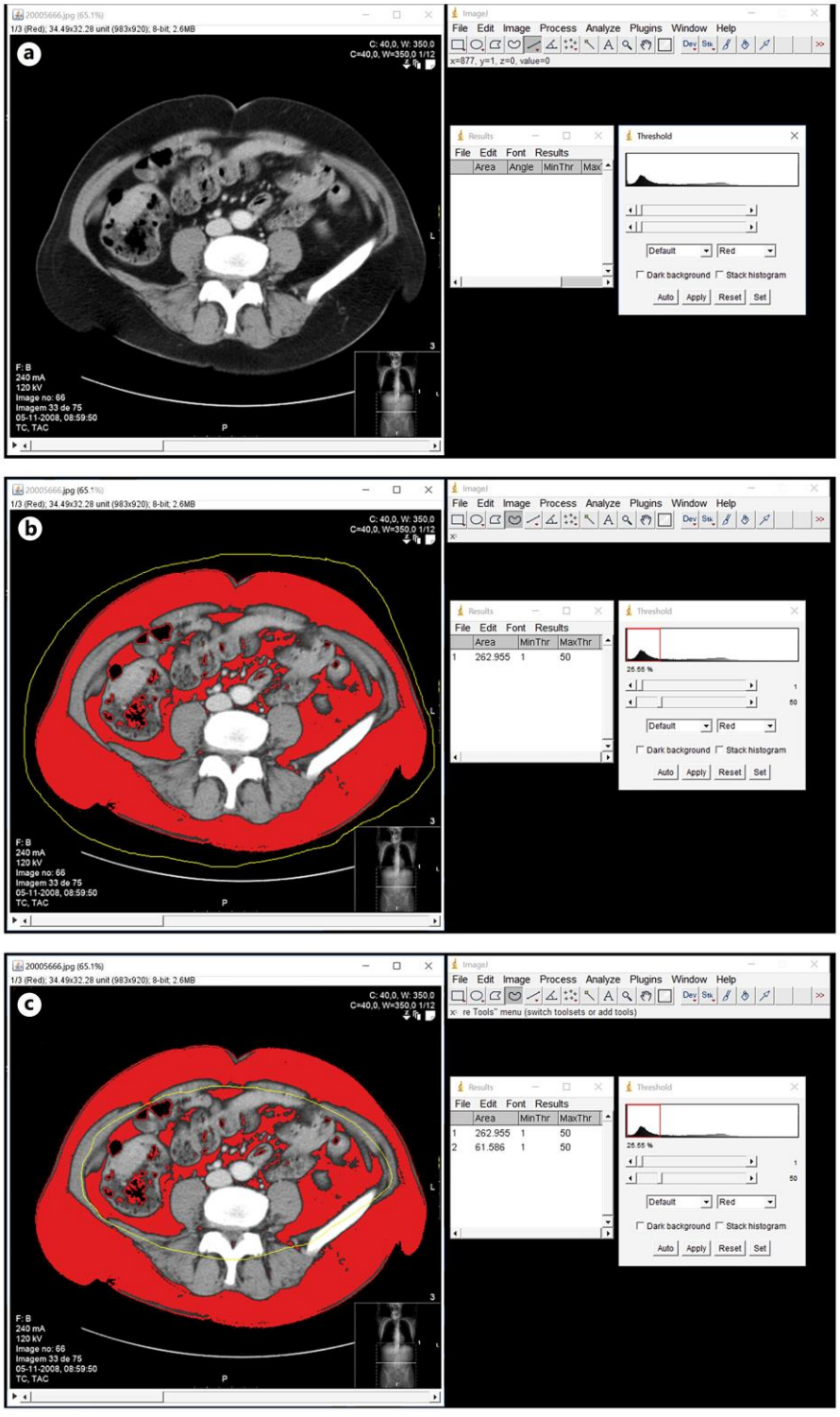

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Fig. 2. Visceral fat (VF) quantification at ImageJ programme. **a** CT-scan. **b** Total fat measure (262 cm^2). **c** VF measure (61 cm^2).

Table 1. Clinicopathological characteristics of patients

Variables	CRC	CC	RC
Number of patients	199	129	70
Age, years	68.2±11.0	69.4±11.2	68.4±10.8
Gender, male, %	62.3	66.7	54.3
ASA, %			
ASA I	11.1	13.2	7.1
ASA II	60.3	55.0	70.0
ASA III	25.1	29.5	17.1
ASA IV	1.0	0.8	1.4
Missing	2.5	1.6	4.3
Laparoscopic surgery, %	3.5	4.7	1.4
Dukes classification, %			
Dukes A	19.1	16.3	24.3
Dukes B	40.7	45.7	31.4
Dukes C	40.2	38.0	44.3
Lymph nodes			
Positive, *n*	1±2.6	1±2.5	1±3.0
Harvest, *n*	11±8.1	11±7.8	11±8.7
≥12 lymph nodes harvested, %	37.7	39.7	33.8
Adjuvant QT, %	57.3	51.9	67.1
Post-operative hospital stay, days	10.0±11.0	9.8±10.8	10.3±11.4
30-days morbidity, %	25.1	24.0	27.1
Minor morbidity	15.1	14.7	15.7
Major morbidity	10.1	9.3	11.4
Anastomotic leak, %	7.5	7.0	8.6
Wound infection, %	17.6	16.3	20.0
30-days re-operation, %	7.0	7.0	7.1
30-days re-admission, %	4.0	3.9	4.3
5-years relapse, %	26.8	24.0	31.9
5-years mortality, %	38.2	37.2	40.0
Overall survival, years	5.2±2.6	5.2±2.6	5.3±2.5
Disease free survival, years	4.6±3.0	4.6±3.0	4.6±2.9
Total fat area, cm^2	303.6±119.3	291.7±114.8	325.6±125.0
Visceral fat area, cm^2	115.7±63.3	113.2±61.7	120.2±66.4

CRC, colorectal cancer; CC, colon cancer; RC, rectal cancer; ASA, American Society of Anesthesiologists Score.

anastomotic leak (p = 0.009) and re-operation (p = 0.005). Despite not being statistically significant, the patients with more VF area present a long post-operative hospital stay, and more wound infections. Pathologic variables (Dukes stage and lymph nodes) were not statistically different between the quartiles of VF area.

The analyses of the CC sub-group revealed significant differences in terms of anastomotic leak (p = 0.008) and re-operation (p = 0.008), like the in the CRC group. However, this sub-group also presented with significant differences on the number of lymph nodes harvested (p = 0.027) and the percentage of patients with at least 12 lymph nodes harvested (p = 0.003).

The RC sub-group presents no statistically different outcomes.

In terms of survival analysis, the Kaplan-Meier curves demonstrate that patients on the first and fourth quartiles have a slight better overall survival (Fig. 3) and disease-free survival (Fig. 4), although this difference is not significant (log-rank p = 0.768 and p = 0.704, respectively).

Discussion

The influence of VF on outcomes in patients who were submitted to curative CRC surgery remains controversial. One of the reasons that explain part of this disagreement

Table 2. Surgical and oncologic outcomes of CRC, CC, RC patients according to visceral fat area distribution into quartiles

	CRC (quartiles)					CC (quartiles)					RC (quartiles)				
	1st	2nd	3rd	4th	*p* value	1st	2nd	3rd	4th	*p* value	1st	2nd	3rd	4th	*p* value
Patients, *n*	50	50	50	49		33	35	34	27		17	15	16	22	
Age, years	66.1	69.8	70.9	69.3	0.155	67.2	69.9	71.7	68.7	0.409	64.0	69.6	69.2	69.9	0.327
ASA, %					0.241					0.497					0.108
ASA I	5.2	3.6	2.1	0.5		4.7	5.5	2.4	0.8		6.0	0	1.5	0	
ASA II	14.4	14.9	16.0	16.5		13.4	13.4	15.7	13.4		16.4	17.9	16.4	22.4	
ASA III	5.2	6.2	6.7	7.7		6.3	7.9	8.7	7.1		3.0	3.0	3.0	9.0	
ASA IV	0.5	0	0.5	0		0.8	0	0	0		0	0	1.5	0	
Postoperative hospital stay, mean, days	8.22	8.68	9.88	13.2	0.100	8.9	7.7	10.4	13.0	0.261	7.0	10.9	8.7	13.5	0.323
Dukes classification, %					0.507					0.733					0.795
Dukes A	3.5	6.0	5.0	4.5		3.9	5.4	3.9	3.1		2.9	7.1	7.1	7.1	
Dukes B	12.1	10.6	10.6	7.5		13.2	13.2	12.4	7.0		10.0	5.7	7.1	8.6	
Dukes C	9.5	8.5	9.5	12.6		8.5	8.5	10.1	10.9		11.4	8.6	8.6	15.7	
Lymph nodes															
Positive, *n*	1	1	2	1	0.790	1	1	1	1	0.998	1	2	2	1	0.586
Harvest, *n*	13	11	9	11	0.166	15	10	10	10	0.027	10	12	9	12	0.750
≥12 lymph nodes harvested, %	13.6	8.9	6.8	8.4	0.091	17.5	7.9	7.1	7.1	0.003	6.2	10.8	6.2	10.8	0.322
30-days morbidity, %	3.5	5.0	7.5	9.0	0.043	3.9	3.9	8.5	7.8	0.071	2.9	7.1	5.7	11.4	0.347
Minor morbidity, %	2.5	3.5	4.0	5.0	0.537	3.1	3.1	4.7	3.9	0.796	1.4	4.3	2.9	7.1	0.495
Major morbidity, %	1.0	1.5	3.5	4.0	0.115	0.8	0.8	3.9	3.9	0.067	1.4	2.9	2.9	4.3	0.875
Anastomotic leak, %	0.0	1.1	2.8	4.5	0.009	0.0	0.0	3.1	3.9	0.008	0.0	4.0	2.0	6.0	0.455
Wound infection, %	2.5	4.0	5.5	5.5	0.315	2.3	2.3	6.2	5.4	0.115	2.9	7.1	4.3	5.7	0.485
30-days reoperation, %	0.0	0.5	2.5	4.0	0.005	0.0	0.0	3.1	3.9	0.008	0.0	1.4	1.4	4.3	0.435
30-days re-admission, %	1.5	2.0	0.5	0.0	0.162	1.6	1.6	0.8	0.0	0.592	1.4	2.9	0.0	0.0	0.187
5-years relapse, %	5.7	7.2	8.2	5.7	0.575	4.8	5.6	8.0	5.6	0.622	7.2	10.1	8.7	5.8	0.271

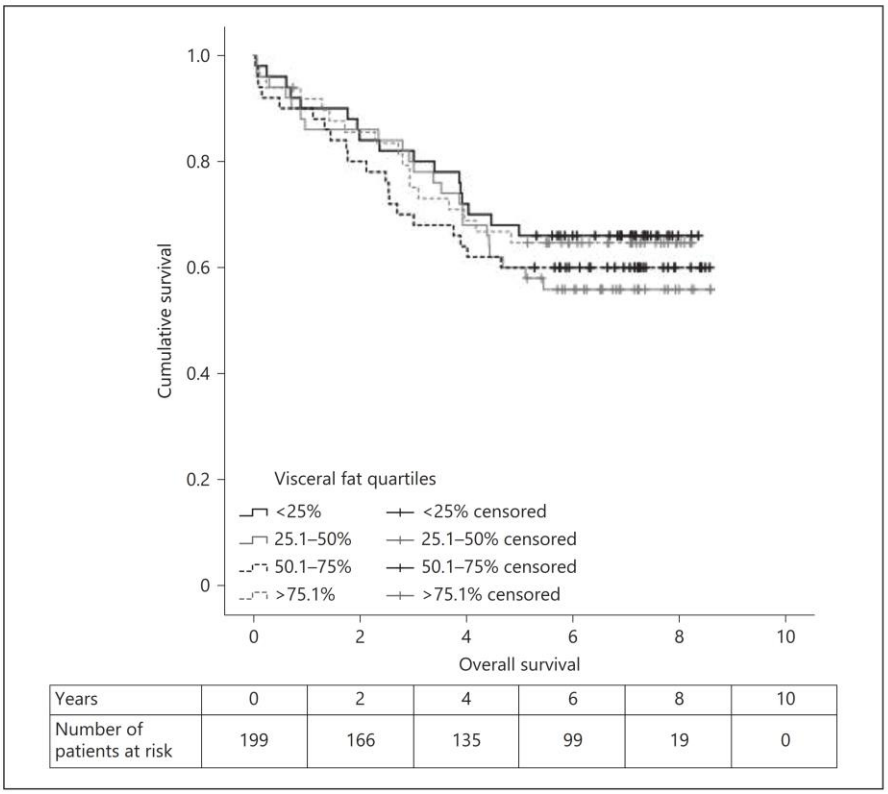
CRC, colorectal cancer; CC, colon cancer; RC, rectal cancer; ASA, American Society of Anesthesiologists Score.

may be the different definitions of VF used in the articles published: VF area or volume [11, 14–17], ratio between VF and subcutaneous fat area [10], ratio between VF and total fat area [12] and ratio between VF and body surface area [9]. In addition, the same definition, like for example VF area, has different cut-off points in different papers [18]. There is a necessity to determine the cut-off point of VF that defines obese patients in order to be able to compare results from different studies. In this paper, we used quartiles because we think that in our population the division of patients into quartiles may be more accurate.

Literature suggests that obese patients have a bigger risk of overall postoperative complications, surgical site infection, anastomotic leakage and colostomy complications [19–22]. A nationwide analysis from Sweden concluded that obese patients have increased post-operative complication rates [6]. A recent meta-analysis that aimed to determine the impact of visceral obesity on laparoscopic CRC surgery concluded that visceral obesity is associated with increased surgical difficulty and post-operative morbidity [18]. However, a recent paper suggested that VF has no influence on intraoperative difficulties, postoperative complications, and postoperative recovery in patients with sigmoid colon or RC [14].

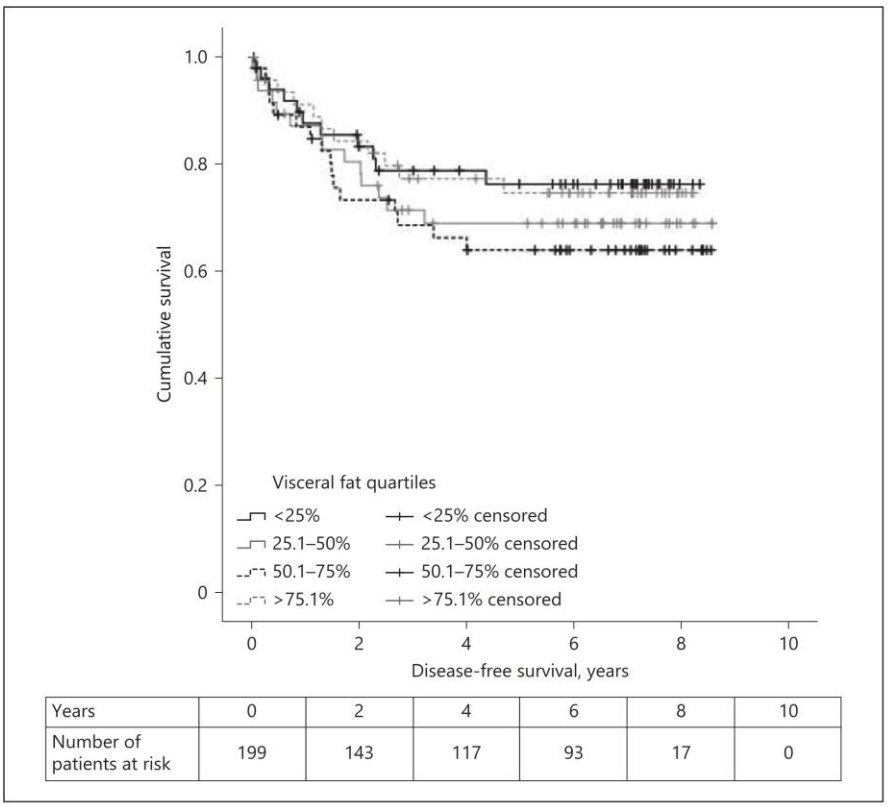
In our study, VF had influence on 30-days morbidity, anastomotic leak and re-operation rates on CRC patients with statistically significant differences. Patients with more VF area appear to have more wound infections and longer hospital stay; however, our results were not statistically significant. These results corroborated most of the literature already published that propose that CRC patients with higher VF are prone to have more complications.

However, the most relevant oncologic result of our data is that CC patients with higher VF have significant differences on the number of lymph nodes harvested and the percentage of patients with at least 12 lymph nodes harvested (accurate tumor staging). Current guidelines, like NCCN, for CC treatment suggest that a minimum of 12 lymph nodes need to be examined to establish N stage [23]. Those guidelines recommend that less than 12



Years	0	2	4	6	8	10
Number of patients at risk	199	166	135	99	19	0

Fig. 3. Overall survival analyzed by quartiles.



Years	0	2	4	6	8	10
Number of patients at risk	199	143	117	93	17	0

Fig. 4. Disease-free survival analyzed by quartiles.

lymph nodes retrieved constitute the high-risk factors for recurrence and adjuvant chemotherapy is beneficial to those patients [23]. Some papers concluded that the number of nodes harvested from patients with higher VF was significantly smaller [14, 17, 21] and others did not agree with this conclusion [10, 12]. This difference of lymph nodes harvested that we found on CC patients can have 2 explanations: VF increases surgical difficulty presenting as a problem for the surgeon to perform an accurate oncologic dissection and fat tissue adhering to the mesentery and makes the identification of the lymph nodes difficult for the pathologist [18].

The RC sub-group analysis did not retrieve any statistical differences. One of the explanations can be the lower number of patients included (70 patients).

The influence of VF on survival remains controversial. One recent review of the literature that analyzes the influence of VF and cancer survival encountered 6 papers of CRC patients, and only 4 of these papers related VF to survival [24]. Another review concluded that the increase in adiposity was associated with an increase of CC-related mortality and decreased the disease-free survival of CC in women and RC in men [25]. Other papers suggested that increased VF was a significant predictor of worst disease-free survival in patients with resectable CRC [10] and reduced overall survival in CRC patients receiving adjuvant chemotherapy [16]. In contrast, other works demonstrated that patients with higher VF tended to have better overall-survival that non-visceral obesity patients [12] and a meta-analyses concluded that there was insufficient evidence to prove the presence of a strong link between adiposity and survival [26]. Emerging literature reports the "obesity paradox" in cancer, which suggests that cancer survival is U-shaped and that extremes of weight may have better survival [27, 28]. Our results, although not statistically significant, show that patients on the first and fourth quartiles have a slight better overall survival and disease-free survival. We cannot explain why this happened, but maybe reflected some of the disagreement that exists around this.

The main limitations of this study include its retrospective design, which bears the issue of incomplete data and potential selection bias, and higher number of laparotomy, which does not reflect the current percentage of laparoscopy approach in CRC surgery.

Conclusion

The results suggest that VF increased likelihood of morbidity, anastomotic leakage and re-operations on CRC patients and lower number of lymph nodes harvest on the sub-group of CC patients.

The influence of VF in the number of lymph nodes harvested on CC patients are particularly interesting and should be verified in prospective trials with a larger set of patients. If the following studies support this difference in lymph nodes harvest, perhaps the most experienced surgeon and pathologist need to be called to deliver the best treatment to those patients.

Disclosure Statement

The authors certify that they have no conflicts of interest to disclose.

References

1 Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin D, Forman D, Bray F: GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide. International Agency for Research on Cancer, 2013. http://globocan.iarc.fr. 2016.

2 Word Health Organization. http://www.who.int/mediacentre/factsheets/fs311/en/ (accessed January 9, 2017).

3 do Carmo I, Dos Santos O, Camolas J, Vieira J, Carreira M, Medina L, Reis L, Myatt J, Galvao-Teles A: Overweight and obesity in Portugal: national prevalence in 2003–2005. Obes Rev 2008;9:11–19.

4 The State of Obesity. http://stateofobesity.org/files/stateofobesity2016.pdf (accessed January 9, 2017).

5 Bouchard C, Despres JP, Mauriege P: Genetic and nongenetic determinants of regional fat distribution. Endocr Rev 1993;14:72–93.

6 Hede P, Sorensson MA, Polleryd P, Persson K, Hallgren T: Influence of BMI on short-term surgical outcome after colorectal cancer surgery: a study based on the Swedish national quality registry. Int J Colorectal Dis 2015;30:1201–1207.

7 Shen W, Punyanitya M, Wang Z, Gallagher D, St-Onge MP, Albu J, Heymsfield SB, Heshka S: Visceral adipose tissue: relations between single-slice areas and total volume. Am J Clin Nutr 2004;80:271–278.

8 Sottier D, Petit JM, Guiu S, Hamza S, Benhamiche H, Hillon P, Cercueil JP, Krause D, Guiu B: Quantification of the visceral and subcutaneous fat by computed tomography: interobserver correlation of a single slice technique. Diagn Interv Imaging 2013;94:879–884.

9 Seki Y, Ohue M, Sekimoto M, Takiguchi S, Takemasa I, Ikeda M, Yamamoto H, Monden M: Evaluation of the technical difficulty performing laparoscopic resection of a rectosigmoid carcinoma: visceral fat reflects technical difficulty more accurately than body mass index. Surg Endosc 2007;21:929–934.

10 Moon HG, Ju YT, Jeong CY, Jung EJ, Lee YJ, Hong SC, Ha WS, Park ST, Choi SK: Visceral obesity may affect oncologic outcome in patients with colorectal cancer. Ann Surg Oncol 2008;15:1918–1922.

11 Tsujinaka S, Konishi F, Kawamura YJ, Saito M, Tajima N, Tanaka O, Lefor AT: Visceral obesity predicts surgical outcomes after laparoscopic colectomy for sigmoid colon cancer. Dis Colon Rectum 2008;51:1757–1765; discussion 1765–1767.
12 Park SW, Lee HL, Doo EY, Lee KN, Jun DW, Lee OY, Han DS, Yoon BC, Choi HS, Lee KH: Visceral obesity predicts fewer lymph node metastases and better overall survival in colon cancer. J Gastrointest Surg 2015;19:1513–1521.
13 Cakir H, Heus C, van der Ploeg TJ, Houdijk AP: Visceral obesity determined by CT scan and outcomes after colorectal surgery; a systematic review and meta-analysis. Int J Colorectal Dis 2015;30:875–882.
14 Yu H, Joh YG, Son GM, Kim HS, Jo HJ, Kim HY: Distribution and impact of the visceral fat area in patients with colorectal cancer. Ann Coloproctol 2016;32:20–26.
15 Park BK, Park JW, Ryoo SB, Jeong SY, Park KJ, Park JG: Effect of visceral obesity on surgical outcomes of patients undergoing laparoscopic colorectal surgery. World J Surg 2015; 39:2343–2353.
16 Lee CS, Murphy DJ, McMahon C, Nolan B, Cullen G, Mulcahy H, Sheahan K, Barnes E, Fennelly D, Ryan EJ, Doherty GA: Visceral adiposity is a risk factor for poor prognosis in colorectal cancer patients receiving adjuvant chemotherapy. J Gastrointest Cancer 2015; 46:243–250.
17 Kang J, Baek SE, Kim T, Hur H, Min BS, Lim JS, Kim NK, Lee KY: Impact of fat obesity on laparoscopic total mesorectal excision: more reliable indicator than body mass index. Int J Colorectal Dis 2012;27:497–505.
18 Yang T, Wei M, He Y, Deng X, Wang Z: Impact of visceral obesity on outcomes of laparoscopic colorectal surgery: a meta-analysis. ANZ J Surg 2015;85:507–513.
19 De Raet J, Delvaux G, Haentjens P, Van Nieuwenhove Y: Waist circumference is an independent risk factor for the development of parastomal hernia after permanent colostomy. Dis Colon Rectum 2008;51:1806–1809.
20 Asteria CR, Gagliardi G, Pucciarelli S, Romano G, Infantino A, La Torre F, Tonelli F, Martin F, Pulica C, Ripetti V, Diana G, Amicucci G, Carlini M, Sommariva A, Vinciguerra G, Poddie DB, Amato A, Bassi R, Galleano R, Veronese E, Mancini S, Pescio G, Occelli GL, Bracchitta S, Castagnola M, Pontillo T, Cimmino G, Prati U, Vincenti R: Anastomotic leaks after anterior resection for mid and low rectal cancer: survey of the Italian society of colorectal surgery. Tech Coloproctol 2008;12: 103–110.
21 Watanabe J, Tatsumi K, Ota M, Suwa Y, Suzuki S, Watanabe A, Ishibe A, Watanabe K, Akiyama H, Ichikawa Y, Morita S, Endo I: The impact of visceral obesity on surgical outcomes of laparoscopic surgery for colon cancer. Int J Colorectal Dis 2014;29:343–351.
22 Wick EC, Hirose K, Shore AD, Clark JM, Gearhart SL, Efron J, Makary MA: Surgical site infections and cost in obese patients undergoing colorectal surgery. Arch Surg 2011; 146:1068–1072.
23 National Comprehensive Cancer Network. Colon Cancer (Version 1.2017). https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf (accessed January 9, 2017).
24 Xiao J, Mazurak VC, Olobatuyi TA, Caan BJ, Prado CM: Visceral adiposity and cancer survival: a review of imaging studies. Eur J Cancer Care (Engl) 2016, Epub ahead of print.
25 Siegel EM, Ulrich CM, Poole EM, Holmes RS, Jacobsen PB, Shibata D: The effects of obesity and obesity-related conditions on colorectal cancer prognosis. Cancer Control 2010;17: 52–57.
26 Parkin E, O'Reilly DA, Sherlock DJ, Manoharan P, Renehan AG: Excess adiposity and survival in patients with colorectal cancer: a systematic review. Obes Rev 2014;15:434–451.
27 Caan BJ, Kroenke CH: Next Steps in Understanding the Obesity Paradox in Cancer. Cancer Epidemiol Biomarkers Prev 2017;26:12.
28 Kroenke CH, Neugebauer R, Meyerhardt J, Prado CM, Weltzien E, Kwan ML, Xiao J, Caan BJ: Analysis of body mass index and mortality in patients with colorectal cancer using causal diagrams. JAMA Oncol 2016;2: 1137–1145.

3.2. The correlation between serum vascular endothelial growth factor (VEGF) and tumor VEGF receptor 3 in colorectal cancer. Goulart A et al. Ann Surg Treat Res. (2019)

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The correlation between serum vascular endothelial growth factor (VEGF) and tumor VEGF receptor 3 in colorectal cancer

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Purpose: Despite plasma biomarkers offering a number of advantages over tissue-based markers, the relationship between serum vascular endothelial growth factor (VEGF) and VEGF receptor (VEGF-R) tumor expression in colorectal cancer (CRC) is still unclear. This study was designed to establish the relationship between the concentration of serum VEGF and tumor VEGF-R expression in patients with CRC.
Methods: A prospective study of consecutive patients undergoing elective colorectal surgery during 1 year. Preoperative VEGF was determined by enzyme-linked immunosorbent assay and VEGF-R3 by immunochemistry.
Results: The initial sample included 134 patients with CRC diagnosis. Results showed significant association of serum values of VEGF with VEGF-R3 expression ($P < 0.001$), even in the presence of confounders (sex, age, body mass index, tumor location, and surgical approach). The estimated effect size was high ($\eta^2 = 0.35$).
Conclusion: Serum VEGF has a significant correlation with tumoral VEGF-R3 expression in CRC.
[Ann Surg Treat Res 2019;97(1):15-20]

Key Words: Colorectal neoplasms, Vascular endothelial growth factors, Enzyme-linked immunosorbent assay, Immunohistochemistry

INTRODUCTION

Vascular endothelial growth factor (VEGF) is the most widely studied angiogenic factor, being considered crucial for tumor angiogenesis [1]. VEGF presents itself as a signal protein that stimulates the growth of new blood vessels and it is thought to facilitate the metastatic spread of tumor cells [1,2]. The pathway for signal transduction of VEGF is composed of 5 glycoproteins belonging to the VEGF family including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor, 3 receptors; VEGF receptor-1 (FTL-1), VEGF-R2 (FLK-1/KDR), and VEGF-R3 (FLT-4), and 2 coreceptors (NRP-1, NRP-2) [2]. The three VEGF-Rs participate differently in angiogenesis and lymphangiogenenis: VEGF-R1 and VEGF-R2 mediate angiogenesis, and VEGF-R3 is mostly involved in lymphangiogenesis [2]. The main inducers of new lymphatic vessels are the principal ligands of VEGF-R3 [2]. In addition to the role in tumor growth, it appears that VEGF also has autocrine functions, acting as a survival factor for tumor cells, protecting them from stresses such as hypoxia, chemotherapy (CT) and radiotherapy (RT) [3]. Exogenous VEGF could attenuate the effect of RT, so combining RT with VEGF inhibitor could be more effective than RT alone [4,5]. A recent review that explores the benefit of adding target therapy to neoadjuvant chemotherapy in locally advanced rectal cancer leaves several open questions in conclusion, such as that patient selection should be based on potential predictive biomarkers

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of response, such as free VEGF, in order to define a subgroup of patients who are more likely to benefit from this form of therapy [6].

VEGF can be measured in the tumor tissue by immunohistochemistry or reverse transcription polymerase chain reaction or in the plasma by enzyme-linked immunosorbent assay (ELISA) method [7]. Detection of serum VEGF has been investigated as a potential serum diagnostic marker for malignant disease, and was found to increase concentrations of free VEGF in various types of cancer, including those of gastrointestinal etiology [8,9]. However, the relationship between the pattern of production of VEGF protein in tumor tissues and its concentration in the circulation remains unclear [10,11].

Plasma biomarkers offer a number of advantages over tissue-based markers. The potential of serum concentration of VEGF being representative of tumor VEGF opens new pathways for further investigation, preoperative prognostic information and treatment response. The absence of papers designed to verify this relationship lead us to design a study with the intention of clarifying the relationship between the concentration of serum VEGF and tumor VEGF-R expression in patients with CRC.

METHODS

Study oversight and patient inclusion

Between August 2015 and August 2016 all patients with a confirmed diagnosis of colorectal adenocarcinoma who underwent elective surgery at our institution were enrolled. Patients who presented evidence of metastasis before or at surgery, necessity of removal of other organs due to tumor invasion, synchronous tumors or history of other malignant tumors within 5 years, history of familial adenomatous polyposis and hereditary nonpolyposis colorectal carcinoma were excluded. We also excluded patients who did not do preoperative assessment, did not have preoperative blood samples collected, malignant polyp excised but without tumor in the colectomy specimen, pathologic completed response after neoadjuvant CT – ypT0, or adenocarcinoma *in situ* and mucinous adenocarcinoma.

Written informed consent was obtained from all participant patients. This study was approved by the Ethics Committee for Health of Hospital de Braga (authorization number 60/2017).

Data collection

At 1 day before surgery, the same nurse performed an anthropometric evaluation of the patients that included body weight and height and obtained a sample for the evaluation of serum VEGF. A biopsy representative of each tumor was taken from Department of Pathology.

Immunohistochemistry

One representative histological specimen of each case at the deepest invaded area of the CRC lesion was selected by same pathologist for immunohistochemistry. Immunohistochemical staining was performed on the samples with a thickness of 2.5 μm, which were cut using the Thermo – MicroM HM355S with a simultaneous water bath at 56℃ for flattening out and drying tissue sections (Medite TFB45). After the water bath, the cut samples were placed on specific slides for a period of 20 minutes at 60℃ in the Memmert Model 100–800. For the removal of paraffin, BondTM Dewax Solution (Catalog number AR9222, Leica Biosystems, Wetzlar, Germany) was used followed by VEGF-R protocol for Mouse Monoclonal Antibody VEGFR-3 (dilution 1:50; clone KLT9; Product code NCL-L-VEGFR-3, Leica Biosystems). The antibody was diluted with Novocastra TM IHC Diluent (Product Code RE7133, Leica Biosystems). All sections were incubated with primary antibody incubation for 60 minutes at 25℃. Staining was performed using the BOND - MAX Automated from Leica following the manufacturer's procedures. It was used with the following products: BondTM Wash Solution 10X Concentrate (Catolog number AR9590, Leica Biosystems), BondTM Epitope Retrival solution 1 (Catolog number AR 9961, Leica Biosystems) and BondTM Polymer Refine Detection (Catolog number DS9800, Leica Biosystems).

Then the slides were washed in distilled water. Afterwards, the slides were dehydrated in an ascending series of alcohols (70%, 96%, and 100%) and made diaphanous with xylene, and finally mounted with Entellan glue.

Microscopic assessment of VEGF-R3 expression

VEGF-R3 staining was graded according to the intensity and extent of staining of the endothelium of the vessels as previously published [12]. The scale presenting hence is the following: 0 = absent (Fig. 1A), 1 = weak/very limited moderate staining (Fig. 1B), 2 = moderate widespread/strong localized staining (Fig. 1C) and 3 = strong widespread (Fig. 1D). This was assessed under ×100 magnification for all of the sections taken.

Serum VEGF determination

For determination of serum VEGF levels, blood samples were collected from the day before surgery. Serum samples were obtained by centrifugation at 3,000 revolutions per minute for 10 minutes and were stored at -80°C until use. Serum levels of VEGF were determined using a commercially available sandwich enzyme immunoassay kit (Human VEGF ELISA kit; Catalog number KHG0111, KHG0112, Life Technologies, Carlsbad, CA, USA). Samples were prepared and tested in duplicate according to the manufacturer's instructions. The reported detection limit is <5 pg/mL.

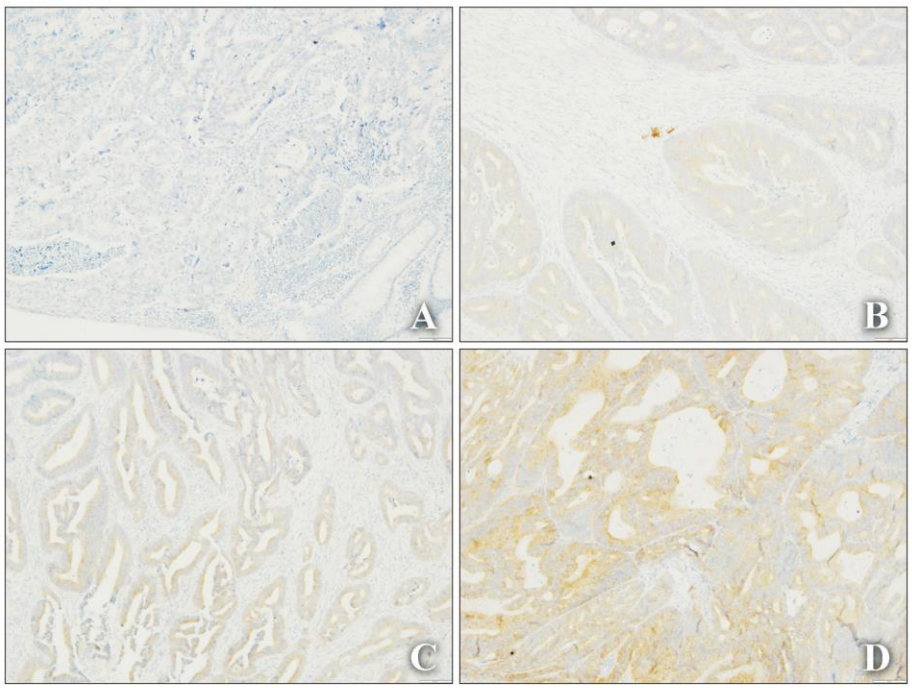

Fig. 1. Immunohistochemical staining for VEGF-R3 in malignant colorectal endothelium. (A) Absent expression of VEGF-R3 in CRC (×100). (B) Weak/very limited moderate staining of VEGF-R3 (×100). (C) Moderate widespread/strong localized staining of VEGF-R3 (×100). (D) Strong widespread staining of VEGF-R3 (×100). VEGF, vascular endothelial growth factor; VEGF-R3, VEGF receptor 3; CRC, colorectal cancer.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics ver. 24.0 (IBM Co., Armonk, NY, USA). Descriptive statistics were presented as means (M) and standard deviations (SD) for quantitative variables; categorical variables we computed frequencies (n) and percentages (%). Normality was checked with Shapiro-Wilks test assuming normality for $P > 0.05$. Levene test was used for assessing the homogeneity of variances, making this assumption when $P > 0.05$. One-way analyses of variance (ANOVA) was used to compare serum values of VEGF. Spearman correlation was used to measure the association between these variables. Confounders were controlled by conducting an analyses of covariance (ANCOVA). Effect size was measured with eta^2 (η^2), considering low effect ($\eta^2 = 0.01$), moderate effect ($\eta^2 = 0.06$) and high effect ($\eta^2 = 0.14$). Significant results were considered for $P < 0.05$.

RESULTS

Study population and baseline characteristics

The initial sample included 134 patients with CRC diagnoses. From these, 60 patients (44.8%) were excluded for the following reasons: no preoperative assessment (26; 19.4%), lack of preoperative blood samples (19; 14.2%), pathologic completed response after neoadjuvant CT – ypT0 (5; 3.7%), malignant polyp excised but without tumor in the colectomy specimen (4; 3.0%), adenocarcinoma *in situ* (4; 3.0%), mucinous adenocarcinoma (1; 0.7%), and lack of sample (1; 0.7%). The final sample was composed of a total of 74 patients with CRC diagnoses, 47

Table 1. Patient and tumor characteristics

Characteristic	Value
Age (yr)	68.34 ± 12.69
Sex	
Male	47 (63.5)
Female	27 (36.5)
Location	
Rectum	18 (24.3)
Colon	56 (75.7)
Surgical approach	
Laparotomy	38 (51.4)
Laparoscopy	33 (44.6)
Conversion from laparoscopy	3 (4.1)
Body mass index (kg/m^2)	26.97 ± 4.08
T classification (TNM)	
T1	5 (6.8)
T2	21 (28.4)
T3	44 (59.5)
T4	4 (5.4)
N classification (TNM)	
N0	51 (68.9)
N+	23 (31.1)
Dukes stage	
Dukes A	20 (27.0)
Dukes B	31 (41.9)
Dukes C	23 (31.1)

Values are presented as mean ± standard deviation or number (%).

Table 2. ANOVA comparison for serum VEGF and tumoral VEGF-R3 expression

	Absent (n = 6)	Weak/very limited moderated (n = 49)	Moderated widespread/strong localized (n = 13)	Strong widespread (n = 6)	ANOVA
Serum VEGF concentration	36.29 ± 12.55	49.68 ± 18.96	65.35 ± 36.21	126.39 ± 77.31	$P < 0.001$ $\eta^2 = 0.34$

Values are presented as mean ± standard deviation.
ANOVA, analysis of variance; VEGF, vascular endothelial growth factor; VEGF-R3, VEGF receptor 3.

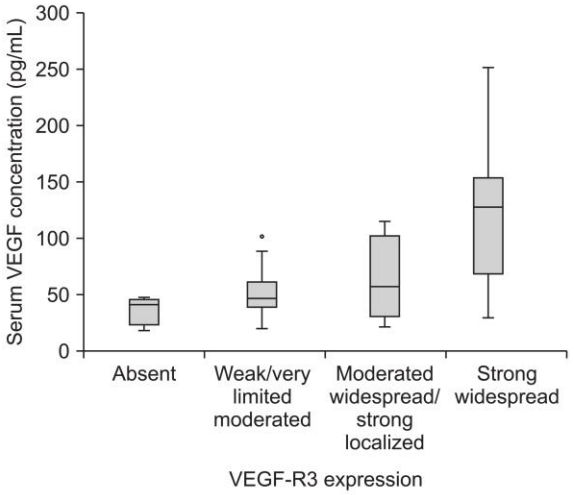

Fig. 2. Serum values of VEGF distribution by immunochemistry VEGF-R3 expression. VEGF, vascular endothelial growth factor; VEGF-R3, VEGF receptor 3; CRC, colorectal cancer.

males (63.5%) and 27 females (36.5%) with a mean age of 68 years. Other patient and pathologic characteristics are described in Table 1.

Analytic results

After checking for normality and variances homogeneity, ANOVA results showed overall significant differences ($P < 0.01$, $\eta^2 = 0.34$). Tukey multiple comparisons test showed significant differences regarding the comparison of strong widespread staining (mean ± SD, 126.39 ± 77.31) with all other categories: moderated widespread/strong localized (mean ± SD, 65.35 ± 36.21), weak/very limited moderated (mean ± SD, 49.68 ± 18.96), and absent (mean ± SD, 36.29 ± 12.55) (Table 2, Fig. 2). Spearman correlation showed significant association between these variables ($\rho = 0.348$, $P = 0.002$).

An ANCOVA analysis intending to control for the confounders: age, sex, body mass index, tumor location, surgical approach, TNM, and Dukes classification was performed. Results showed significant association of serum values of VEGF with VEGF-R3 expression ($P < 0.01$), even in the presence of all referred confounders. Effect size estimate was $\eta^2 = 0.35$ (Table 3).

Table 3. ANCOVA analysis of covariance for serum values of VEGF by VEGF-R3 expression adjusted for age, sex, body mass index, location, surgical approach, T classification, N classification, and Dukes classification

Variable	P-value	η^2
VEGF-R3 expression	<0.001	0.35
Age	0.315	0.02
Sex	0.197	0.03
Body mass index	0.280	0.02
Location	0.747	0.02
Surgical approach	0.809	0.01
T classification (TNM)	0.218	0.02
N classification (TNM)	0.260	0.02
Dukes classification	0.185	0.03

ANCOVA, analysis of covariance; VEGF, vascular endothelial growth factor; VEGF-R3, VEGF receptor 3.

DISCUSSION

The relationship between VEFG and CRC outcomes is controversial. Some studies showed that VEGF has no significant prognostic value in CRC [13], but many others have demonstrated an association between overexpression of VEGF and poor CRC outcomes: the overexpression of VEGF and VEGF-R in CRC tissue indicates poor prognosis [4,14,15], predicts early relapse [16], and preoperative VEGF serum concentration predicts poor disease-specific survival and disease-free survival in colon cancer patients [17]. When comparing patients who had metastatic tumors compared with patients who had nonmetastatic tumors, it was found that VEGF expression was higher in the first group [18-20]. Takahashi et al. [21] demonstrated that VEGF expression levels in patients with lymph node negative CRC was significantly associated with time to recurrence, and Cascinu et al. [22] verified the association between positive VEGF tumor status with a significant reduction in the 5-year disease-free survival rate. Some studies have shown that VEGF is also a useful prognostic marker, by significantly correlating with angio-lymphatic invasion, lymph node status, and depth of invasion, although it is not an independent prognostic factor [10].

Regardless of serum VEGF collected before the surgery theoretically presenting many advantages over tumoral VEGF,

only a few studies evaluated the prognostic impact of VEGF serum levels in patients with CRC. The Danish Colorectal Cancer Study Group conducted a large study which suggested a biologically relevant role for serum VEGF concentrations in patients with CRC, after having found that high preoperative VEGF concentrations were associated with a reduced overall survival [23-25]. In the same way, Nakayama et al. [26] reported on elevated circulating levels of VEGF in patients with CRC who had more advanced disease and in patients who experienced tumor recurrence.

Probably one of the reasons for serum VEGF being used as prognostic marker in only a few papers is related to the absence of studies designed with the purpose of clarifying the relationship between serum and tumoral VEGF. Our work showed a strong association ($P < 0.001$, $\eta^2 = 0.34$) between preoperative serum VEGF and VEGF-R3 tumor expression even after controlling for potential confounders. This association could be explained by previous studies that show that VEGF is expressed in a wide variety of human tissues, particularly high quantity in tumors, and that, *in vitro*, many tumor cell lines secrete VEGF [17]. Thus, it seems logical that patients with tumor cells with a higher expression of VEGF-R present higher levels of serum VEGF in comparison with tumors with lesser VEGF-R expression.

These results open up new horizons in terms of investigation as a potential biomarker in selection of treatment and prognostic information. In the near future, it may be possible to implement a patient selection strategy to effectively identify those patients who are most likely to benefit from neoadjuvant treatment with VEGF inhibitors based on this confirmed relationship between serum and tumoral VEGF expression. However, due to limitation of sample size, our work needs to be considered as a pilot study. Further studies with a larger sample need to be developed in order to confirm these results. Moreover, our study only focuses on the relationship between serum VEGF and tumor VEGF-R expression, so the prognostic significance of serum VEGF still requires further investigation.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Larijani LV, Ghasemi M, Charati JY, Mehrabian-Fard M, Saravi NS. Evaluation of VEGF immunohistochemical expression and correlation with clinicopathologic features in colorectal cancer. Govaresh 2015;20:199-204.
2. Al-Rawi MA, Mansel RE, Jiang WG. Molecular and cellular mechanisms of lymphangiogenesis. Eur J Surg Oncol 2005;31:117-21.
3. Jurgensmeier JM, Schmoll HJ, Robertson JD, Brooks L, Taboada M, Morgan SR, et al. Prognostic and predictive value of VEGF, sVEGFR-2 and CEA in mCRC studies comparing cediranib, bevacizumab and chemotherapy. Br J Cancer 2013;108:1316-23.
4. Peng Y, Wang L, Du C, Gu J. Expression of vascular endothelial growth factor can predict distant metastasis and disease-free survival for clinical stage III rectal cancer following 30-Gy/10-f preoperative radiotherapy. Int J Colorectal Dis 2012;27:1555-60.
5. Gupta VK, Jaskowiak NT, Beckett MA, Mauceri HJ, Grunstein J, Johnson RS, et al. Vascular endothelial growth factor enhances endothelial cell survival and tumor radioresistance. Cancer J 2002;8:47-54.
6. Benevento I, De Felice F, Musio D, Tombolini V. The addition of target therapy to neoadjuvant chemoradiotherapy in locally advanced rectal cancer: a review. Chemotherapy 2017;62:314-22.
7. Byrne AM, Bouchier-Hayes DJ, Harmey JH. Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). J Cell Mol Med 2005;9:777-94.
8. Bottomley MJ, Webb NJ, Watson CJ, Holt L, Bukhari M, Denton J, et al. Placenta growth factor (PlGF) induces vascular endothelial growth factor (VEGF) secretion from mononuclear cells and is co-expressed with VEGF in synovial fluid. Clin Exp Immunol 2000;119:182-8.
9. Jelkmann W. Pitfalls in the measurement of circulating vascular endothelial growth factor. Clin Chem 2001;47:617-23.
10. Martins SF, Reis RM, Rodrigues AM, Baltazar F, Filho AL. Role of endoglin and VEGF family expression in colorectal cancer prognosis and anti-angiogenic therapies. World J Clin Oncol 2011;2:272-80.
11. Cressey R, Wattananupong O, Lertprasertsuke N, Vinitketkumnuen U. Alteration of protein expression pattern of vascular endothelial growth factor (VEGF) from soluble to cell-associated isoform during tumourigenesis. BMC Cancer 2005;5:128.
12. White JD, Hewett PW, Kosuge D, McCulloch T, Enholm BC, Carmichael J, et al. Vascular endothelial growth factor-D expression is an independent prognostic marker for survival in colorectal carcinoma. Cancer Res 2002;62:1669-75.
13. Zheng S, Han MY, Xiao ZX, Peng JP, Dong Q. Clinical significance of vascular endothelial growth factor expression and neovascularization in colorectal

carcinoma. World J Gastroenterol 2003;9:1227-30.
14. Martins SF, Garcia EA, Luz MA, Pardal F, Rodrigues M, Filho AL. Clinicopathological correlation and prognostic significance of VEGF-A, VEGF-C, VEGFR-2 and VEGFR-3 expression in colorectal cancer. Cancer Genomics Proteomics 2013;10:55-67.
15. Des Guetz G, Uzzan B, Nicolas P, Cucherat M, Morere JF, Benamouzig R, et al. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. Br J Cancer 2006;94:1823-32.
16. Tsai HL, Yang IP, Lin CH, Chai CY, Huang YH, Chen CF, et al. Predictive value of vascular endothelial growth factor overexpression in early relapse of colorectal cancer patients after curative resection. Int J Colorectal Dis 2013;28:415-24.
17. De Vita F, Orditura M, Lieto E, Infusino S, Morgillo F, Martinelli E, et al. Elevated perioperative serum vascular endothelial growth factor levels in patients with colon carcinoma. Cancer 2004;100:270-8.
18. Takahashi Y, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res 1995;55:3964-8.
19. Tokunaga T, Oshika Y, Abe Y, Ozeki Y, Sadahiro S, Kijima H, et al. Vascular endothelial growth factor (VEGF) mRNA isoform expression pattern is correlated with liver metastasis and poor prognosis in colon cancer. Br J Cancer 1998;77:998-1002.
20. Ishigami SI, Arii S, Furutani M, Niwano M, Harada T, Mizumoto M, et al. Predictive value of vascular endothelial growth factor (VEGF) in metastasis and prognosis of human colorectal cancer. Br J Cancer 1998;78:1379-84.
21. Takahashi Y, Tucker SL, Kitadai Y, Koura AN, Bucana CD, Cleary KR, et al. Vessel counts and expression of vascular endothelial growth factor as prognostic factors in node-negative colon cancer. Arch Surg 1997;132:541-6.
22. Cascinu S, Staccioli MP, Gasparini G, Giordani P, Catalano V, Ghiselli R, et al. Expression of vascular endothelial growth factor can predict event-free survival in stage II colon cancer. Clin Cancer Res 2000;6:2803-7.
23. Werther K, Christensen IJ, Brunner N, Nielsen HJ. Soluble vascular endothelial growth factor levels in patients with primary colorectal carcinoma. The Danish RANX05 Colorectal Cancer Study Group. Eur J Surg Oncol 2000;26:657-62.
24. Werther K, Christensen IJ, Nielsen HJ; Danish RANX05 Colorectal Cancer Study Group. The association between preoperative concentration of soluble vascular endothelial growth factor, perioperative blood transfusion, and survival in patients with primary colorectal cancer. Eur J Surg 2001;167:287-92.
25. Werther K, Christensen IJ, Nielsen HJ; Danish RANX05 Colorectal Cancer Study Group. Prognostic impact of matched preoperative plasma and serum VEGF in patients with primary colorectal carcinoma. Br J Cancer 2002;86:417-23.
26. Nakayama Y, Sako T, Shibao K, Okazaki K, Rempo N, Onitsuka K, et al. Prognostic value of plasma vascular endothelial growth factor in patients with colorectal cancer. Anticancer Res 2002;22:2437-42.

3.3. Early Inflammatory Biomarkers as Predictive Factors for Freedom from Infection after Colorectal Cancer Surgery: A Prospective Cohort Study. Goulart et al. Surg Infect (Larchmt). (2018)

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Early Inflammatory Biomarkers as Predictive Factors for Freedom from Infection after Colorectal Cancer Surgery: A Prospective Cohort Study

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Abstract

Purpose: Different biomarkers are useful in diagnosing infections. The aim of this work was to clarify the relation between different inflammatory biomarkers (white blood cell [WBC] count, C-reactive protein [CRP], procalcitonin [PCT], and C-reactive protein-to-albumin ratio [CAR]) and early infectious complications after colorectal surgery.
Methods: This prospective single-center cohort study included 130 patients undergoing elective colorectal surgery. The WBC count, CRP, and PCT were measured at post-operative day one (POD1) and POD3 and albumin on POD3.
Results: Patients with surgical site infections (SSI) exhibited significantly higher CRP concentrations on POD1 and CRP and CAR on POD3 than did patients without SSI. According to receiver operating characteristic analysis, the CRP concentration on POD1 and the CRP and CAR on POD3 showed the highest area under the curve (AUC) for predicting SSI (AUC 0.639, 0.736, and 0.729, respectively). Multivariable logistic regression analysis showed that CRP on POD1 and CRP and CAR on POD3 were independent predictors of SSI (odds ratio 7.355, 7.605, and 8.337, respectively).
Conclusions: The CRP concentration on PO1 and CRP and CAR on POD3 can positively identify patients at low risk of SSI. They can be used as a prognostic tool to predict an uneventful post-operative period and therefore have been incorporate into our discharge criteria after elective colorectal resection, improving clinical decision-making.

Keywords: colorectal cancer; C-reactive protein; C-reactive protein-to-albumin ratio; surgical site infection

RECENT ADVANCES in colorectal cancer surgery technique and peri-operative care had little impact in the overall complication rate, which remains at about 30% [1]. Infectious complications remain a major clinical problem in colorectal surgery, contributing to significant post-operative morbidity, prolonged hospital stay, additional cost, and more deaths [2].

Despite the importance of early diagnosis of infections in order to initiate the treatment as soon as possible, their diagnosis usually is difficult, delaying their resolution. Therefore, there is a clear necessity for early sensitive and specific markers for post-operative infections [2]. Several biomarkers of infection, namely white blood cell (WBC) count and C-reactive protein (CRP) and procalcitonin (PCT) concentrations, have proved useful in the diagnosis of infection in different clinical settings as well as in the assessment of its response to antibiotic therapy [2–4]. Also, a low serum albumin concentration is associated with post-operative complications [5, 6].

In the presence of inflammation, the liver synthesizes an acute-phase reactant called CRP, which can be found in the blood as a result of stimulation by interleukin-6 and tumor necrosis factor-alfa [7]. It probably plays an important role in innate immunity by assisting complement binding to foreign and damaged cells and enhancing phagocytosis by macrophages, thus acting as an early defense against infection [8]. Production of CRP is part of a nonspecific acute-phase response to most forms of infection, tissue damage, inflammation, and malignant disease [7].

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Also, PCT is the inactive 116-amino-acid pro-peptide of the hormone calcitonin. Patients with sepsis or less severe bacterial and fungal infectiond show an increase in the concentration of PCT [9]. The concentration is a valuable marker for inflammation, and the central role of PCT as an early and reliable diagnostic and prognostic tool in contexts such as infection and sepsis and both infectious and non-infectious systemic inflammatory response syndrome (SIRS) has been demonstrated [9].

An early decrease in the post-operative serum albumin concentration is associated with adverse clinical outcomes and results from surgical insult [10]. The greater capillary leakage of albumin is one of the characteristics of SIRS, leading to a low plasma albumin concentration in patients undergoing major abdominal surgery [11].

The WBC count, CRP, and PCT have been studied by several authors as early predictors of infectious complications. However, there still is no consensus regarding the diagnostic accuracy of each one for early detection of complications in patients undergoing colorectal surgery [3,9,12,13]. However, the CRP-to-albumin ratio (CAR) has received almost no studies to determine its diagnostic accuracy for post-operative complications in patients undergoing colorectal surgery [5]. The aim of this work was to clarify the relation between different inflammatory biomarkers (WBC, CRP, PCT, and CAR) and early infectious complications of colorectal surgery.

Patients and Methods

Study design and selection of participants

This study was approved by the local ethics committee. It was a prospective single-center cohort study that included all patients with colorectal adenocarcinoma who underwent elective surgery in our institution from August 2015 to August 2016. Exclusion criteria were evidence of metastasis before or at surgery, synchronous tumors, and absence of colorectal adenocarcinoma in the surgical specimen.

Data collection

The range of data collected included baseline characteristics such as age and sex, co-morbidities such as arterial hypertension and dyslipidemia, and type of surgery (laparoscopy vs laparotomy). The laboratory data collected were WBC count, CRP (mg/L), and PCT (ng/mL) on post-operative day (POD) 1 and POD3 and albumin (g/dL) on POD 3.

All post-operative infectious complications were recorded according to the Surgical Site Infection (SSIs) Guidelines and were classified as superficial incisional (involves only skin and subcutaneous tissue of the incision), deep incisional (involves deep soft tissues of the incision such as fascial and muscle layers), and organ/space (involves any part of the body deeper than the fascial/muscle layers that is opened or manipulated during the operative procedure). In this study, SSIs were all confirmed by the same attending physician. The follow-up period was 30 days after surgery.

Data analysis

Statistical analysis was performed using the 2016 Statistical Package for the Social Science Program (SPSS) version 24 (IBM Corporation, Armonk, NY). Descriptive statistics are presented as frequencies (n) and percentages (%) for categorical variables and as means (M) and standard deviations (SD) for quantitative variables, after checking to see if the asymmetry coefficient fell inside the [−2; 2] interval. Univariable analysis between laboratory data and SSI were performed with the independent-sample *t*-test.

Receiving operator characteristic (ROC) curves were built in order to assess the sensitivity and specificity of the biomarkers WBC count, CRP, and PCT one and three days after surgery. The CAR was calculated three days after surgery. Precision was calculated as the area under the curve (AUC) (null hypothesis—H0—was AUC = 0.5). We also calculated the positive and negative predictive values for the significant early biomarkers.

Finally, adjusted odds ratios (ORs) were determined for the significant early biomarkers using adjusted unconditional logistic regression. The confounders tested were sex, age, arterial hypertension, type II diabetes mellitus, dyslipidemia, and surgical approach. Significant results were considered as $p < 0.05$.

This work has been reported in line with the STROCSS criteria [14].

Results

Study population and baseline characteristics

From August 2015 to August 2016, a total of 138 patients were enrolled, and eight patients were excluded, four because of high-grade dysplasia and one each for serrated adenoma, lipoma, metastasis at surgery, and adenocarcinoma of the appendix. There were 81 men (62.3%) and 49 women with a mean age of 67.83 years (SD 13.07) and a median age of 68.5

Table 1. Patient, Tumor Characteristics, and Treatment Complications

Variable	N *(%)*
Arterial hypertension	74 (56.9)
Type II diabetes mellitus	35 (26.9)
Dyslipidemia	53 (40.8)
Location of the tumor	
Colon	97 (74.6)
Rectum	33 (25.4)
If rectum, neoadjuvant treatment	14 (42.4)
Surgical approach	
Laparoscopy	59 (45.4)
Converted laparoscopy	8 (6.2)
Laparotomy	63 (48.5)
30-day complication grade (Clavien Dindo)	
None	95 (73.1)
I	8 (6.2)
II	13 (10.0)
IIIa	3 (2.3)
IIIb	7 (5.4)
IVa	1 (0.8)
IVb	2 (1.5)
V	1 (0.8)
Anastomotic leak[a]	10 (8.3)
Dukes stage	
Not classifiable (Tis or yT0)	6 (4.5)
A	41 (31.5)
B	43 (33.1)
C	40 (30.8)

[a]Anastomotic leak occurred in 10 of 121 patients with anastomoses.

Table 2. Statistical Results Comparing Laboratory Variables with Surgical Site Infection

	Percent of patients evaluated	*SSI (mean)*	*No SSI (mean)*	p
Post-operative day 1				
White blood cells (cells/mm^3)	100	10.6	10.7	0.823
C-reactive protein (mg/L)	100	101.7	77.5	0.024
Procalcitonin (ng/mL)	93	2.2	1.6	0.545
Post-operative day 3				
White blood cells (cells/mm^3)	96	9.3	9.2	0.983
C-reactive protein (mg/L)	96	177.9	106.9	0.000
Procalcitonin (ng/mL)	96	2.6	1.7	0.407
Albumin (g/dL)	96	2.6	2.9	0.063
C-reactive protein-to-albumin ratio	96	74.8	38.8	0.000

years (range 34–91 years). The rest of the baseline characteristics, surgery and treatment performed, and pathologic characteristics of the tumor are summarized in Table 1. There were 26 patients (20.0%) who had detectable SSIs. Of these, 12 patients (9.2% of the total series) had superficial incisional SSI, 1 (0.8%) had deep incisional SSI, and 13 (10.0%) had organ/space SSI.

Predictive clinical features

To assess the utility of clinical features for the early assessment of SSIs, analysis of the WBC count, CRP concentration (mg/L), and PCT concentration (ng/mL) was made on PODs 1 and 3, whereas the concentration of albumin (g/dL) was measured only on POD 3 (Table 2). On POD1, patients with SSI exhibited significantly higher CRP concentrations than did those without SSI ($p < 0.05$). On POD3, patients with SSI exhibited higher CRP concentrations and CAR values than did those in the non-SSI group ($p < 0.05$). There were no statistically significant differences between the two groups in WBC count or PCT on PODs 1 and 3. The surgical approach (laparoscopy vs. laparotomy) was associated with a statistical difference in the development of SSI ($p = 0.035$) and exhibited differences in the laboratory values of the inflammatory markers (Table 3).

To compare each marker and to determine the optimum cut-off for SSI diagnosis, ROC analysis was performed. On POD1, CRP was the most reliable predictor of SSI (cut-off value 73 mg/L; AUC 0.639). On POD3, the CAR was a significant predictor of SSI (cut-off value 43; AUC 0.736) as was CRP (cut-off value 123 mg/L; AUC 0.729) (Fig. 1).

The optimum cut-off and the corresponding sensitivity, specificity, and positive and negative predictive values are summarized in Table 4.

Risk and predictive factors for SSI after colorectal cancer surgery

Adjusted logistic regressions showed significant results for CRP on POD1 and CRP and CAR on POD3 for the risk of infection. For CRP on POD1, our proposed cut-off value was associated with a risk 7.355 times higher for patients with SSI (odds ratio [OR] 7.355; 95% confidence interval [CI] 12.076–26.066; $p = 0.002$). For CRP on POD3, our proposed cut-off was associated with a risk 7.605 times higher for patients with SSI (OR 7.605; 95% CI 2.313–25.007; $p = 0.001$). For CAR, our proposed cut-off showed a risk 8.337 times higher for patients with SSI (OR 8.337; 95% CI 2.202–20.168; $p = 0.001$) (Table 5).

Discussion

After colorectal cancer surgery, the most significant source of morbidity and delay of discharge is infectious complications. Through the use of newer biochemical markers (CPR and PCT), it may be possible to predict accurately a group of patients at higher risk of poor outcome because of infections [8] and, at the same time, help to identify patients for safe early discharge [7].

For many surgeons, the early identification of SSI is still a challenge. In the period after elective colorectal surgery, SSI can lead to prolonged hospital stays, increased morbidity and

Table 3. Statistical Results Comparing Laboratory Variables with Surgical Approach

	Percent of patients evaluated	*Laparotomy (mean)*	*Laparoscopy (mean)*	p
Post-operative day 1				
White blood cells (cells/mm^3)	100	10.9	10.4	0.327
C-reactive protein (mg/L)	100	102.5	56.7	0.000
Procalcitonin (ng/mL)	93	3.0	0.2	0.001
Post-operative day 3				
White blood cells (cells/mm^3)	96	8.7	9.9	0.489
C-reactive protein (mg/L)	96	145.7	94.2	0.002
Procalcitonin (ng/mL)	96	2.9	0.6	0.022
Albumin (g/dL)	96	2.6	3.1	0.000
C-reactive protein-to-albumin ratio	96	59.2	31.2	0.000

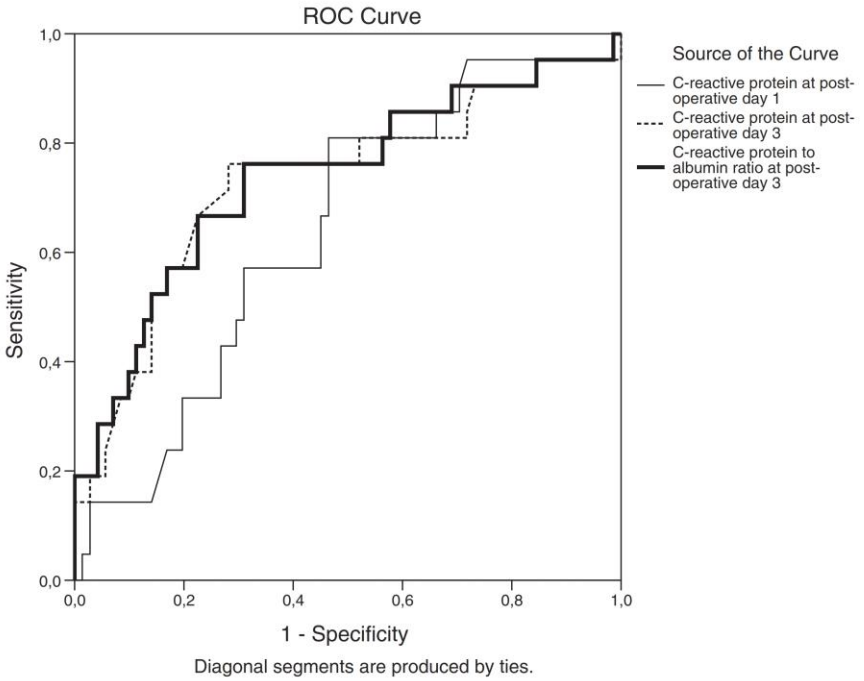

FIG. 1. Receiver operating characteristics curves of C-reactive protein (CRP) on POD1 and CRP and C-reactive protein-to-albumin ratio (CAR) on POD3.

mortality rates, and higher medical costs [5]. Despite the combined evaluation of clinical, laboratory, and radiologic findings, early diagnosis of SSI cannot be made reliably by any known individual feature or even by experienced clinical judgment [9].

When most studies try to find a predictive factor for infection, few have attempted to identify predictors of an uneventful post-operative course in terms of infection. But such information can be valuable in developing algorithms for discharge. This study demonstrated an increase in CRP on POD1 and in CRP and CAR on POD3 with significant differences between patients with and without complications. The ROC curve analysis showed that the CRP concentration on POD1 and CRP and CAR on POD3 predict the development of post-operative infectious complications. In adjusted logistic regressions, both CRP on POD1 and CRP and CAR on POD3 were identified as independent predictors of SSI.

Our study shows that using a cut-off value for CRP of 73 mg/L on POD1 may have sensitivity and specificity adequate to be used for clinical decision making, but the most important finding is that, in our population, CRP ≤73 mg/L on POD1 predicted an uncomplicated course in 91.5% of patients. Although most studies agree that CRP on PO1 is influenced by surgical insult [2,8,15] and its post-operative peak occurs at 48 hours after surgery [2], the lack of increase in the CRP concentration on the first post-operative day is a predictor of a complication-free post-operative period.

At POD3, most studies suggest that CRP is a predictor of infectious complications [2, 7,15]. In our study, as on POD1, CRP at a cut-off 123 mg/L on POD3 had a high negative predictive value (91.2%).

Similarly, we studied CAR, which is based on circulating concentrations of two acute-phase proteins, CRP and albumin, which are associated with inflammation caused by surgical insult. In this way, we concluded that almost all patients with CAR ≤43 on POD3 will not develop an SSI (negative predictive value 90.7%).

The research on CAR is still in development. Ge et al. [5] found that patients with CAR >2.2 on POD3 should be monitored intensively for early detection of post-operative

Table 4. Sensitivity, Specificity, Negative Predictive Value (NPV), Positive Predictive Value (PPV), and Optimum Cut-Off of Each Marker Based on ROC Curve

	Cut-off	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPV (%)*
CRP POD1	73	81.8	55.1	34.0	91.5
CRP POD3	123	77.3	70.3	43.6	91.2
CAR POD3	43	77.3	66.2	40.5	90.7

CAR = C-reactive protein-to-albumin ratio; CRP = C-reactive protein; POD = post-operative day.

Table 5. Adjusted Logistic Regressions (Adjusted for Confounders Sex, Age, Arterial Hypertension, Type II Diabetes Mellitus, Dyslipidemia, and Surgical Approach)

	Adjusted OR	*95% CI*	p
CRP POD1	7.355	2.076–26.066	0.002
CRP POD3	7.605	2.313–25.007	0.001
CAR POD3	8.337	2.202–20.168	0.001

CI = confidence interval; OR = odds ratio; POD = post-operative day.

complications. Shibutani et al. [16] found pre-operative CAR to be a useful prognostic marker in patients with colorectal cancer undergoing potentially curative surgery. In patients with sepsis, Ranzani et al. [17] reported that residual inflammation at ICU discharge, as assessed by CAR, was an independent risk factor for a poor outcome.

Elevated WBC count is a nonspecific inflammatory marker and one of the SIRS criteria. Therefore, it is not surprising that the count had a poor diagnostic performance for infection in patients in the intensive care unit and post-operatively [15]. However, some studies have reported a late increase in WBC counts in patients with infectious complications of colorectal surgery, correlating with the clinical diagnosis of complications [15]. We could have missed this late increase, as we assessed inflammatory markers only on PODs 1 and 3. Furthermore, in our study, PCT failed as a predictor of early post-operative infection on both PODs 1 and 3. We could not find a correlation between SSI and the PCT concentration. Silvestre et al. [2] recently described finding that in critically ill surgical patients, an increase in PCT did not predict complications.

Our results show that the surgical approach influences the development of SSI and the mean values of inflammatory markers are different statistically according to the approach by laparoscopy or laparotomy. However, the clinical relevance of these findings cannot be assessed from these data because our study was not designed to evaluate the influence of the surgical approach on SSI and inflammatory markers. The study design did not assign patients to the laparotomy or laparoscopy groups at baseline. Therefore, these groups are not equally distributed, for example in terms of patients' age, and cannot be compared.

Finally, there are important limitations we should make note of. First, with an observational design and moderate sample size, this study requires validation in large-scale prospective multi-center trials. Second, because of the small sample, we combined patients with organ/space SSIs with those with incisional SSIs and analyzed them as one group. Although there are significant differences in CRP on POD1 and CRP and CAR on POD3 in patients with and without SSI, further studies are necessary to investigate the usefulness of those markers.

In conclusion, CRP on PO1 and CRP and CAR on POD3 can identify patients at low risk of SSI. Our study suggests that they can be used as a prognostic tool to forecast an uneventful post-operative period. They have been incorporate into our fast-track discharge criteria after elective colorectal resection, improving clinical decision-making.

Author Disclosure Statement

The authors have no conflicts of interest with regard to this manuscript.

References

1. Brown SR, Mathew R, Keding A, et al. The impact of postoperative complications on long-term quality of life after curative colorectal cancer surgery. Ann Surg 2014; 259:916–923.
2. Silvestre J, Rebanda J, Lourenco C, Povoa P. Diagnostic accuracy of C-reactive protein and procalcitonin in the early detection of infection after elective colorectal surgery: A pilot study. BMC Infect Dis 2014;14:444.
3. McSorley ST, Khor BY, MacKay GJ, et al. Examination of a CRP first approach for the detection of postoperative complications in patients undergoing surgery for colorectal cancer: A pragmatic study. Medicine 2017;96:e6133.
4. Holl S, Fournel I, Orry D, et al. Should CT scan be performed when CRP is elevated after colorectal surgery? Results from the Inflammatory Markers After Colorectal Surgery study. J Vasc Surg 2017;154:5–9.
5. Ge X, Cao Y, Wang H, et al. Diagnostic accuracy of the postoperative ratio of C-reactive protein to albumin for complications after colorectal surgery. World J Surg Oncol 2017;15:15. Pubmed Central PMCID: 5223565.
6. Tanaka T, Sato T, Yamashita K, et al. Effect of preoperative nutritional status on surgical site infection in colorectal cancer resection. Dig Surg 2017;34:68–77.
7. Zawadzki M, Czarnecki R, Rzaca M, et al. C-reactive protein and procalcitonin predict anastomotic leaks following colorectal cancer resections: A prospective study. Wideochir Inne Tech Maloinwazyjne 2016;10:567–573. Pubmed Central PMCID: 4729737.
8. MacKay GJ, Molloy RG, O'Dwyer PJ. C-reactive protein as a predictor of postoperative infective complications following elective colorectal resection. Colorectal Dis 2011;13:583–587.
9. Takakura Y, Hinoi T, Egi H, et al. Procalcitonin as a predictive marker for surgical site infection in elective colorectal cancer surgery. Langenbecks Arch Surg 2013;398:833–839.
10. Hubner M, Mantziari S, Demartines N, et al. Postoperative albumin drop is a marker for surgical stress and a predictor for clinical outcome: A pilot study. Gastroenterol Res Pract 2016;2016:8743187. Pubmed Central PMCID: 4736779.
11. Norberg A, Rooyackers O, Segersvard R, Wernerman J. Albumin kinetics in patients undergoing major abdominal surgery. PLoS ONE 2015;10:e0136371.
12. Giaccaglia V, Salvi PF, Cunsolo GV, et al. Procalcitonin, as an early biomarker of colorectal anastomotic leak, facilitates enhanced recovery after surgery. J Crit Care 2014; 29:528–532.
13. Selby J, Prabhudesai A. Can C-reactive protein predict the severity of a post-operative complication after elective resection of colorectal cancer? Int J Colorectal Dis 2014;29:1211–1215.
14. Agha RA, Borrelli MR, Vella-Baldacchino M, et al. The STROCSS statement: Strengthening the reporting of cohort studies in surgery. Int J Surg 2017;46:198–202.
15. Oberhofer D, Juras J, Pavicic AM, et al. Comparison of C-reactive protein and procalcitonin as predictors of postoperative infectious complications after elective colorectal surgery. Croat Med J 2012;53:612–619. Pubmed Central PMCID: 3541587.
16. Shibutani M, Maeda K, Nagahara H, et al. Prognostic significance of the preoperative ratio of C-reactive protein to albumin in patients with colorectal cancer. Anticancer Res 2016;36:995–1001.
17. Ranzani OT, Zampieri FG, Forte DN, et al. C-reactive protein/albumin ratio predicts 90-day mortality of septic patients. PLoS ONE 2013;8:e59321.

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Original Article

The influence of metabolic syndrome in the outcomes of colorectal cancer patients

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ABSTRACT

Aims: Determine the influence of metabolic syndrome and its different components in the outcomes of colorectal cancer surgery at 30 days.
Materials and methods: Prospective study that included all patients submitted to elective colorectal cancer surgery between August 2015 and August 2016 at Hospital de Braga. Clinical and laboratory parameters evaluated pre-operatively were: central obesity, blood pressure, fasting glucose, triglycerides levels and HDL cholesterol levels. Any complications during the first 30-days after surgery were recorded (readmission, reintervention, anastomotic dehiscence, morbimortality).
Results: One hundred and thirty-four patients were included. Metabolic syndrome was diagnostic in 40.7% of patients with the ATPIII definition, 67.5% with the AHA definition and 67.0% with the IDF definition. At 30 days after colorectal cancer surgery, 73.1% patients don't have any complication, 15.7% have minor complications (grade I/II of Clavien-Dindo classification), 11.1% have major complications (grade III/IV/V of Clavien-Dindo classification) and 1.5% have died from surgical complications (grade V of Clavien-Dindo classification). The statistic analysis didn't reveal any association between MS, or it's different components, and surgical outcomes.
Conclusion: This study seems to indicate that metabolic syndrome don't have any influence in surgical outcomes of colorectal cancer surgery.

1. Introduction

The terms "metabolic syndrome" (MS) stand for a cluster of interrelated risk factors of metabolic origin that have been proved to predict a higher risk of atherosclerotic cardiovascular disease as well as type 2 diabetes mellitus expressing, this way, its clinical importance. With a prevalence of approximately 24,6–30.9% in Europe [1] the development of this syndrome appears to be directly related to abdominal obesity and insulin resistance [2].

Since its initial description (approximately 80 years ago) [1] many different definitions have been proposed by several institutions, but they all agree on the same basic components, namely hypertension, dyslipidemia, insulin resistance and central obesity.

The most popular definitions are those from National Cholesterol Education Program – Third Adult Treatment Panel (NCEP-ATPIII), or just ATPIII, from 2001 [3], the International Diabetes Federation (IDF), from 2005 [4], and, finally, the American Heart Association/National Heart Lung and Blood Institute (AHA/NHLBI), from also 2005 [2].

Besides the cardiovascular consequences of the metabolic syndrome, in several cohort studies and meta-analyses, this entity has been proven to increase cancer risk in general [5,6] with major effects on the gastrointestinal tract, namely increasing the risk of non-neoplastic gastrointestinal disorders, precursor lesions and CRC itself [7]. This relationship between metabolic syndrome and the risk of colorectal cancer is at the moment supported by a large number of studies [1,8,9], and this linkage is mainly explained by abdominal obesity and insulin resistance with a multifactorial mechanism of carcinogenesis involving the action of adipokines, inflammatory cytokines, adiponectin, leptin, IGF-1 and others [1].

Colorectal cancer represents an important health issue, as being the third most common cancer in men and the second in women worldwide (10.0% and 9.2% of the total, respectively) [10]. These

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numbers seem to be rising dramatically worldwide due to urbanization, aging, diet changes and lifestyle [11].

At least one study of our knowledge has already proved the deleterious influence of MS on CRC prognosis, with significantly shorter survival and higher recurrence and liver metastasizing rates, implying that MS is an important prognostic factor for CRC [12]. Moreover, this deleterious influence was also proved for the outcomes of CRC surgery at 30 days, showing a higher rate of postoperative complications and a longer hospital stay in patients classified with the AHA/NHLBI definition of MS. Also in this study from 2009, high blood pressure and high triglyceride levels were as well considered important risk factors for severe complications after CRC surgery, but only the presence of the cluster of metabolic abnormalities that constitute MS was proven to be an independent variable in the multivariate analysis, and not each individual component [13].

Concerning the influence of obesity in surgery outcomes, WC proved to be an independent risk factor for the development of parastomal hernia after permanent colostomy [14]. Another study published in 2013 proved that waist-hip-ration (as a measure of central obesity) had a significant influence in negative outcomes after CRC surgery, namely reoperation, medical complications, intraoperative complications and conversion to open approach, being this prediction effect superior to the one verified when measuring BMI and or WC [15]. Obesity was also associated with an increase in anastomotic leakage after rectal cancer resection [16].

Furthermore, a study concerning the financial implications of CRC surgery in obese patients has detected a significant increase in hospital expenses due to a higher rate of severe complications. The major contributors for these costs were wards stay, operations, and intensive care units [17].

The aim of this study is to determine the influence of MS and different components of MS (high fasting glucose, central obesity, high blood pressure, high triglycerides levels and low HDL cholesterol levels) in the outcomes of CRC surgery at 30 days (reintervention, readmission, dehiscence and morbimortality).

2. Methods

2.1. Study oversight and patients inclusion

This study included all the patients with a confirmed diagnosis of colorectal adenocarcinoma who underwent elective surgery at Hospital de Braga during August 2015 till August 2016. Patients who presented evidence of metastasis before or at surgery, necessity of removal of other organs due to tumor invasion, synchronous tumors or history of other malignant tumors within 5 years, history of familial adenomatous polyposis and hereditary non-polyposis colorectal carcinoma were excluded.

2.2. Data collection

At the first moment of evaluation, during pre-operative consultation, data were collected concerning patient's age, gender, history of arterial hypertension, diabetes mellitus, dyslipidemia, prior neoplasms, usual medication (with special concern for hypertension, diabetes, high triglycerides and low HDL cholesterol specific treatments) and family history of neoplasms. On the day prior to their admission to the hospital for surgery, the patients were requested to present themselves at Clinical Academic Center at Hospital de Braga for an anthropometric evaluation, carried out, all times, by the same nurse, which included height, weight and waist circumference measurement, as well as collection of blood samples for evaluation of fasting glucose, HDL cholesterol and triglycerides levels. During a period of 30 days following surgery, data about complications were collected and registered, namely morbimortality, readmission, reintervention and anastomotic dehiscence. Morbimortality was posteriorly classified according to Clavien-Dindo's classification [18].

2.3. Statistical analysis

Statistical analysis was performed using SPSS – Statistical Package for the Social Science Program, version 23.

Descriptive statistics are presented as absolute frequencies (n) and relative (%) for categorical variables, and mean (M) and standard deviation (SD), or median (Mdn) and interquartile ranges, for quantitative variables, depending on whether or not symmetry of the distributions was ensured. The chi-square test was used to identify associations between dichotomous outcomes (morbimortality, dehiscence, reintervention and readmission) and the independent variables. When the maximum assumed 20% of cells with the expected frequency of less than 5 was exceeded, Fisher's test (2×2 tables) was used. The Mann-Whitney test was used to compare the outcome measured on an ordinal scale (Morbimortality according to Clavien-Dindo classification) with the independent variables. Finally, logistic regressions were used to measure the risk of the independent variables on the dichotomous outcomes. The significance level for rejection of H_0 was 5% ($p < 0.05$).

2.4. Ethical issues

The present study was approved by the Ethic Committee of Hospital de Braga. The investigators made sure to safeguard the anonymity and confidentiality of all the participants. A written informed consent was obtained from each patient.

3. Results

The present study included a sample of 134 patients with CRC diagnoses, with a mean age of 67.91 years old, 82 males and 52 females. 46 (40.7% in 113) of these patients were diagnoses with MS according to the ATPIII definition, 79 (67.5% in 117) according to the AHA definition and 71 (67.0% in 106) according to the IDF definition. These data are shown in Table 1. We were not able to collect the necessary information for these diagnoses in some of the patients (ND in the table), most often by absence of the patient from the appointments with the nurse for measurement of anthropometric parameters and blood samples collection.

Table 1
Sample characterization; ND = No data.

	Statistics
Age M (SD)	67.91 (12.94)
Gender n (%)	
M	82 (61.2%)
F	52 (38.8%)
MS n (%)	
ATPIII Definition	n = 113
No	67 (59.3%)
Yes	46 (40.7%)
ND	21
AHA Definition	n = 117
No	38 (32.5%)
Yes	79 (67.5%)
ND	17
IDF Definition	n = 106
No	35 (33.0%)
Yes	71 (67.0%)
ND	28

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Thirty days after CRC surgery, 98 patients (73.1%) did not suffer from any complication. 11 patients were readmitted to the hospital, 10 patients suffered anastomotic dehiscence and 10 patients were again submitted to surgery. With a total morbimortality percentage of 26.9%, only 2 deaths were registered during this period. Complications distribution according to Clavien-Dindo Classification and the previous data about 30 days after surgery outcomes are shown in Table 2.

Table 2
Sample surgical outcomes.

	n (%)
Readmission	11 (8.3%)
Dehiscence	10 (7.5%)
Reintervention	10 (7.5%)
Morbimortality	36 (26.9%)
Clavien-Dindo Classif.	
No complications	98 (73.1%)
Grade I	8 (6.0%)
Grade II	13 (9.7%)
Grade IIIa	3 (2.2%)
Grade IIIb	6 (4.5%)
Grade IVa	1 (0.7%)
Grade IVb	3 (2.2%)
Grade V	2 (1.5%)
Length of Hospital Stay Mdn (IQR)	5.00 (3.00)

After applying chi-square tests, the results suggest no evidence of association between any of the MS definition diagnosis (or their different components) and the surgical outcomes studied, except for low HDL cholesterol levels by ATPIII definition (HDL – c < 40 mg/dL in men or <50 mg/dL in women) as a predictor of morbimortality at 30 days, (p = 0.037) (Table 3).

Logistic regression was computed in order to calculate Odds ratio (OR) for the risk of having morbimortality having low HDL cholesterol (by ATPIII definition) as a predictor.

The risk of morbimortality for patients with low HDL cholesterol is 2.42 times increased when compared with patients with high HDL cholesterol (95% CI = [1.04, 5.62]). This result was statistically significant for p = 0.039 (Table 4).

As preforming Mann-Whitney Test for ordinal outcomes (Morbimortality according to Clavien-Dindo Classification) we were able to also stablish a statistically significant result, which states that patients with low HDL-cholesterol (by ATPIII definition)

Table 4
Logistic Model; In this case: Yes = HDL – C < 40 mg/dL in men or <50 mg/dL in women and No = HDL – C ≥ 40 mg/dL in men or ≥50 mg/dL in women.

	OR	95% CI	p-value
Low HDL cholesterol			
No	1	1	1
Yes	2.42	(1.04–5.62)	p = 0.039

Table 3
Chi square tests results. relation between dichotomous outcomes and independent variables.

	Readmission			Reintervention			Dehiscence			Morbimortality		
	No	Yes	p value	No	Yes	p value	No	Yes	p value	No	Yes	p value
MS ATPIII Definition	43 (93.5%)	3 (6.5%)	0.456	42 (91.3%)	4 (8.7%)	0.361	40 (93.0%)	3 (7%)	0.854	34 (73.9%)	12 (26.1%)	0.790
Central Obesity (ATPIII Definition)	44 (91.7%)	4 (8.3%)	0.787	45 (93.8%)	3 (6.3%)	0.462	44 (93.6%)	3 (6.4%)	0.861	40 (83.3%)	8 (16.7%)	0.056
High Triglycerides level (ATPIII Definition)	42 (91.3)	4 (8.7%)	0.870	45 (97.8%)	1 (2.2%)	0.120	41 (97.6%)	1 (2.4%)	0.088	33 (71.7%)	13 (28.3%)	0.883
Low HDL Cholesterol level (ATPIII Definition)	53 (89.8%)	6 (10.2%)	0.797	54 (90.0%)	6 (10.0%)	0.164	49 (89.1%)	6 (10.9%)	0.297	38 (63.3%)	22 (36.7%)	**.037**
High Blood Pressure (ATPIII Definition)	82 (91.1%)	8 (8.9%)	0.463	82 (91.1%)	8 (8.9%)	0.185	77 (90.6%)	8 (9.4%)	0.204	62 (68.9%)	28 (31.1%)	0.106
High Fasting Glucose (ATPIII Definition)	44 (91.7%)	4 (8.3%)	0.787	45 (93.8%)	3 (6.3%)	0.462	44 (93.6%)	3 (6.4%)	0.861	40 (83.3%)	8 (16.7%)	0.056
MS AHA Definition	73 (92.4%)	6 (7.6%)	0.595	75 (94.9%)	4 (5.1%)	0.963	70 (94.6%)	4 (5.4%)	0.529	63 (79.7%)	16 (20.3%)	0.054
Central Obesity (AHA Definition)	44 (91.7%)	4 (8.3%)	0.787	45 (93.8%)	3 (6.3%)	0.462	44 (93.6%)	3 (6.4%)	0.861	40 (83.3%)	8 (16.7%)	0.056
High Triglycerides level (AHA Definition)	42 (91.3%)	4 (8.7%)	0.870	45 (97.8%)	1 (2.2%)	0.120	41 (97.6%)	1 (2.4%)	0.088	33 (71.7%)	13 (28.3%)	0.883
Low HDL Cholesterol level (AHA Definition)	85 (90.4%)	9 (9.6%)	0.595	87 (91.6%)	8 (8.4%)	0.095	80 (90.9%)	8 (9.1%)	0.095	68 (71.6%)	27 (28.4%)	0.319
High Blood Pressure (AHA Definition)	105 (92.1%)	9 (7.9%)	0.771	106 (92.2%)	9 (7.8%)	0.232	99 (91.7%)	9 (8.3%)	0.246	86 (74.8%)	29 (25.2%)	0.380
High Fasting Glucose (AHA Definition)	52 (89.7%)	6 (10.3%)	0.686	54 (93.1%)	4 (6.9%)	0.922	50 (94.3%)	3 (5.7%)	0.381	44 (75.9%)	14 (24.1%)	0.425
MS IDF Definition	64 (90.1%)	7 (9.9%)	0.831	66 (93.0%)	5 (7.0%)	0.108	60 (92.3%)	5(7.7%)	0.363	54 (76.1%)	17 (26.9%)	0.411
Central Obesity (IDF Definition)	75 (90.4%)	8 (9.6%)	0.764	78 (94.0%)	5 (6.0%)	0.200	71 (92.2%)	6 (7.8%)	0.159	65 (78.3%)	18 (21.7%)	0.088
High Triglycerides level (IDF Definition)	42 (91.3%)	4 (8.7%)	0.870	45 (97.8%)	1 (2.2%)	0.120	41 (97.6%)	1 (2.4%)	0.088	33 (71.7%)	13 (28.3%)	0.883
HDL Cholesterol level (IDF Definition)	85 (90.4%)	9 (9.6%)	0.595	87 (91.6%)	8 (8.4%)	0.095	80 (90.9%)	8 (9.1%)	0.323	68 (71.6%)	27 (28.4%)	0.319
High Blood Pressure (IDF Definition)	105 (92.1%)	9 (7.9%)	0.771	106 (92.2%)	9 (7.8%)	0.232	99 (91.7%)	9 (8.3%)	0.246	86 (74.8%)	29 (25.2%)	0.380
High Fasting Glucose (IDF Definition)	52 (89.7%)	6 (10.3%)	0.686	54 (93.1%)	4 (6.9%)	0.922	50 (94.3%)	3 (5.7%)	0.381	44 (75.9%)	14 (24.1%)	0.425

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Table 5
Mann-Whitney Test Results. Relation between ordinal outcome (Morbimortality according to Clavien-Dindo classification) and Independent Variables.

	Man- Whitney Test				
	Morbimortality According to Clavien-dindo Classification				
	Mdn (IQR)				
	No	Yes	p value	U	Z
MS ATPIII Definition	0 (1)	0 (1)	0.777	1503.00	−0.283
Central Obesity (ATPIII Definition)	0 (2)	0 (0)	0.091	1251.50	−1.691
High Triglycerides level (ATPIII Definition)	0 (1)	0 (1)	0.880	1680.00	−0.151
Low HDL Cholesterol level (ATPIII Definition)	0 (0)	0 (2)	**.033**	**1400.50**	**−2.129**
High Blood Pressure (ATPIII Definition)	0 (0)	0 (1)	0.133	15670.50	−1.504
High Fasting Glucose (ATPIII Definition)	0 (2)	0 (0)	0.091	1251.50	−1.691
MS AHA Definition	0 (2)	0 (0)	0.067	1260.00	−1.830
Central Obesity (AHA Definition)	0 (2)	0 (0)	0.091	1251.50	−1.691
High Triglycerides level (AHA Definition)	0 (1)	0 (1)	0.880	1680.00	−0.151
Low HDL Cholesterol level (AHA Definition)	0 (0)	0 (2)	0.239	1312.00	0.239
High Blood Pressure (AHA Definition)	0 (2)	0 (1)	0.398	881.00	−0.845
High Fasting Glucose (AHA Definition)	0 (1)	0 (1)	0.452	1685.50	−0.752
MS IDF Definition	0 (1)	0 (1)	0.538	1171.50	−0.616
Central Obesity (IDF Definition)	0 (2)	0 (0)	0.137	918.50	−1.488
High Triglycerides level (IDF Definition)	0 (1)	0 (1)	0.880	1680.00	−0.151
HDL Cholesterol level (IDF Definition)	0 (0)	0 (2)	0.239	1312.00	−1.177
High Blood Pressure (IDF Definition)	0 (2)	0 (1)	0.398	881.00	−0.845
High Fasting Glucose (IDF Definition)	0 (1)	0 (1)	0.452	1685.50	−0.752

present higher grades of complications, according to Clavien-Dion classification (Mdn = 0; IQR = 2; p = 0.033), (Table 5).

4. Discussion

It is well known nowadays that MS is a risk factor for the development of CRC, as proven, between many others, by a large cohort study from 2006 [9] or by a recent meta-analysis (reporting 17 studies) from 2013 [8] being the pathophysiological mechanism pointed has responsible mostly related to insulin resistance and abdominal obesity [1]. This fact may perhaps explain the high incidence of MS (in all its definitions) in our sample (constituted only by CRC diagnosed patients) in comparison, for example, with a report that included 1433 inhabitants of Porto city (Portugal). In this study, the investigators obtained an estimated prevalence of MS of 24.0% with the ATPIII criteria, 37.2% with the AHA criteria and, 41.9% with the IDF criteria [19], in contrast with our results of 40.7%, 67.5% and 67.0%, respectively.

On the other hand, the relationship between MS and CRC surgical outcomes, recurrence and survival has been a source of disagreement with discording studies being published over the time.

For instance, a study from 2010 showed that the presence of metabolic syndrome was associated with a significant increase of hepatic metastasis and tumor recurrence [12]. In the same year another prospective study from Thailand peremptorily concluded that MS was an independent rick factor for postoperative complications and longer hospital stay in CRC patients submitted to surgery, with a 30 days follow-up [13]. On the other hand, in 2013, a retrospective cohort investigation concluded that MS had no influence on recurrence and overall survival of CRC patients, perhaps explained by the combined effects of elevated blood glucose and hypertension and the protective effect of dyslipidemia, has proven in the same study [20]. Moreover, two survival analysis from 2016 were able to, again, prove a deleterious effect of MS. On one of them, a prospective study involving 1318 CRC patients, it was proved that MS, specially hyperglycemia, were robust predictors of CRC mortality [21]. On the other one, from October 2016, patients were divided into 4 categories (defined by the presence of MS and/or obesity) and, as a result, the group with MS and obesity combined obtained a worse survival, overall and CRC related [22].

The pointed mechanism for this interaction might be explain by several theories: first, the insulin-resistant state present in MS influences an abnormal metabolism in adipocytes (especially visceral fat adipocytes) with subsequent increase in levels of Interleukin-6 and tumor necrosis factor-α (both pro-inflammatory) and low levels of adiponectin (protective adipokine) leading to an excessive systemic inflammatory response. Secondly, MS has been correlated with a situation of impaired microvascular circulation, which may cause diminished perfusion and poor tissue healing. And finally, alterations in polymorphonuclear cells has been noticed in patients with MS, which might be caused by the low levels of Leukotriene B4, essential in these cells function, leading this way to alterations in innate immune defense [13].

In our study there was no statistically significant influence of any of the MS definitions or it's components on the studied outcomes (no p value < 0.05), and the only finding that proves influence of HDL-cholesterol (by ATPIII definition) with 30 days morbimortality and with Clavien-Dindo Classification Grade appears to us a statistical finding instead of a valuable finding concerning the context.

5. Conclusion

This study seems to indicate that there is no apparent association between MS, in its different definitions and components, and CRC surgical outcomes (reintervention, readmission, dehiscence and morbimortality) at 30 days.

While a deeper understanding of this relationship could lead to a better clinical management, data remains inconclusive. The question of whether or not a better control of metabolic status could improve CRC patients' prognosis waits for further studies with larger sample sizes and longer follow-up timings.

Conflicts of interest

None.

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References

[1] Pais R, Silaghi H, Silaghi AC, Rusu ML, Dumitrascu DL. Metabolic syndrome and risk of subsequent colorectal cancer. World J Gastroenterol 2009;15(41):5141–8.
[2] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation 2005;112(17):2735–52, doi:http://dx.doi.org/10.1161/CIRCULATIONAHA.105.169404.
[3] Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol In adults (Adult treatment panel III). JAMA 2001;285(19):2486–97.
[4] Alberti KG, Zimmet P, Shaw J. Metabolic syndrome-a new world-wide definition. a consensus statement from the international diabetes federation. Diabet Med 2006;23(5):469–80, doi:http://dx.doi.org/10.1111/j.1464-5491.2006.01858.x.
[5] Uzunlulu M, Telci Caklili O, Oguz A. Association between metabolic syndrome and cancer. Ann Nutr Metab 2016;68(3):173–9, doi:http://dx.doi.org/10.1159/000443743.
[6] Jaggers JR, Sui X, Hooker SP, LaMonte MJ, Matthews CE, Hand GA, et al. Metabolic syndrome and risk of cancer mortality in men. Eur J Cancer 2009;45(10):1831–8, doi:http://dx.doi.org/10.1016/j.ejca.2009.01.031.
[7] Feakins RM. Obesity and metabolic syndrome: pathological effects on the gastrointestinal tract. Histopathology 2016;68(5):630–40, doi:http://dx.doi.org/10.1111/his.12907.
[8] Esposito K, Chiodini P, Capuano A, Bellastella G, Maiorino MI, Rafaniello C, et al. Colorectal cancer association with metabolic syndrome and its components: a systematic review with meta-analysis. Endocrine 2013;44(3):634–47, doi:http://dx.doi.org/10.1007/s12020-013-9939-5.
[9] Ahmed RL, Schmitz KH, Anderson KE, Rosamond WD, Folsom AR. The metabolic syndrome and risk of incident colorectal cancer. Cancer 2006;107(1):28–36, doi:http://dx.doi.org/10.1002/cncr.21950.
[10] GLOBOCAN. Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. International Agency for Research on Cancer; 2012 [Internet]. 2012. Available from: http://globocan.iarc.fr/.
[11] Ahmadi A, Noroozi M, Pourhoseingholi MA, Hashemi-Nazari SS. Effect of metabolic syndrome and its components on survival in colorectal cancer: a prospective study. J Renal Injury Prev 2015;4(1):15–9, doi:http://dx.doi.org/10.12861/jrip.2015.05.
[12] Shen Z, Ye Y, Bin L, Yin M, Yang X, Jiang K, et al. Metabolic syndrome is an important factor for the evolution of prognosis of colorectal cancer: survival, recurrence, and liver metastasis. Am J Surg 2010;200(1):59–63, doi:http://dx.doi.org/10.1016/j.amjsurg.2009.05.005.
[13] Lohsiriwat V, Pongsanguansuk W, Lertakyamanee N, Lohsiriwat D. Impact of metabolic syndrome on the short-term outcomes of colorectal cancer surgery. Dis Colon Rectum 2010;53(2):186–91, doi:http://dx.doi.org/10.1007/DCR.0b013e3181bdbc32.
[14] De Raet J, Delvaux G, Haentjens P, Van Nieuwenhove Y. Waist circumference is an independent risk factor for the development of parastomal hernia after permanent colostomy. Dis Colon Rectum 2008;51(12):1806–9, doi:http://dx.doi.org/10.1007/s10350-008-9366-5.
[15] Kartheuser AH, Leonard DF, Penninckx F, Paterson HM, Brandt D, Remue C, et al. Waist circumference and waist/hip ratio are better predictive risk factors for mortality and morbidity after colorectal surgery than body mass index and body surface area. Ann Surg 2013;258(5):722–30, doi:http://dx.doi.org/10.1097/SLA.0b013e3182a6605a.
[16] Rullier E, Laurent C, Garrelon JL, Michel P, Saric J, Parneix M. Risk factors for anastomotic leakage after resection of rectal cancer. Br J Surg 1998;85(3):355–8, doi:http://dx.doi.org/10.1046/j.1365-2168.1998.00615.x.
[17] Govaert JA, Lijftogt N, van Dijk WA, Tseng LN, Liem RS, Tollenaar RA, et al. Colorectal cancer surgery for obese patients: financial and clinical outcomes of a Dutch population-based registry. J Surg Oncol 2016;113(5):489–95, doi:http://dx.doi.org/10.1002/jso.24187.
[18] Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg 2004;240(2):205–13.
[19] Santos AC, Barros H. Impact of metabolic syndrome definitions on prevalence estimates: a study in a Portuguese community. Diabetes Vasc Dis Res 2007;4(4):320–7, doi:http://dx.doi.org/10.3132/dvdr.2007.059.
[20] Yang Y, Mauldin PD, Ebeling M, Hulsey TC, Liu B, Thomas MB, et al. Effect of metabolic syndrome and its components on recurrence and survival in colon cancer patients. Cancer 2013;119(8):1512–20.
[21] Peng F, Hu D, Lin X, Chen G, Liang B, Zhang H, et al. Preoperative metabolic syndrome and prognosis after radical resection for colorectal cancer: the Fujian prospective investigation of cancer (FIESTA) study. Int J Cancer 2016;139(12):2705–13, doi:http://dx.doi.org/10.1002/ijc.30404.
[22] Cespedes Feliciano EM, Kroenke CH, Meyerhardt JA, Prado CM, Bradshaw PT, Dannenberg AJ, et al. Metabolic dysfunction, obesity, and survival among patients with early-stage colorectal cancer. J Clin Oncol 2016, doi:http://dx.doi.org/10.1200/JCO.2016.67.4473 Sep 6. pii: JCO674473. [Epub ahead of print].

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Chapter 4: General Discussion

4.1. Metabolic Syndrome

Empirically, surgeons believe that obese patients and patients with comorbidities, such as arterial hypertension and diabetes, have an increased risk of postoperative complications and worst outcomes than normal weight and no comorbidities patients. Despite MetS being a well-known risk factor for CRC development,(50, 51), the literature is controversial regarding the influence in short term outcomes (30-days complications, anastomotic leak, reinterventions and readmissions)(55, 56) and long term outcomes (recurrence and survival).(53, 57-63)

The MetS is an association of obesity and comorbidities and its prevalence in the Portuguese population was estimated as 36.5% using the NCEP-ATPIII criteria and 49.6% using the IDF criteria (PORMETS study).(13) A quarter of the patients submitted to a colorectal surgery actually may develop postoperative complications.(89, 90) Given the prevalence of MetS and the outcome of complication being common, herein we tried to clarify the influence of MetS in the CRC short term outcomes. We analyzed the MetS in the different definitions as a whole and the different components individually.

Our study included 134 patients submitted to a CRC surgery between August 2015 and August 2016. MetS diagnosis occurred in 40.7% of these patients according to the NCEP-ATPIII definition, 67.5% according to the AHA definition and 67.0% according to the IDF definition. Those numbers are higher than the PORMETS study, probably because our sample included patients with the diagnosis of CRC and the PORMETS study included adults from primary health care centers lists.

The incidence of postoperative complications in our study was 26.9%, with 8.3% rate of readmissions, 7.5% of reinterventions and 7.5% of anastomotic leak. The statistical analysis revealed no evidence of association between any of the MetS definition diagnosis (or their different components) with postoperative complication rates. We found that low HDL cholesterol levels using NCEP-ATPIII definition was a predictor of 30-days complication as a dichotomous and ordinal variable ($p= 0.037$ and $p = 0.033$, respectively). Despite its statistical significance, as an isolated finding it probably doesn't reflect clinical significance and, therefore, we conclude that MetS does not lead to impaired postoperative outcome following CRC surgery.

One of the reasons for the obesity criteria in MetS not being significantly correlated to postoperative outcomes may be the fact that central obesity is not equal to VF (the most active fat in metabolic terms).

Importantly, waist circumference was proven to be insufficient to distinguish between subcutaneous and VF.(108)

During a mean period of 28.3 months follow-up, our study registered 12 relapses and 16 deaths in 130 patients. The Kaplan-Meier analysis for DFS was not statistically significant for any of the MetS definitions (NCEP-ATPIII definition had p=0.180; AHA definition had p=0.335; IDF definition had p=0.811). In terms of OS, the Kaplan-Meier analysis did not reveal any statistically significant differences for any of the criteria of MetS definition (NCEP-ATPIII definition had p=0.908; AHA definition had p=0.062; IDF definition had p=0.461). Performing the same statistical analysis for each of the components of MetS in the different definitions, we have reached the same results. For those reasons, we concluded that MetS and its different components were not a prognostic factor for DFS or OS in patients submitted to a curative CRC surgery. However, our results, despite been prospectively collected, are based in a small sample size with a rare outcome (relapse or death), and for those reasons they need to be validated in a larger sample size prospective study.

4.1.1 Visceral Fat

Like previously stated, VF is more metabolically active than subcutaneous fat and has been shown to be central to the pathogenesis of the MetS.(21) The increase of VF has been associated, in some studies, with a significant increase of surgical wound infection, anastomotic leak, reintervention rates and an increase in postoperative hospital stay when compared to low VF patients.(40, 46, 109, 110) However, other studies did not reach the same conclusions and do not agree with the influence of VF on CRC outcomes.(111)

In order to clarify this relationship between VF and CRC outcomes (post-operative complications, oncologic results and survival), we developed a retrospective study that included 199 patients with CRC submitted to surgery with curative intent that were followed for a minimum of 5 years. The lack of consensus in the definition of VF is one of the limitations of the studies that use this variable. In our study, patients were divided into quartiles of VF area; however, other authors use VF volume,(112) ratio between VF and subcutaneous fat area,(46) ratio between VF and total fat area (109) and ratio between VF and body surface area.(113)

In our study, VF had a negative influence in 30-days morbidity (p=0.043), anastomotic leak (p=0.009) and re-operation rates (p=0.005) on CRC patients. In sub-group analysis (colon vs rectal cancer), patients with higher VF submitted to surgery with colon cancer had fewer lymph nodes harvested compared with patients with less VF (p=0.027); moreover, the percentage of patients with at least 12 lymph nodes harvested was also smaller in higher VF patients (p=0.003). In terms of DFS and OS, our results showed that patients on the first and fourth quartiles had a trend for a slight better survival than the patients in the middle quartiles, but the differences encountered were not significant in terms of statistical analysis (log-rank p=0.768 for OS and p=0.704 for DFS).

Collecting at least 12 lymph nodes (accurate tumor staging) is crucial because it can determine the need of adjuvant chemotherapy (less than 12 lymph nodes identified is a risk factor taken in consideration at multidisciplinary oncology meetings). Our results revealed that patients with higher VF have fewer lymph nodes retrieved. Indeed, VF increases surgical difficulty, presenting as a problem for the surgeon to perform an accurate oncologic dissection near the origin of the vessels (D3 dissection); in addition, the amount of fat tissue in the mesentery makes the identification of the lymph nodes difficult for the pathologist. This hypothesis was already described in a previous study.(42)

4.2. Biomarkers

4.2.1. Inflammatory markers

About 30% of patients develop complications after CRC surgery.(114) The most significant source of morbidity is attributable to infectious complications that contribute to a significant increase of hospitalization costs, prolonged hospital stay and post-operative morbimortality.(91)

A prompt diagnosis of infectious complication has been found to improve surgical outcomes.(115) An easy, cheap and standardized method for early identification of the patients that will develop complications is the ambition of every surgeon as it would allow a quick identification of those high-risk patients and early initiation of treatment in the hope of decreasing the morbidity associated with delayed diagnosis. Several biomarkers of infection such as WBC, CRP, PCT and albumin are the most studied predictors of infection after CRC surgery.

Elevated WBC count is a nonspecific inflammatory marker and one of the systemic inflammatory response syndrome (SIRS) criteria. Individually, the WBC count has a low sensitivity, specificity and positive predictive value (PPV), having a lower contribution to the early detection of inflammatory complications.(116)

CRP plays a major role in innate immunity by assisting complement binding foreign and damaged cells, and enhancing phagocytosis by macrophages, thus acting as an early defense against infection. With only 19 hours of half-life, CRP is a valuable marker of systemic inflammatory secondary response to the surgical procedure or even a marker of complications, tending to normalize rapidly with the patient's recovery.(117)

In the setting of a bacterial infection, cytokines and lipopolysaccharide stimulate neutrophils and cells in the lungs, liver, intestine, and brain to produce PCT. Viral infections do not have the same influence in rising PCT concentration like bacterial infections. This particularity, in theory, makes this biomarker superior to others in predicting a bacterial infection.(118)

Albumin is the most abundant protein in humans (55-60% of protein in human plasma). Albumin is exclusively produced by the liver and about two thirds of the total body albumin pool is in the extravascular space. The serum albumin concentration depends basically on three factors: synthesis, distribution, and degradation. In the presence of any kind of traumatic event (like a surgery), an albumin concentration drop is observed in the first hours because of three events: 1) decrease of hepatic albumin synthesis in favor of production of acute phase molecules by the liver (CRP, fibrinogen and macroglobin); 2) increase of basal energy expenditure, which can consume up to 20% of the body proteins within ten days; 3) increase in the capillary leak that transfers the albumin from plasma to the third space. This increase in capillary leak is the most important mechanism for the decrease of plasma albumin concentration after a surgery.(119, 120).

At the time of development of this research work, most published studies attempted to find positive predictive factors for infection. The opposite, the identification of the patients that will not complicate remained less investigated, particularly when it came to suggesting a cut-off point for selecting patients to securely discharge.

With respect to predicting infection, the studies were controversial regarding the best marker. As expected, WBC was not a reliable predictor of septic complications, especially in the first few postoperative days.(121-123) CRP showed mix results, with some authors concluding that this marker presents

insufficient value to predict infectious complications,(121) while in other studies it showed a good predictive value (81, 122) and was even concluded that is would be better than PCT.(124, 125) PCT is a more consensual marker to predict infections, generally more accurate than CRP, but with different cut-off points.(84, 123, 126) However, the value of PCT as a predictor of infections was not demonstrated in all studies.(91)

In our work, CRP at post-operative day (POD) 1 and 3 and the CRP to albumin ratio (CAR) at POD 3 were the most accurate predictors of infection. The optimum cut-off point determined by the receiver operating characteristic (ROC) curve was 73mg/L for CRP at POD 1, 123mg/L for CRP at POD 3 and 43 for CRP to albumin ratio (CAR) at POD 3. Their sensitivity and PPV were low (sensitivity of 55.1, 70.3 and 66.2; PPV of 34.0, 43.6 and 40.5, respectively) but the NPV was high (91.5, 91.2 and 90.7, respectively). This high NPV makes these markers of particular interest in predicting an uncomplicated postoperative course, in more than 90% of the patients. Both PCT and WBC failed as predictors of early postoperative infection on POD 1 and 3.

Recently many studies and meta-analysis have been published to validate the importance of the NPV of different inflammatory markers. In 2016, Giaccaglia published the results of the multicentric PREDICTS study that revealed a good NPV of CRP and PCT for anastomotic leakage at POD 3 and 5.(96) Facy (2017) concluded that CRP <100mg/L at POD 4 can be safely discharged regardless of the surgical approach (laparoscopy or laparotomy).(127) Domínguez-Comesaña (2018) reach a NPV of 100% for PCT at POD 1 and 3 and for CRP at POD 3.(92) A meta-analysis concluded that a laboratory value of CRP less than 159mg/L at POD 3 has a very high NPV (90%) of infectious complications.(97) A more recent meta-analysis concluded that PCT is a useful negative test for anastomotic leakage following elective colorectal surgery (NPV 90-100%) but is unable to accurately predict an anastomotic leakage (PPV 34%).(128)

Inflammatory markers, like the WBC, CRP and PCT, increase with the surgical insult regardless of the presence of an infectious complication and are not specific of any complication. Those findings reflect the controversy in attempting to define a cut-off point to predict septic complications. However, as seen in recent papers, a NPV is more consensual and is more important in clinical practice. In the era of laparoscopy, the majority of the patients submitted to a CRC surgery will not develop a complication, tolerate oral food and have their pain controlled with oral analgesics in the first postoperative day. Those patients with low levels of inflammatory markers (such as CRP or CAR that were tested in our work) can be safely discharged earlier, which can allow for a reduction of costs, an increase in patient

satisfaction and can, probably, reduce the development of infectious complications associated with prolonged hospitalization.

4.2.2. Vascular Endothelial Growth Factor

Increased tumoral expression of VEGF is associated with poor prognosis in several obesity-related cancers.(73, 78) The relationship between VEFG and CRC outcomes is controversial. While some studies showed that VEGF has no significant prognostic value in CRC,(129) others have demonstrated an association between overexpression of VEGF and poor CRC outcomes: overexpression of VEGF and VEGFR in CRC tissue may indicate poor prognosis,(78, 102, 103) and predict early relapse,(105) while preoperative VEGF serum concentration may predict poor disease-specific survival and DFS in colon cancer patients.(106)

One of the treatment options for metastatic CRC is the combination of bevacizumab (anti-VEGF therapy) with conventional chemotherapy.(130) Decreased efficacy in obese patients has been reported and has been speculated to be associated with increased levels of VEGF (and other proangiogenic factors) produced by visceral adipose tissue.(73) However, the potential for VEGF levels to be a predictive or prognostic biomarker for anti-VEGF therapy is not clear. The majority of studies failed to demonstrate the benefit for anti-VEGF therapy,(131-133) but others have showed that VEGF expression can be prognostic for anti-VEGF treatment outcomes in metastatic CRC.(77) In the neoadjuvant setting, the results of a meta-analysis concluded that, based on phase I/II studies, adding bevacizumab to conventional neoadjuvant treatment may increase pathological complete response but also increases the incidence of severe adverse events.(82) This work also leaves several open questions in its discussion , like the fact that patient selection should be based on potential predictive response biomarkers, such as free-VEGF, in order to define a subgroup of patients, who would most likely benefit from this form of therapy.(82)

Plasma biomarkers offer a number of advantages over tissue-based markers. The potential of serum concentration of VEGF being representative of tumor VEGF expression opens new pathways for further investigation, preoperative prognostic information and treatment response. At the time of the development of our work, the absence of studies designed to verify this relationship led us to design a study with the intention of clarifying the relationship between the concentration of serum VEGF and tumor VEGF-R expression in patients with CRC.

The work started with 134 patients submitted to CRC surgery, and 74 of those patients were included. Serum VEGF concentration were determined by ELISA and tumor VEGF-R3 expression was graded according to the intensity and extent of staining of the endothelium of the vessels as absent, weak/very limited moderate staining, moderate widespread/strong localized staining and strong widespread staining. A strong association was found between serum VEGF and tumoral VEGF-R3 expression ($p < 0.001$; $\eta^2 = 0.34$), even after controlling for potential confounders ($p < 0.001$; $\eta^2 = 0.35$).

VEGF is expressed in a wide variety of human tissues, being particularly high but not exclusive of tumors.(106) For this reason, even patients whose tumors didn't stain for VEGF-R3 presented low levels of serum VEGF (36.29pg/mL). In turn, serum VEGF progressively increased as higher intensity of the staining for VEGF-R3 (49.68pg/mL in weak/very limited moderate staining, 65.35pg/mL in moderate widespread/strong localized staining; 126.39pg/mL in strong widespread staining).

The ability to predict tumor expression of VEGF by collecting a blood sample from the patient may open new horizons in terms of identifying a potential biomarker that can help in selection of treatment and determining prognosis. Based on the relationship between serum and tumoral VEGF expression established in this work, it may be possible, in the near future, to implement a patient selection strategy that effectively identifies those patients who are most likely to benefit from neoadjuvant treatment with VEGF inhibitors.

Chapter 5: Conclusions

MetS is a complex cluster of conditions and diseases, each of those having different influences on the prognosis of patients affected by CRC. This may explain the controversy we found in the literature and also why our results did not reveal any influence in terms of short-term outcomes and prognosis in patients submitted to a curative CRC surgery. Even when we analyzed obesity separately from the other risk factors, we did not obtain statistically significant results. One of the reasons for this may be related with the use of waist circumference for classification of obesity in all of the MetS definitions. It is known that patients with the same waist circumference can have different proportions of visceral and subcutaneous fat and also that VF is more active than subcutaneous fat. When we looked at VF, we found a negative influence in surgical and oncologic outcomes. In our opinion, the most important influence is related to the number of lymph nodes harvested: in an obese patient, especially with higher VF, harvest of lymph nodes is demanding, in terms of surgical skills. This highlights the need for patients to be operated by experienced surgical teams in order to achieve the best possible results.

Pursuing predictors of outcomes is of high importance in the context of developing surgical protocols. With our work, we defined the cut-off for CRP and CAR to predict an uneventful postoperative period. We find the ability to early identify the majority of patients who will not develop a complication more important than trying to predict what patients will have morbidity. In the context of hospital admission, surgeons want to discharge patients that will not complicate as quickly as possible and focus on close monitoring of the rest in order to rapidly intervene in case a complication develops. Predictors of complications are not specific because they can identify whose patients will develop a complication but fail to precisely define the type of complication that will occur. For example, high PCT can predict an anastomotic leak but can also indicate a risk of wound infection or pneumonia. Currently, we do not have different cut-offs or predictors for each complication. This is another reason why we believe that predictors of uneventful postoperative period are more useful in clinical practice.

With our work, we were able to demonstrate the relationship between serum and tumor VEGF. The ability to predict the tumoral concentration of a marker based on its serum concentration can be the base to further investigations.

Chapter 6: Future Perspective

We believe that, in the setting of CRC surgery, the NPV of the inflammatory markers is more relevant to clinical practice than the PPV. The next step would be to verify if adopting a cut-off point of inflammatory markers to the discharge criteria represents a significant reduction in admission time, in costs and an increase in patient satisfaction.

In this work, we were able to prove the relationship between serum and tumor VEGF. The implications of this relationship, such as predicting the pathology of the tumors (high serum VEGF concentration implies high tumor grade or nodal metastasis?), disease outcome (worst DFS or OS?) and benefit of adding anti-VEGF to neoadjuvant treatment (patients with higher serum VEGF concentration benefit of adding anti-VEGF drugs?) remain to be studied.

References

1. International Agency for Research on Cancer - World Health Organization. Globocan 2018.
2. RORENO. Instituto Português de Oncologia do Porto Francisco Gentil – EPE. Registo Oncológico Nacional 2010. 2016.
3. World Health Organization. Obesity and overweight 2018 [Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.
4. Oliveira A, Araujo J, Severo M, Correia D, Ramos E, Torres D, et al. Prevalence of general and abdominal obesity in Portugal: comprehensive results from the National Food, nutrition and physical activity survey 2015-2016. BMC public health. 2018;18(1):614.
5. Kylin E. Studien ueber das Hypertonie-Hyperglyca "mieHyperurika" miesyndrom. Zentralblatt fuer Innere Medizin 1923;44:105-27.
6. Kaur J. A comprehensive review on metabolic syndrome. Cardiol Res Pract. 2014;2014:943162.
7. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15(7):539-53.
8. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001;285(19):2486-97.
9. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005;112(17):2735-52.
10. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome–a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med. 2006;23(5):469-80.
11. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640-5.
12. Samson SL, Garber AJ. Metabolic syndrome. Endocrinol Metab Clin North Am. 2014;43(1):1-23.
13. Raposo L, Severo M, Barros H, Santos AC. The prevalence of the metabolic syndrome in Portugal: the PORMETS study. BMC public health. 2017;17(1):555.
14. Scuteri A, Laurent S, Cucca F, Cockcroft J, Cunha PG, Manas LR, et al. Metabolic syndrome across Europe: different clusters of risk factors. Eur J Prev Cardiol. 2015;22(4):486-91.
15. Moore JX, Chaudhary N, Akinyemiju T. Metabolic Syndrome Prevalence by Race/Ethnicity and Sex in the United States, National Health and Nutrition Examination Survey, 1988-2012. Prev Chronic Dis. 2017;14:E24.
16. Slaughter KN, Thai T, Penaroza S, Benbrook DM, Thavathiru E, Ding K, et al. Measurements of adiposity as clinical biomarkers for first-line bevacizumab-based chemotherapy in epithelial ovarian cancer. Gynecol Oncol. 2014;133(1):11-5.
17. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med. 2003;348(17):1625-38.
18. Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon DA, Smeeth L. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5.24 million UK adults. Lancet. 2014;384(9945):755-65.

19. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet. 2008;371(9612):569-78.
20. Ligibel JA, Alfano CM, Courneya KS, Demark-Wahnefried W, Burger RA, Chlebowski RT, et al. American Society of Clinical Oncology position statement on obesity and cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2014;32(31):3568-74.
21. Doyle SL, Donohoe CL, Lysaght J, Reynolds JV. Visceral obesity, metabolic syndrome, insulin resistance and cancer. Proc Nutr Soc. 2012;71(1):181-9.
22. Vongsuvanh R, George J, Qiao L, van der Poorten D. Visceral adiposity in gastrointestinal and hepatic carcinogenesis. Cancer Lett. 2013;330(1):1-10.
23. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis. 2009;30(7):1073-81.
24. Unamuno X, Gomez-Ambrosi J, Rodriguez A, Becerril S, Fruhbeck G, Catalan V. Adipokine dysregulation and adipose tissue inflammation in human obesity. Eur J Clin Invest. 2018;48(9):e12997.
25. Ackerman SE, Blackburn OA, Marchildon F, Cohen P. Insights into the Link Between Obesity and Cancer. Curr Obes Rep. 2017;6(2):195-203.
26. Fan Y, Gan Y, Shen Y, Cai X, Song Y, Zhao F, et al. Leptin signaling enhances cell invasion and promotes the metastasis of human pancreatic cancer via increasing MMP-13 production. Oncotarget. 2015;6(18):16120-34.
27. Cao Y. Angiogenesis modulates adipogenesis and obesity. J Clin Invest. 2007;117(9):2362-8.
28. Brakenhielm E, Veitonmaki N, Cao R, Kihara S, Matsuzawa Y, Zhivotovsky B, et al. Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. Proc Natl Acad Sci U S A. 2004;101(8):2476-81.
29. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. Nat Med. 2012;18(3):363-74.
30. Miyazawa-Hoshimoto S, Takahashi K, Bujo H, Hashimoto N, Saito Y. Elevated serum vascular endothelial growth factor is associated with visceral fat accumulation in human obese subjects. Diabetologia. 2003;46(11):1483-8.
31. Modesitt SC, Hsu JY, Chowbina SR, Lawrence RT, Hoehn KL. Not all fat is equal: differential gene expression and potential therapeutic targets in subcutaneous adipose, visceral adipose, and endometrium of obese women with and without endometrial cancer. Int J Gynecol Cancer. 2012;22(5):732-41.
32. Ottaiano A, De Divitiis C, Capozzi M, Avallone A, Pisano C, Pignata S, et al. Obesity and Cancer: Biological Links and Treatment Implications. Curr Cancer Drug Targets. 2018;18(3):231-8.
33. Larsson SC, Wolk A. Obesity and colon and rectal cancer risk: a meta-analysis of prospective studies. Am J Clin Nutr. 2007;86(3):556-65.
34. Wang Y, Jacobs EJ, Patel AV, Rodriguez C, McCullough ML, Thun MJ, et al. A prospective study of waist circumference and body mass index in relation to colorectal cancer incidence. Cancer causes & control : CCC. 2008;19(7):783-92.
35. Schoen RE, Tangen CM, Kuller LH, Burke GL, Cushman M, Tracy RP, et al. Increased blood glucose and insulin, body size, and incident colorectal cancer. J Natl Cancer Inst. 1999;91(13):1147-54.
36. Moore LL, Bradlee ML, Singer MR, Splansky GL, Proctor MH, Ellison RC, et al. BMI and waist circumference as predictors of lifetime colon cancer risk in Framingham Study adults. Int J Obes Relat Metab Disord. 2004;28(4):559-67.
37. Siegel EM, Ulrich CM, Poole EM, Holmes RS, Jacobsen PB, Shibata D. The effects of obesity and obesity-related conditions on colorectal cancer prognosis. Cancer Control. 2010;17(1):52-7.
38. Im JP, Kim D, Chung SJ, Jin EH, Han YM, Park MJ, et al. Visceral obesity as a risk factor for colorectal adenoma occurrence in surveillance colonoscopy. Gastrointestinal endoscopy. 2018;88(1):119-27 e4.

39. Lee JY, Lee HS, Lee DC, Chu SH, Jeon JY, Kim NK, et al. Visceral fat accumulation is associated with colorectal cancer in postmenopausal women. PloS one. 2014;9(11):e110587.
40. Tsujinaka S, Konishi F, Kawamura YJ, Saito M, Tajima N, Tanaka O, et al. Visceral obesity predicts surgical outcomes after laparoscopic colectomy for sigmoid colon cancer. Diseases of the colon and rectum. 2008;51(12):1757-65; discussion 65-7.
41. Ozoya OO, Siegel EM, Srikumar T, Bloomer AM, DeRenzis A, Shibata D. Quantitative Assessment of Visceral Obesity and Postoperative Colon Cancer Outcomes. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2017;21(3):534-42.
42. Yang T, Wei M, He Y, Deng X, Wang Z. Impact of visceral obesity on outcomes of laparoscopic colorectal surgery: a meta-analysis. ANZ journal of surgery. 2015;85(7-8):507-13.
43. Kuritzkes BA, Pappou EP, Kiran RP, Baser O, Fan L, Guo X, et al. Visceral fat area, not body mass index, predicts postoperative 30-day morbidity in patients undergoing colon resection for cancer. International journal of colorectal disease. 2018;33(8):1019-28.
44. Watanabe J, Tatsumi K, Ota M, Suwa Y, Suzuki S, Watanabe A, et al. The impact of visceral obesity on surgical outcomes of laparoscopic surgery for colon cancer. International journal of colorectal disease. 2014;29(3):343-51.
45. Yu H, Joh YG, Son GM, Kim HS, Jo HJ, Kim HY. Distribution and Impact of the Visceral Fat Area in Patients With Colorectal Cancer. Annals of coloproctology. 2016;32(1):20-6.
46. Moon HG, Ju YT, Jeong CY, Jung EJ, Lee YJ, Hong SC, et al. Visceral obesity may affect oncologic outcome in patients with colorectal cancer. Annals of surgical oncology. 2008;15(7):1918-22.
47. Lee CS, Murphy DJ, McMahon C, Nolan B, Cullen G, Mulcahy H, et al. Visceral Adiposity is a Risk Factor for Poor Prognosis in Colorectal Cancer Patients Receiving Adjuvant Chemotherapy. Journal of gastrointestinal cancer. 2015;46(3):243-50.
48. Guiu B, Petit JM, Bonnetain F, Ladoire S, Guiu S, Cercueil JP, et al. Visceral fat area is an independent predictive biomarker of outcome after first-line bevacizumab-based treatment in metastatic colorectal cancer. Gut. 2010;59(3):341-7.
49. Miyamoto Y, Oki E, Emi Y, Tokunaga S, Shimokawa M, Ogata Y, et al. Low Visceral Fat Content Is a Negative Predictive Marker for Bevacizumab in Metastatic Colorectal Cancer. Anticancer research. 2018;38(1):491-9.
50. Esposito K, Chiodini P, Capuano A, Bellastella G, Maiorino MI, Rafaniello C, et al. Colorectal cancer association with metabolic syndrome and its components: a systematic review with meta-analysis. Endocrine. 2013.
51. Choi YJ, Lee DH, Han KD, Shin CM, Kim N. Abdominal obesity, glucose intolerance and decreased high-density lipoprotein cholesterol as components of the metabolic syndrome are associated with the development of colorectal cancer. European journal of epidemiology. 2018;33(11):1077-85.
52. Ahmed RL, Schmitz KH, Anderson KE, Rosamond WD, Folsom AR. The metabolic syndrome and risk of incident colorectal cancer. Cancer. 2006;107(1):28-36.
53. Jaggers JR, Sui X, Hooker SP, LaMonte MJ, Matthews CE, Hand GA, et al. Metabolic syndrome and risk of cancer mortality in men. Eur J Cancer. 2009;45(10):1831-8.
54. Pais R, Silaghi H, Silaghi AC, Rusu ML, Dumitrascu DL. Metabolic syndrome and risk of subsequent colorectal cancer. World J Gastroenterol. 2009;15(41):5141-8.
55. Shariq OA, Hanson KT, McKenna NP, Kelley SR, Dozois EJ, Lightner AL, et al. Does Metabolic Syndrome Increase the Risk of Postoperative Complications in Patients Undergoing Colorectal Cancer Surgery? Diseases of the colon and rectum. 2019.
56. Zarzavadjian Le Bian A, Denet C, Tabchouri N, Levard H, Besson R, Perniceni T, et al. The effect of metabolic syndrome on postoperative outcomes following laparoscopic colectomy. Techniques in coloproctology. 2018;22(3):215-21.

57. Shen Z, Ye Y, Bin L, Yin M, Yang X, Jiang K, et al. Metabolic syndrome is an important factor for the evolution of prognosis of colorectal cancer: survival, recurrence, and liver metastasis. Am J Surg. 2010;200(1):59-63.
58. You J, Liu WY, Zhu GQ, Wang OC, Ma RM, Huang GQ, et al. Metabolic syndrome contributes to an increased recurrence risk of non-metastatic colorectal cancer. Oncotarget. 2015;6(23):19880-90.
59. Peng F, Hu D, Lin X, Chen G, Liang B, Zhang H, et al. Preoperative metabolic syndrome and prognosis after radical resection for colorectal cancer: The Fujian prospective investigation of cancer (FIESTA) study. International journal of cancer. 2016;139(12):2705-13.
60. Cespedes Feliciano EM, Kroenke CH, Meyerhardt JA, Prado CM, Bradshaw PT, Dannenberg AJ, et al. Metabolic Dysfunction, Obesity, and Survival Among Patients With Early-Stage Colorectal Cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2016;34(30):3664-71.
61. Yang Y, Mauldin PD, Ebeling M, Hulsey TC, Liu B, Thomas MB, et al. Effect of metabolic syndrome and its components on recurrence and survival in colon cancer patients. Cancer. 2013;119(8):1512-20.
62. Ahmadi A, Noroozi M, Pourhoseingholi MA, Hashemi-Nazari SS. Effect of metabolic syndrome and its components on survival in colorectal cancer: a prospective study. Journal of renal injury prevention. 2015;4(1):15-9.
63. Reed M, Patrick C, Croft B, Walde N, Voutsadakis IA. The metabolic syndrome and its components as prognostic factors in metastatic colorectal cancer. Indian journal of gastroenterology : official journal of the Indian Society of Gastroenterology. 2019.
64. Lohsiriwat V, Pongsanguansuk W, Lertakyamanee N, Lohsiriwat D. Impact of metabolic syndrome on the short-term outcomes of colorectal cancer surgery. Diseases of the colon and rectum. 2010;53(2):186-91.
65. Tsai IJ, Beilin LJ, Puddey IB, Croft KD, Barden A. Impaired ex vivo leukotriene B4 production characterizes the metabolic syndrome and is improved after weight reduction. J Clin Endocrinol Metab. 2007;92(12):4747-52.
66. Huang C, Chen Y. Lymphangiogenesis and colorectal cancer. Saudi medical journal. 2017;38(3):237-44.
67. Karkkainen MJ, Alitalo K. Lymphatic endothelial regulation, lymphoedema, and lymph node metastasis. Semin Cell Dev Biol. 2002;13(1):9-18.
68. Al-Rawi MA, Mansel RE, Jiang WG. Molecular and cellular mechanisms of lymphangiogenesis. Eur J Surg Oncol. 2005;31(2):117-21.
69. Martins SF, Reis RM, Rodrigues AM, Baltazar F, Filho AL. Role of endoglin and VEGF family expression in colorectal cancer prognosis and anti-angiogenic therapies. World J Clin Oncol. 2011;2(6):272-80.
70. Matsumoto K, Nakayama Y, Inoue Y, Minagawa N, Katsuki T, Shibao K, et al. Lymphatic microvessel density is an independent prognostic factor in colorectal cancer. Diseases of the colon and rectum. 2007;50(3):308-14.
71. Duff SE, Jeziorska M, Kumar S, Haboubi N, Sherlock D, O'Dwyer ST, et al. Lymphatic vessel density, microvessel density and lymphangiogenic growth factor expression in colorectal cancer. Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland. 2007;9(9):793-800.
72. Byrne AM, Bouchier-Hayes DJ, Harmey JH. Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). J Cell Mol Med. 2005;9(4):777-94.
73. Hursting SD, Hursting MJ. Growth signals, inflammation, and vascular perturbations: mechanistic links between obesity, metabolic syndrome, and cancer. Arterioscler Thromb Vasc Biol. 2012;32(8):1766-70.

74. Saranadasa M, Wang ES. Vascular endothelial growth factor inhibition: conflicting roles in tumor growth. Cytokine. 2011;53(2):115-29.
75. Silha JV, Krsek M, Sucharda P, Murphy LJ. Angiogenic factors are elevated in overweight and obese individuals. Int J Obes (Lond). 2005;29(11):1308-14.
76. Himbert C, Delphan M, Scherer D, Bowers LW, Hursting S, Ulrich CM. Signals from the Adipose Microenvironment and the Obesity-Cancer Link-A Systematic Review. Cancer prevention research. 2017;10(9):494-506.
77. Jurgensmeier JM, Schmoll HJ, Robertson JD, Brooks L, Taboada M, Morgan SR, et al. Prognostic and predictive value of VEGF, sVEGFR-2 and CEA in mCRC studies comparing cediranib, bevacizumab and chemotherapy. Br J Cancer. 2013;108(6):1316-23.
78. Martins SF, Garcia EA, Luz MA, Pardal F, Rodrigues M, Filho AL. Clinicopathological correlation and prognostic significance of VEGF-A, VEGF-C, VEGFR-2 and VEGFR-3 expression in colorectal cancer. Cancer Genomics Proteomics. 2013;10(2):55-67.
79. Zawadzki M, Czarnecki R, Rzaca M, Obuszko Z, Velchuru VR, Witkiewicz W. C-reactive protein and procalcitonin predict anastomotic leaks following colorectal cancer resections - a prospective study. Wideochir Inne Tech Maloinwazyjne. 2016;10(4):567-73.
80. Allin KH, Nordestgaard BG. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. Critical reviews in clinical laboratory sciences. 2011;48(4):155-70.
81. MacKay GJ, Molloy RG, O'Dwyer PJ. C-reactive protein as a predictor of postoperative infective complications following elective colorectal resection. Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland. 2011;13(5):583-7.
82. Benevento I, De Felice F, Musio D, Tombolini V. The Addition of Target Therapy to Neoadjuvant Chemoradiotherapy in Locally Advanced Rectal Cancer: A Review. Chemotherapy. 2017;62(5):314-22.
83. Schuetz P, Bretscher C, Bernasconi L, Mueller B. Overview of procalcitonin assays and procalcitonin-guided protocols for the management of patients with infections and sepsis. Expert review of molecular diagnostics. 2017;17(6):593-601.
84. Takakura Y, Hinoi T, Egi H, Shimomura M, Adachi T, Saito Y, et al. Procalcitonin as a predictive marker for surgical site infection in elective colorectal cancer surgery. Langenbeck's archives of surgery. 2013;398(6):833-9.
85. Muller B, White JC, Nylen ES, Snider RH, Becker KL, Habener JF. Ubiquitous expression of the calcitonin-i gene in multiple tissues in response to sepsis. J Clin Endocrinol Metab. 2001;86(1):396-404.
86. Hayati F, Mohd Azman ZA, Nasuruddin DN, Mazlan L, Zakaria AD, Sagap I. Serum Procalcitonin Predicts Anastomotic Leaks in Colorectal Surgery. Asian Pac J Cancer Prev. 2017;18(7):1821-5.
87. Fanali G, di Masi A, Trezza V, Marino M, Fasano M, Ascenzi P. Human serum albumin: from bench to bedside. Molecular aspects of medicine. 2012;33(3):209-90.
88. Kratz F. Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. Journal of controlled release : official journal of the Controlled Release Society. 2008;132(3):171-83.
89. Greco M, Capretti G, Beretta L, Gemma M, Pecorelli N, Braga M. Enhanced recovery program in colorectal surgery: a meta-analysis of randomized controlled trials. World journal of surgery. 2014;38(6):1531-41.
90. Watt DG, McSorley ST, Park JH, Horgan PG, McMillan DC. A Postoperative Systemic Inflammation Score Predicts Short- and Long-Term Outcomes in Patients Undergoing Surgery for Colorectal Cancer. Annals of surgical oncology. 2017;24(4):1100-9.
91. Silvestre J, Rebanda J, Lourenco C, Povoa P. Diagnostic accuracy of C-reactive protein and procalcitonin in the early detection of infection after elective colorectal surgery - a pilot study. BMC infectious diseases. 2014;14:444.
92. Dominguez-Comesana E, Estevez-Fernandez SM, Lopez-Gomez V, Ballinas-Miranda J, Dominguez-Fernandez R. Procalcitonin and C-reactive protein as early markers of postoperative intra-

abdominal infection in patients operated on colorectal cancer. International journal of colorectal disease. 2017;32(12):1771-4.
93. Giaccaglia V, Salvi PF, Cunsolo GV, Sparagna A, Antonelli MS, Nigri G, et al. Procalcitonin, as an early biomarker of colorectal anastomotic leak, facilitates enhanced recovery after surgery. Journal of critical care. 2014;29(4):528-32.
94. Medina-Fernandez FJ, Diaz-Lopez C, Briceno J. A Different Approach to the Use of C-Reactive Protein and Procalcitonin in Postoperative Infectious Complications. Annals of surgery. 2017;266(6):e98-e9.
95. Medina-Fernandez FJ, Garcilazo-Arismendi DJ, Garcia-Martin R, Rodriguez-Ortiz L, Gomez-Barbadillo J, Gallardo-Valverde JM, et al. Validation in colorectal procedures of a useful novel approach for the use of C-reactive protein in postoperative infectious complications. Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland. 2016;18(3):O111-8.
96. Giaccaglia V, Salvi PF, Antonelli MS, Nigri G, Pirozzi F, Casagranda B, et al. Procalcitonin Reveals Early Dehiscence in Colorectal Surgery: The PREDICS Study. Annals of surgery. 2016;263(5):967-72.
97. Gans SL, Atema JJ, van Dieren S, Groot Koerkamp B, Boermeester MA. Diagnostic value of C-reactive protein to rule out infectious complications after major abdominal surgery: a systematic review and meta-analysis. International journal of colorectal disease. 2015;30(7):861-73.
98. Egenvall M, Morner M, Martling A, Gunnarsson U. Prediction of outcome after curative surgery for colorectal cancer: preoperative haemoglobin, C-reactive protein and albumin. Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland. 2018;20(1):26-34.
99. Woo HD, Kim K, Kim J. Association between preoperative C-reactive protein level and colorectal cancer survival: a meta-analysis. Cancer causes & control : CCC. 2015;26(11):1661-70.
100. Rossi S, Basso M, Strippoli A, Schinzari G, D'Argento E, Larocca M, et al. Are Markers of Systemic Inflammation Good Prognostic Indicators in Colorectal Cancer? Clinical colorectal cancer. 2017;16(4):264-74.
101. Okugawa Y, Toiyama Y, Yamamoto A, Shigemori T, Ide S, Kitajima T, et al. Lymphocyte-C-reactive Protein Ratio as Promising New Marker for Predicting Surgical and Oncological Outcomes in Colorectal Cancer. Annals of surgery. 2019.
102. Des Guetz G, Uzzan B, Nicolas P, Cucherat M, Morere JF, Benamouzig R, et al. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. Br J Cancer. 2006;94(12):1823-32.
103. Peng Y, Wang L, Du C, Gu J. Expression of vascular endothelial growth factor can predict distant metastasis and disease-free survival for clinical stage III rectal cancer following 30-Gy/10-f preoperative radiotherapy. International journal of colorectal disease. 2012;27(12):1555-60.
104. Wang Y, Yao X, Ge J, Hu F, Zhao Y. Can vascular endothelial growth factor and microvessel density be used as prognostic biomarkers for colorectal cancer? A systematic review and meta-analysis. TheScientificWorldJournal. 2014;2014:102736.
105. Tsai HL, Yang IP, Lin CH, Chai CY, Huang YH, Chen CF, et al. Predictive value of vascular endothelial growth factor overexpression in early relapse of colorectal cancer patients after curative resection. International journal of colorectal disease. 2013;28(3):415-24.
106. De Vita F, Orditura M, Lieto E, Infusino S, Morgillo F, Martinelli E, et al. Elevated perioperative serum vascular endothelial growth factor levels in patients with colon carcinoma. Cancer. 2004;100(2):270-8.
107. White JD, Hewett PW, Kosuge D, McCulloch T, Enholm BC, Carmichael J, et al. Vascular endothelial growth factor-D expression is an independent prognostic marker for survival in colorectal carcinoma. Cancer Res. 2002;62(6):1669-75.

108. Hede P, Sorensson MA, Polleryd P, Persson K, Hallgren T. Influence of BMI on short-term surgical outcome after colorectal cancer surgery: a study based on the Swedish national quality registry. International journal of colorectal disease. 2015;30(9):1201-7.
109. Park SW, Lee HL, Doo EY, Lee KN, Jun DW, Lee OY, et al. Visceral Obesity Predicts Fewer Lymph Node Metastases and Better Overall Survival in Colon Cancer. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2015;19(8):1513-21.
110. Cakir H, Heus C, van der Ploeg TJ, Houdijk AP. Visceral obesity determined by CT scan and outcomes after colorectal surgery; a systematic review and meta-analysis. International journal of colorectal disease. 2015;30(7):875-82.
111. Kim JY. Impact of Visceral Fat Area in Colorectal Surgery. Annals of coloproctology. 2016;32(1):3-4.
112. Park BK, Park JW, Ryoo SB, Jeong SY, Park KJ, Park JG. Effect of Visceral Obesity on Surgical Outcomes of Patients Undergoing Laparoscopic Colorectal Surgery. World journal of surgery. 2015;39(9):2343-53.
113. Seki Y, Ohue M, Sekimoto M, Takiguchi S, Takemasa I, Ikeda M, et al. Evaluation of the technical difficulty performing laparoscopic resection of a rectosigmoid carcinoma: visceral fat reflects technical difficulty more accurately than body mass index. Surgical endoscopy. 2007;21(6):929-34.
114. Brown SR, Mathew R, Keding A, Marshall HC, Brown JM, Jayne DG. The impact of postoperative complications on long-term quality of life after curative colorectal cancer surgery. Annals of surgery. 2014;259(5):916-23.
115. Macarthur DC, Nixon SJ, Aitken RJ. Avoidable deaths still occur after large bowel surgery. Scottish Audit of Surgical Mortality, Royal College of Surgeons of Edinburgh. The British journal of surgery. 1998;85(1):80-3.
116. Warschkow R, Tarantino I, Torzewski M, Naf F, Lange J, Steffen T. Diagnostic accuracy of C-reactive protein and white blood cell counts in the early detection of inflammatory complications after open resection of colorectal cancer: a retrospective study of 1,187 patients. International journal of colorectal disease. 2011;26(11):1405-13.
117. Welsch T, Muller SA, Ulrich A, Kischlat A, Hinz U, Kienle P, et al. C-reactive protein as early predictor for infectious postoperative complications in rectal surgery. International journal of colorectal disease. 2007;22(12):1499-507.
118. Sridharan P, Chamberlain RS. The efficacy of procalcitonin as a biomarker in the management of sepsis: slaying dragons or tilting at windmills? Surgical infections. 2013;14(6):489-511.
119. Hubner M, Mantziari S, Demartines N, Pralong F, Coti-Bertrand P, Schafer M. Postoperative Albumin Drop Is a Marker for Surgical Stress and a Predictor for Clinical Outcome: A Pilot Study. Gastroenterology research and practice. 2016;2016:8743187.
120. Wierdak M, Pisarska M, Kusnierz-Cabala B, Witowski J, Dworak J, Major P, et al. Changes in plasma albumin levels in early detection of infectious complications after laparoscopic colorectal cancer surgery with ERAS protocol. Surgical endoscopy. 2018;32(7):3225-33.
121. Pedersen T, Roikjaer O, Jess P. Increased levels of C-reactive protein and leukocyte count are poor predictors of anastomotic leakage following laparoscopic colorectal resection. Danish medical journal. 2012;59(12):A4552.
122. Almeida AB, Faria G, Moreira H, Pinto-de-Sousa J, Correia-da-Silva P, Maia JC. Elevated serum C-reactive protein as a predictive factor for anastomotic leakage in colorectal surgery. International journal of surgery. 2012;10(2):87-91.
123. Munoz JL, Alvarez MO, Cuquerella V, Miranda E, Pico C, Flores R, et al. Procalcitonin and C-reactive protein as early markers of anastomotic leak after laparoscopic colorectal surgery within an enhanced recovery after surgery (ERAS) program. Surgical endoscopy. 2018;32(9):4003-10.

124. Lagoutte N, Facy O, Ravoire A, Chalumeau C, Jonval L, Rat P, et al. C-reactive protein and procalcitonin for the early detection of anastomotic leakage after elective colorectal surgery: pilot study in 100 patients. Journal of visceral surgery. 2012;149(5):e345-9.
125. Oberhofer D, Juras J, Pavicic AM, Rancic Zuric I, Rumenjak V. Comparison of C-reactive protein and procalcitonin as predictors of postoperative infectious complications after elective colorectal surgery. Croatian medical journal. 2012;53(6):612-9.
126. Tan WJ, Ng WQ, Sultana R, de Souza NN, Chew MH, Foo FJ, et al. Systematic review and meta-analysis of the use of serum procalcitonin levels to predict intra-abdominal infections after colorectal surgery. International journal of colorectal disease. 2018;33(2):171-80.
127. Facy O, Paquette B, Orry D, Santucci N, Rat P, Rat P, et al. Inflammatory markers as early predictors of infection after colorectal surgery: the same cut-off values in laparoscopy and laparotomy? International journal of colorectal disease. 2017;32(6):857-63.
128. Su'a B, Tutone S, MacFater W, Barazanchi A, Xia W, Zeng I, et al. Diagnostic accuracy of procalcitonin for the early diagnosis of anastomotic leakage after colorectal surgery: a meta-analysis. ANZ journal of surgery. 2019.
129. Zheng S, Han MY, Xiao ZX, Peng JP, Dong Q. Clinical significance of vascular endothelial growth factor expression and neovascularization in colorectal carcinoma. World J Gastroenterol. 2003;9(6):1227-30.
130. National Comprehensive Cancer Network. Rectal Cancer (version 2.2020) 2020 [Available from: https://www.nccn.org/professionals/physician_gls/pdf/rectal.pdf.
131. Bruhn MA, Townsend AR, Khoon Lee C, Shivasami A, Price TJ, Wrin J, et al. Proangiogenic tumor proteins as potential predictive or prognostic biomarkers for bevacizumab therapy in metastatic colorectal cancer. International journal of cancer. 2014;135(3):731-41.
132. Hegde PS, Jubb AM, Chen D, Li NF, Meng YG, Bernaards C, et al. Predictive impact of circulating vascular endothelial growth factor in four phase III trials evaluating bevacizumab. Clin Cancer Res. 2013;19(4):929-37.
133. Jubb AM, Hurwitz HI, Bai W, Holmgren EB, Tobin P, Guerrero AS, et al. Impact of vascular endothelial growth factor-A expression, thrombospondin-2 expression, and microvessel density on the treatment effect of bevacizumab in metastatic colorectal cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2006;24(2):217-27.