ORIGINAL ARTICLE

Side-effects related to adjuvant CAPOX treatment for colorectal cancer are associated with intermuscular fat area, not with total skeletal muscle or fat, a retrospective observational study

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Abstract

Aims Chemotherapeutic treatment is regularly accompanied by side-effects. Hydrophilic chemotherapeutics such as capecitabine and oxaliplatin (CAPOX), often used in colorectal cancer treatment, predominantly accumulate in non-adipose tissues. Therefore the aim of this paper was to investigate whether body composition and fat infiltration in the muscle (muscle attenuation and intermuscular-adipose-tissue [IMAT] content) are associated with chemotherapy-induced toxicities.

Methods In this retrospective observational study, we collected data from 115 colorectal cancer patients receiving adjuvant CAPOX chemotherapy between 2006 and 2015. Information on cancer characteristics were obtained from the Netherlands Cancer Registry. Diagnostic CT scans were retrieved to assess cross-sectional areas of skeletal muscle and adipose tissue at the third lumbar vertebrae. Information on dose-limiting toxicity [DLT] and relative administered dose (as % of BSA-based-planned-dose) were retrieved from medical charts. Associations between body composition, muscle quality and chemotherapy-induced toxicities were determined using Cox-regression and linear-regression analyses.

Results We found that DLT incidence was 90% in our cohort: 50% had their dose reduced, 30% their next cycle postponed, 4% a full treatment stop and 6% was hospitalized at their first DLT. Most common were reductions in oxaliplatin dose whilst keeping the capecitabine dose constant. Cox regression analysis indicated no association between body composition or muscle quality and DLT during the first treatment cycle or time to the first DLT. Multiple linear regression showed that higher IMAT-index and IMAT muscle percentage were associated with a lower relative administered dose of oxaliplatin.

Conclusions In conclusion; only IMAT, not skeletal or fat area was associated with dose-limiting toxicities among these CRC patients who received CAPOX treatment.

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Introduction

Surgical removal of the tumour is the standard treatment of colorectal cancer (CRC). Depending on the stage of cancer, this may be followed by adjuvant chemotherapy. The therapeutic window of most drugs used in adjuvant therapy is very narrow making underdosing (leading to poor antitumor effects) and overdosing (leading to toxicities) common problems [1]. Severe adverse drug reactions can necessitate dose reduction, postponement of the next treatment cycle, hospitalization or even termination of treatment.

Most CRC patients on chemotherapy receive a combination of different drugs, usually a fluorouracil (FU) based and platinum based drug (e.g. capecitabine and oxaliplatin (CAPOX)). Capecitabine is a highly water-soluble pro-drug which is enzymatically metabolized into the anti-metabolite fluorouracil (FU) in the body [2]. This conversion is performed by thymidine phosphorylase found in several tumour tissues at relatively high levels. The cytotoxic effects of FU are

due to incorporation of its metabolites into the DNA and RNA or inhibition of thymidine synthesis (crucial for DNA synthesis) [2]. Oxaliplatin is a platinum coordination complex, which undergoes a series of spontaneous, non-enzymatic conversions in the body. It can form several reactive species which form complexes with amino acids, proteins, DNA and other macromolecules in plasma and tissues [3]. Platinum-DNA adducts disrupt DNA replication and transcription [4]. In clinical practice, the chemotherapeutic dose is based on a patient's body surface area BSA, calculated with the Mosteller [5] formula:

$$\mathrm{BSA}\ (\mathrm{m}^2) = \sqrt{\mathrm{height}\ (\mathrm{cm}) \times \mathrm{weight}\ (\mathrm{kg}) / 3600}$$

As can be seen from this formula, only height and weight are used as input, and body composition is not taken into account. Considering the pharmacokinetic determinants of capecitabine and oxaliplatin (i.e. water solubility and binding to amino acids, proteins and DNA), it is expected that both will distribute mainly over the lean body (fat-free) compartments of the patient. Therefore, not taking skeletal muscle mass into account, could lead to under- or overdosing.

Body composition can be determined by measuring the total surface area of muscle, visceral fat, subcutaneous fat and intermuscular fat in a single slice CT scan analysis at L3 level [6]. Commonly, muscle mass is expressed as skeletal muscle index (SMI [cm^2/m^2]; muscle surface area indexed for length) [6]. Shen et al. reported that single slice CT scan tissue areas at L3 level are a good indicator for whole body fat and muscle volume as determined by MRI [7]. Mourtzakis et al. found that CT derived muscle surface at L3 was strongly related to whole-body fat free mass measured by DXA making it possible to estimate lean body mass (LBM) [6]. Previous research suggests an association between a low muscle mass and a decreased survival rate in colorectal cancer patients [8]. Moreover, low lean body mass or muscle mass have been associated with a higher risk of chemotherapy-induced toxicities in colorectal cancer patients [9–12]. In these studies different methods to assess lean body mass were used and for these different outcomes, different cut-off points for low lean mass were used. The study of Jung [10], for example, focussed on the cross-sectional area of the psoas muscle instead of the more common measurement of abdominal muscles at L3 level. Additionally these studies included patients with different diseases and therapeutic backgrounds. For example, the study of Barrett [9] included metastatic colorectal cancer patients, while others focussed on patients with a primary colorectal tumour [10–12]. Some studies included patients on 5-FU-based chemotherapy [10,12], while others included patients on multiple different treatment regiments [9,11]. Despite the differences in design of the studies, outcomes confirm each-other and therefore, there is an overall consensus that low SMI is prognostic of chemo-therapy induced toxicity outcomes [13]. However, most studies focus on SMI or lean body mass only, without taking the fat mass, or muscle to fat ratio into account. Moreover, no study has been performed to assess the association between low muscle mass and toxicities in CRC patients receiving adjuvant CAPOX treatment.

Besides skeletal muscle mass, fat infiltration in the muscle might influence adjuvant chemotherapy. Fat infiltration can be assessed by studying the radio density of muscle tissue (muscle attenuation), which is considered a measure of intramyocellular lipid droplets in the muscle [14], and by assessing intermuscular adipose tissue areas [6] (IMAT) using CT scans. Low muscle attenuation was found to be associated with poor prognosis in metastatic renal cell carcinoma [15]. It was also found to be associated with high-grade adverse events during immunotherapy treatment in metastatic melanoma patients [16]. Moreover, IMAT has been shown to be associated with an altered systemic inflammatory response in patients with primary operable CRC [17]. At this moment the role of low muscle attenuation or IMAT has not been studied in the context of CAPOX induced toxicities.

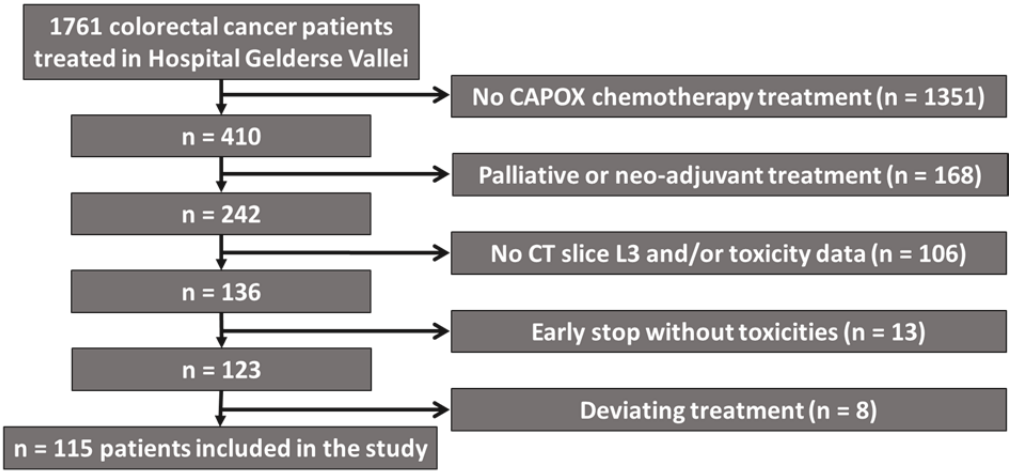
Therefore, the aim of the current study was to investigate the association between body composition, muscle attenuation and IMAT content and chemotherapy-induced toxicities in a retrospective cohort among colorectal cancer patients receiving adjuvant CAPOX treatment. Moreover, we aimed to get insight into the prognostic relevance of treatment toxicities for the patient. Therefore, the secondary aim of this study was to explore whether high dose reductions or an early stop of the treatment were associated with survival.

Methods

Patient characteristics/inclusion

Patients treated with chemotherapy for CRC were selected from all CRC patients treated in hospital Gelderse Vallei (Ede, The Netherlands), a peripheral mid-sized hospital, located centrally in the Netherlands, between June 2006 and July 2015. Information on cancer location, stage and treatment were obtained from the Netherlands Cancer Registry, data on chemotherapy toxicity and survival were extracted from medical records. Inclusion criteria were: adjuvant treatment with CAPOX chemotherapy; availability of abdominal CT scan made within 100 days before start of chemotherapy treatment; and availability of toxicity data. Patients were excluded from the study if their treatment was stopped prematurely without experiencing toxicities or if the patient did not receive the standard chemotherapeutic dose due to a dihydropyrimidine dehydrogenase deficiency (a key enzyme involved in the metabolism of capacetabine) (n=8) or comorbidities. A large part of the patients was excluded from the cohort as they did not receive chemotherapy treatment because of the stage of their cancer (n = 1351, 77%). Of the 410 CRC patients who received CAPOX treatment, 168 were excluded because they had a palliative or neo-adjuvant treatment regime, 106 were excluded for missing data, 13 for an early stop of treatment without toxicities and 8 for deviating treatment without toxicities. As a result, we included 115 patients in this retrospective study (Figure 1).

Figure 1 Flow-chart of colorectal cancer patients, from hospital Gelderse Vallei (Ede, The Netherlands), included in this retrospective study.



Treatment protocol

The CAPOX dose that was administered was based on the BSA calculated with the Mosteller formula [5]. The treatment protocol consisted of eight cycles of 21 days. On day 1, patients were planned to receive 130 mg/m^2 of oxaliplatin. On day 1 to day 14, patients were planned to receive 1000 mg/m^2 of capecitabine twice daily, and from day 15 to 21 patients had a resting period.

Body composition

Cross-sectional areas of skeletal muscle and fat tissue were determined in single slice CT scans (made for diagnostic purposes) at the third lumbar vertebra (L3) as described by others [6]. Image analysis software was used for the analysis of the CT slices (SliceOmatic version 5.0, Tomovision). Different tissue areas were characterised based on Hounsfield units (HU) [6]; HU of -190 to -30 represent intermuscular adipose tissue (IMAT) and subcutaneous adipose tissue (SAT), -150 to -50 visceral adipose tissue (VAT), and -29 to 150 skeletal muscle tissue (SM). Muscle attenuation (MA) was determined by taking the mean HU in the entire skeletal muscle area at the third lumbar vertebra. Total adipose tissue (TAT) was defined as IMAT + VAT + SAT. SM and IMAT were indexed by height (in m^2) and named skeletal muscle index (SMI) and IMAT index, respectively. Furthermore, the following relative values were calculated: Muscle percentage (SM area as a percentage of total area of SM + TAT at L3 level) and IMAT muscle

percentage (IMAT area as a percentage of muscle + IMAT area at L3 level). BSA divided by the LBM was used as measure for the chemotherapeutic dose per kg LBM at the start of the treatment. Whole lean body mass was determined using the following formula [6]: $LBM\ (kg) = (SM \times 0.3) + 6.06$. Sarcopenia was determined using sex and BMI specific cut-offs which have been shown to be significantly negatively associated with mortality in patients with solid tumours determined by Martin et al. (i.e. 41 cm^2/m^2 for women, 43 cm^2/m^2 for men with a BMI < 25 kg/m^2 and 53 cm^2/m^2 for men with a BMI ≥ 25 kg/m^2) [18]. CT-scans were analysed by 3 different researchers who were trained to quantify muscle mass, intermuscular adipose tissue, visceral adipose tissue and subcutaneous adipose tissue at the L3 level. The inter-observer variation was 1.43% for SM, 6.15% for IMAT, 4.74% for VAT and 2.82% for SAT.

Chemotherapy-induced toxicities and survival

Side-effects and survival data were extracted from patients' medical records. Administered dose, deviation from treatment cycle and chemotherapeutic side effects were scored for each treatment cycle. Toxicities per chemotherapy cycle were defined as dose limiting toxicity (DLT) if there was: 1) dose reduction, 2) cycle delay, 3) cycle stop or 4) hospitalization because of side-effects. From these data we defined our output parameters: 1) First cycle DLT, which is a DLT during/resulting from the first cycle of chemotherapy 2) time to first DLT (expressed in cycles) and 3) the total relative administered dose of oxaliplatin and capecitabine (total administered dose as % of the BSA-based planned dose). A low total relative administered dose implies that the patient experienced more frequent, or more severe toxicities compared to a high relative administered dose. Survival was defined as time between start of chemotherapy treatment and date of death. Patients with no record of death were censored on October 20, 2016.

Data-analysis

Prevalence ratios were calculated to assess the association between body composition and DLT (yes/no) using the Cox regression model with time as a fixed variable. Hazard ratios were calculated to assess the association between body composition and the time to the first dose-limiting toxicity using the Cox regression model with time expressed as the number of treatment cycles. Regression coefficients were calculated to assess the association between body composition and relative administered dose using a multiple linear regression model. All body composition parameters were assessed as continuous variables. In addition, BMI and sarcopenia were included as categorical variables. A separate model was constructed for each body composition parameter. Next to the separate models, we also made a multivariate model containing all CT-scan derived measures.

To explore the effect of dose reduction on survival, hazard ratios were calculated for patients receiving less than the median relative administered dose vs those receiving the median dose or more, using Cox regression.

For all analyses, the factors age and gender were added to each model-based on associations found in literature [19]. Moreover, factors BMI and tumour stage were tested for possible confounding.

Results

Patients' characteristics and toxicity incidence

Slightly more men than women were included in the study (54%) (Table 1). The mean age at CRC diagnosis was 61.9 years (± 9.4 SD). The majority of the patients were diagnosed with tumour stage III (84%). There were no patients with tumour stage I receiving chemotherapy; tumour stage II and IV were present in 9% and 7% of the study population, respectively. More than half of the patients were overweight [BMI 25-29] or obese [BMI>30] (57%) at diagnosis.

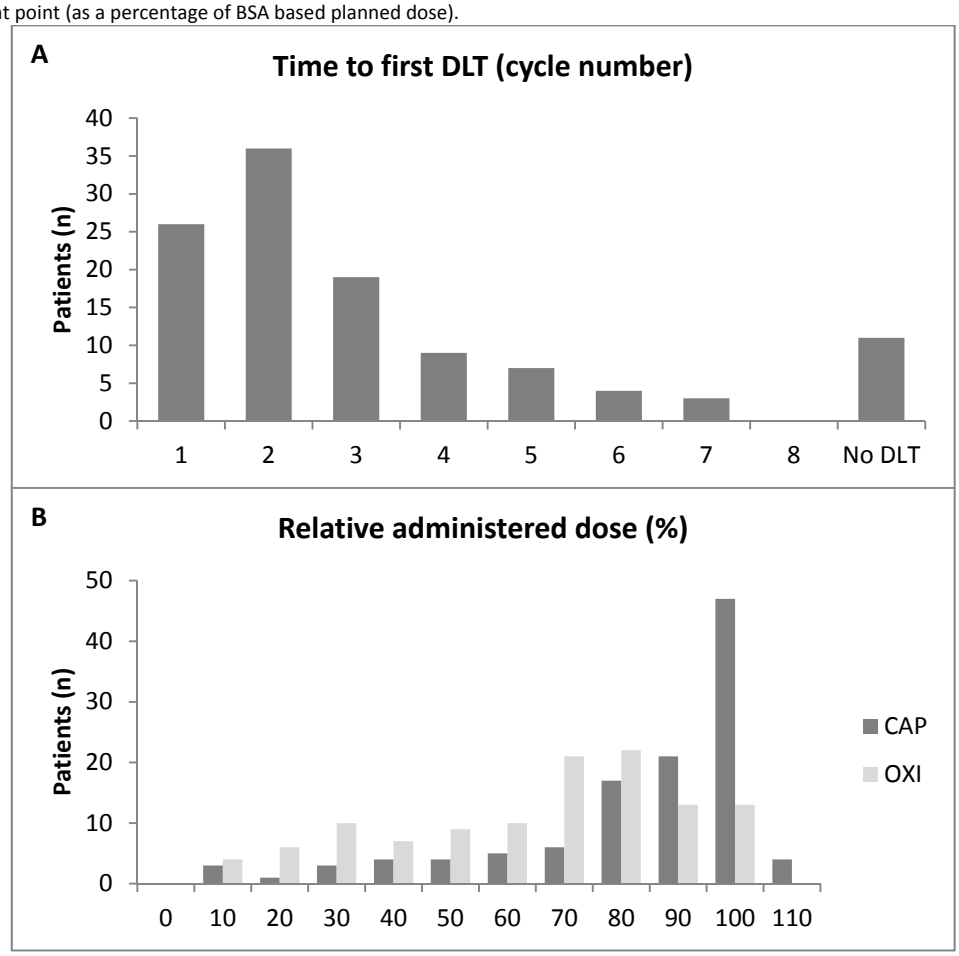
DLT incidence was high with 90% of all patients experiencing a DLT (Figure 2A). Half of the patients (50%) had their dose reduced, 30% had their next cycle postponed, 4% had a full treatment stop and 6% was hospitalized because of side-effects at their first DLT. The relative administered dose of capecitabine and oxaliplatin varied greatly between patients (Figure 2B). Most commonly, oxaliplatin was reduced, less often capecitabine. In some instances, capecitabine dosis was even increased slightly leading to relative administered doses of > 100%. However, it has to be noted that these relative doses cannot be seen as completely

independent outcome measures since a full stop leads to a low relative administered dose of both drugs. Unfortunately, we were not able to determine whether such a stop was caused by toxicities by either one or both of the drugs. In the analyses of relative administered dose, we therefore only focussed on oxaliplatin. In none of the performed analyses, BMI or tumour stage affected the outcome of the analysis, therefore all models were only adjusted for sex and age.

Table 1 Patient characteristics

Patient Characteristics	Total	DLT cycle 1	No DLT cycle 1
Number of patients	**115**	**26**	**89**
Age	**61.9 ± 9.4**	**64.4 ± 9.4**	**61.2 ± 9.4**
Sex			
Male	**62 [54]**	**14 [54]**	**48 [54]**
Female	**53 [46]**	**12 [46]**	**41 [46]**
Tumour stage (n [%])			
Stage I	**0 [0]**	**0 [0]**	**0 [0]**
Stage II	**10 [9]**	**3 [12]**	**7 [8]**
Stage III	**97 [84]**	**22 [85]**	**75 [84]**
Stage IV	**8 [7]**	**1 [4]**	**7 [8]**
Cancer site (n [%])			
Colon	**105 [91]**	**25 [96]**	**80 [90]**
Rectum	**10 [9]**	**1 [4]**	**9 [10]**
BMI (n [%])			
Underweight	**2 [2]**	**0 [0]**	**2 [2]**
Normal weight	**48 [42]**	**11 [42.3]**	**37 [42]**
Overweight	**44 [38]**	**11 [42.3]**	**33 [37]**
Obese	**21 [18]**	**4 [15.4]**	**17 [19]**
Sarcopenia (n [%])			
Non Sarcopenic	**75 [65]**	**19 [73]**	**56 [63]**
Sarcopenic	**40 [35]**	**7 [27]**	**33 [37]**
Sarcopenic Overweight (n [%])			
Non Sarcopenic/Non Overweight	**30 [26]**	**8 [31]**	**22 [25]**
Non Sarcopenic/Overweight	**45 [39]**	**11 [42]**	**34 [38]**
Sarcopenic/Non Overweight	**20 [17]**	**3 [12]**	**17 [19]**
Sarcopenic/Overweight	**20 [17]**	**4 [15]**	**16 [18]**
Skeletal Muscle Index (cm^2/m^2)	**47.3 ± 7.8**	**47.9 ± 7.0**	**47.1 ± 8.1**
TAT Surface (VAT+SAT+IMAT, cm^2)	**353.8 ± 171.2**	**357.7 ± 173.4**	**352.6 ± 171.5**
Muscle Percentage (%)	**32.7 ± 14.4**	**33.3 ± 16.6**	**32.5 ± 13.8**
BSA / LBM (cm^2/kg)	**399.9 ± 62.3**	**392.8 ± 54.3**	**402.0 ± 64.6**
Muscle Attenuation (HU)	**35.0 ± 7.8**	**34.2 ± 8.3**	**35.3 ± 7.7**
IMAT Index (cm^2/m^2)	**4.9 ± 3.4**	**5.7 ± 4.1**	**4.7 ± 3.2**
IMAT Muscle Percentage (%)	**9.2 ± 5.5**	**10.3 ± 5.8**	**8.9 ± 5.4**

Figure 2A number of people experiencing first DLT per cycle. **Figure 2B** number of people receiving relative administered dose rounded to the closest 10th percent point (as a percentage of BSA based planned dose).



Dose limiting toxicities in the first cycle of chemotherapy, and time to first DLT

None of the indicators of body composition were associated with DLT during the first cycle of chemotherapy, as indicated by the prevalence ratios in Table 2. Moreover, the hazard ratios in table 2 show that none of the indicators of body composition was associated with time to first DLT. These indicators included BMI, the body composition parameters SMI and muscle percentage, the muscle fat infiltration parameters MA and IMAT and the dose per lean body mass.

Dose limiting toxicities expressed as the relative administered dose

Muscle percentage, IMAT index and IMAT muscle percentage were log transformed to comply with assumptions of the regression analysis. Regression coefficients indicated that a higher IMAT index was associated with a lower relative administered dose of oxaliplatin both in the model with only the IMAT index, as well as in the multivariate model containing all CT-scan derived body composition variables. A higher IMAT muscle percentage was associated with a lower relative administered dose of oxaliplatin. No associations were observed for any of the other parameters and relative administered dose of oxaliplatin (Table 3).

For visualisation of the results, two heat-map plots were produced. The first one shows the relative administered dose of oxaliplatin plotted against BMI and SMI. The figure shows scattered data indicating no relation between plotted parameters (Figure 3A). The second plot shows the relative administered dose plotted against BMI and IMAT muscle percentage, showing a clear pattern over the IMAT-SM percentage axis illustrating the association found with the multiple linear regression (Figure 3B).

Table 2 Association between body composition parameters and first cycle DLT or time to first DLT. Prevalence and Hazards ratios (including 95% CI) determined using COX Proportional Hazards modelling, adjusting for sex and age.

Variable	First Cycle DLT PR	(95% CI)		Time to first DLT HR	(95% CI)	
BMI	1.00	0.91	1.10	1.01	0.97	1.06
Skeletal Muscle Index (cm^2/m^2)	1.02	0.96	1.08	1.01	0.98	1.04
TAT Surface (VAT+SAT+IMAT, cm^2)	1.00	1.00	1.00	1.00	1.00	1.00
Muscle Percentage (%)	1.01	0.98	1.04	1.00	0.98	1.01
BSA / LBM (cm^2/kg)	1.00	0.99	1.00	1.00	1.00	1.00
Muscle Attenuation (HU)	1.00	0.95	1.06	1.00	0.97	1.02
IMAT Index (cm^2/m^2)	1.03	0.93	1.15	1.04	0.98	1.10
IMAT Muscle Percentage (%)	1.02	0.95	1.10	1.03	0.99	1.07
BMI						
Underweight	*n<1*			0.35	0.05	2.56
Normal weight	*ref*			*ref*		
Overweight	1.17	0.50	2.74	0.89	0.57	1.40
Obese	0.85	0.27	2.72	0.97	0.57	1.67
Sarcopenia						
No	*ref*			*ref*		
Yes	0.67	0.28	1.60	1.14	0.76	1.72
Sarcopenic Overweight (n [%])						
Non Sarcopenic/Non Overweight	*ref*			*ref*		
Non Sarcopenic/Overweight	0.91	0.34	2.44	0.93	0.54	1.61
Sarcopenic/Non Overweight	0.52	0.13	2.02	1.07	0.57	2.02
Sarcopenic/Overweight	0.78	0.23	2.60	1.12	0.60	2.08

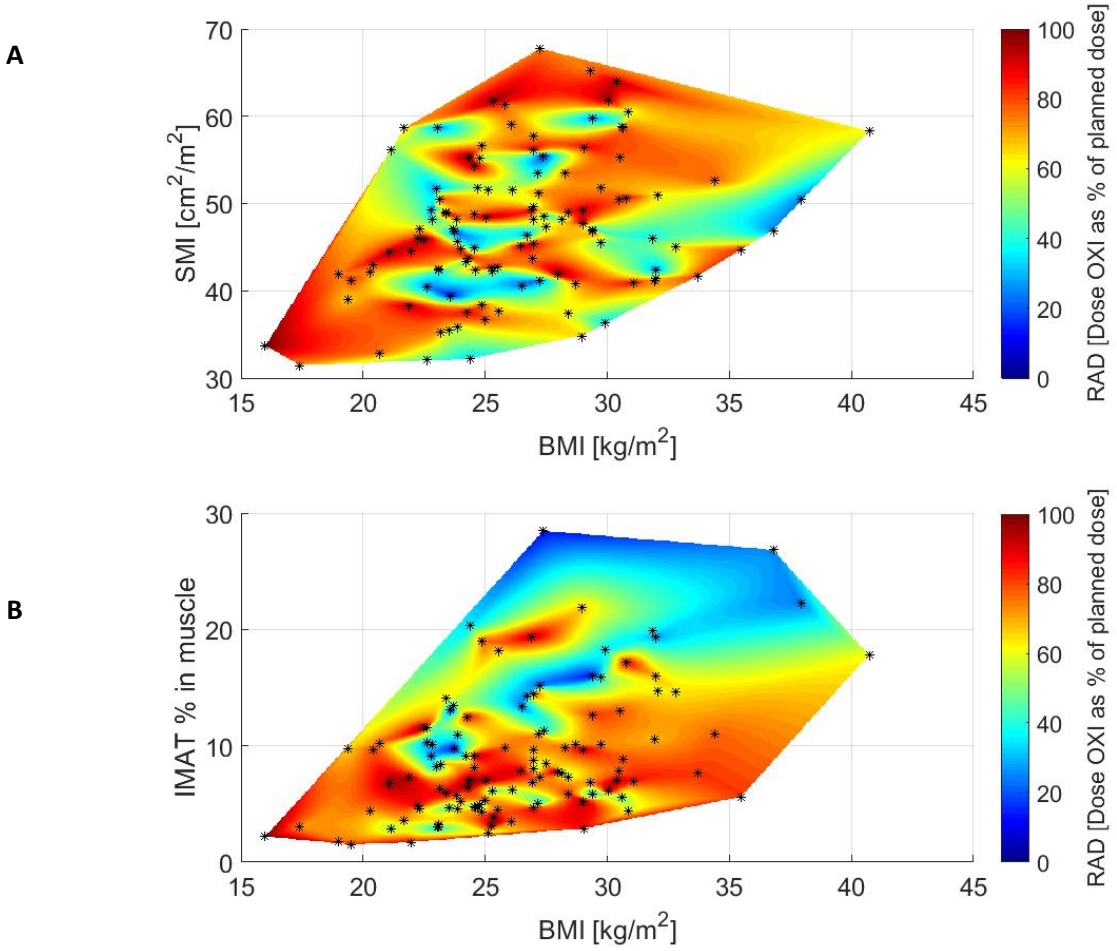
Multivariate Variable	TOTAL PR	(95% CI)		TOTAL HR	(95% CI)	
Skeletal Muscle Index (cm^2/m^2)	1.01	0.95	1.08	1.00	0.97	1.03
VAT Surface (cm^2)	1.00	1.00	1.01	1.00	1.00	1.00
SAT Surface (cm^2)	1.00	0.99	1.01	1.00	1.00	1.00
Muscle Attenuation (HU)	1.05	0.91	1.22	1.05	0.96	1.14
IMAT Index (cm^2/m^2)	0.75	0.23	2.41	1.01	0.98	1.04

Table 3: Association between body composition parameters and the relative administered dose of oxaliplatin.

	Relative Administered Dose OXI		
Variable	B	(95% CI)	
BMI	-0.38	-1.43	0.67
Skeletal Muscle Index (cm^2/m^2)	-0.18	-0.84	0.48
TAT Surface (VAT+SAT+IMAT, cm^2)	-0.02	-0.04	0.01
Muscle Percentage (%)$^+$	12.77	-15.95	41.49
BSA / LBM (cm^2/kg)	0.04	-0.06	0.14
Muscle Attenuation (HU)	0.11	-0.51	0.73
IMAT Index (cm^2/m^2)$^+$	*-17.04**	*-32.62*	*-1.45*
IMAT Muscle Percentage (%)$^+$	*-17.92**	*-35.64*	*-0.21*
BMI			
Underweight	22.08	-11.34	55.49
Normal weight	*ref.*		
Overweight	1.51	-8.25	11.28
Obese	4.26	-7.96	16.49
Sarcopenia			
No	*ref.*		
Yes	1.19	-7.87	10.26
Sarcopenic Overweight (n [%])			
Non Sarcopenic/Non Overweight	*ref.*		
Non Sarcopenic/Overweight	3.99	-7.49	15.47
Sarcopenic/Non Overweight	4.80	-9.01	18.68
Sarcopenic/Overweight	2.44	-11.05	15.92
Multivariate			
Variable			
Skeletal Muscle Index (cm^2/m^2)	0.11	-0.60	0.82
VAT Surface (cm^2)	-0.01	-0.08	0.05
SAT Surface (cm^2)	0.02	-0.04	0.09
Muscle Attenuation (HU)	-0.64	-1.48	0.20
IMAT Index (cm^2/m^2)$^+$	*-30.89**	*-56.65*	*-5.14*

Beta's (including 95% CI) determined using the Multiple Linear Regression model. All betas were adjusted for sex and age. $^+$Data is log-transformed * $p<0.05$

Figure 3 heat map indicating the relative administered dose plotted against **A.** SMI/BMI and **B.** IMAT-SM Percentage/BMI. Asterisks indicate patients. Figure **A** shows a clearly scattered pattern whereas in figure **B** a gradient can be seen on the y-axis.



Dose reductions and survival

Two groups were made for the survival analysis; patients receiving equal or more than the median relative administered dose (71.7%) of oxaliplatin (n=60) and patients receiving less than the median administered dose (n=55). In these subgroups, survival was 76.7% (n=46) and 74.5% (n=41), respectively. Mean follow up time was 58.0 months (± 27.2 SD) and 48.7 months (± 28.8 SD), respectively. No association was found for a dose below median versus a dose equal or above median and overall survival (adjusted HR 1.08, 95% CI: 0.48 - 2.40).

Discussion

In this study we found no association between either low skeletal muscle mass, or relatively low skeletal muscle mass (relative to amount of fat) and CAPOX-induced toxicities. However, we found that during the course of their treatment program, patients with more intermuscular fat received a significantly lower percentage of the planned dose of oxaliplatin. This dose was planned based on the BSA of the patient at the start of treatment. This means that in our study, patients with more IMAT were likely to experience more often dose reduction or curtailment of their chemotherapy.

In this study, the incidence of toxicity was very high: 90% of patients apparently experienced serious side-effects. We found no associations between body composition and chemotherapy-induced toxicities during the first cycle of the treatment or the time to first DLT. Often, oxaliplatin dose was reduced whilst maintaining the capecitabine dose constant. To get an indication of

the consequence of dose reductions or early stop of the treatment, we studied the association between the relative administered dose of oxaliplatin and survival rate. No significant association between receiving a dose lower than the median relative administered dose and survival rate was observed.

Unlike previous studies, we did not find an association between low skeletal muscle mass and chemotherapy-induced toxicities in colorectal cancer patients [9–12]. There are three factors that may explain this discrepancy; 1) treatment protocol, 2) measurement/analysis methods and 3) population. Regarding 1), Treatment protocol; patients in this retrospective study received a combination of capecitabine and oxaliplatin, whereas some previously published studies applied other chemotherapeutics [10,12] or included multiple treatment strategies [9,11]. Possibly, the association between SMI and chemotherapy-induced toxicities is stronger in these other chemotherapeutic treatments than in the CAPOX treatment used in our study. With respect to 2), measurement/analysis methods: we abstracted toxicity data from medical records; in these records severity of side-effects was not systematically scored. As we did not have this information, we decided to focus on the information that was systematically recorded in the medical records including dose reduction, postponement or termination of treatment or hospitalization information. In our study, toxicity incidence was very high (91%) compared to studies (max 61.3% [12]) that did find an association between muscle mass and chemotherapy-induced toxicities in colorectal cancer patients. Possibly this is due to our way of toxicity recording; we recorded all DLT's where in other studies mild toxicities leading to small dose reductions or postponements might not be included. Furthermore, for determining muscle mass, not all studies use the same variable. In some studies, only the psoas muscle [10] was measured not taking the full muscle surface into account. Moreover, different studies use different cut-off levels for sarcopenia [9,11,12,18]. We decided to use those cut-offs points available in literature that we felt were most appropriate to use [18]. Those cut-off levels were defined in a Canadian population - which may differ with regards to lifestyle factors from our Dutch population. However, as patient characteristics in that study (age, BMI and body composition parameters) are similar to our patient characteristics, we felt it was reasonable to use those cut-offs. Another factor is that not all previous studies have used the same covariates to correct for potential confounding. Some use no correction for confounding, whereas others correct for multiple factors including tumour stage or obesity. We adjusted for sex and age in all our models based on previous findings published in literature [19]. Nevertheless, adjustment for additional confounding did not importantly affect our regression estimates. Regarding 3) study population; in our study, we included only patients receiving adjuvant chemotherapy who were predominantly classified as stage III colorectal cancer patients. This deviates from some other studies where predominantly [11] or only [9] stage IV colorectal cancer patients were included. Maybe the associations across different cancer stages differ. It is likely that patients classified stage IV will more often have cachexia where a decreased muscle mass is often accompanied by a general poor condition. Moreover, populations used in cited studies are Northern American, French and South Korean. Populations may differ in their physical fitness resulting from differences in lifestyle habits. Fitness may also be reflected by IMAT but this parameter was not commonly reported in earlier studies. To conclude, there are some differences between our study and the cited studies that might explain why we did not find an association between muscle mass and toxicities in our population. However, the exact cause for this difference in findings remains unclear.

Low muscle attenuation is associated with excess fat deposition in the tissue [20]. This has been shown to be independently associated with poor survival rates in a large cohort of patients with solid tumours of the lung and gastrointestinal tract [18]. However, in our study we did not find an association of muscle attenuation with chemotherapy-induced toxicities. To the best of our knowledge the association between IMAT and chemotherapy-induced toxicities has not been described earlier. In literature, IMAT is associated with adverse clinical outcomes like insulin resistance, loss of muscle strength and mobility dysfunction [21]. Possibly, the explanation for our finding that patients with more IMAT are prone to more frequent/more severe toxicities might be that having a high IMAT content is a generic marker for

poor health indicative for patients who are less able to cope with the impact of the treatment.

In our study we had limited statistical power to study the association between dose-limitations and survival, and we did not find indications that dose-limitation was associated with higher mortality. Previous research suggests that early discontinuation of FU-based chemotherapy is associated with increased mortality rate for stage III colorectal cancer patients (n=3,733) [22]. Another study indicates a trend that discontinuation of CAPOX treatment in an elderly colorectal cancer population is associated to lower crude 3-year recurrence-free and overall survival (n=191) [23]. However, these studies are not directly comparable to our study since we used a different measure for chemotherapy compliance as mentioned earlier.

Our study is the first to examine the association between the relative muscle mass (relative to fat mass) and fat infiltration in the muscle and CAPOX induced toxicities in colorectal cancer patients. This was done in a retrospective study with a homogeneous patient group. We only included colorectal cancer patients with the same treatment policy and dosing strategy. Due to the retrospective nature of the study, we had to work with data that were available in medical records. Although toxicity data were not scored in a standardized way in those records, the toxicity parameters used in our study (DLT and relative administered dose) are based on objective data (dosing, treatment timing and hospitalization), which will not be affected by the way they are reported. Strength of our outcome parameters is that these indicate several different aspects of the toxicities experienced by patients; 1) toxicity in the first cycle 2) the number of toxicities experienced at different points in the treatment (time to first DLT) and 3) the severity of toxicities (relative administered dose). Body composition was determined using L3 single slice CT scan analysis. CT scans are considered as the golden standard for body composition analysis, and L3 skeletal muscle tissue and total adipose tissue are strongly correlated with respectively LBM and total fat mass [7]. Moreover, a study on cadaver material showed a highly significant correlation between the amount of IMAT measured by CT scan and the actual amount of IMAT [24].

In conclusion, we could not confirm our hypothesis that relatively low skeletal muscle mass (relative to amount of fat) is associated with chemotherapy-induced toxicities in a retrospective study of CRC patients receiving adjuvant CAPOX treatment. At the same time, we did find that patients with more IMAT were more likely to receive a lower relative administered dose of oxaliplatin. In exploratory analyses, receiving a lower relative administered dose however was not associated with a decreased survival rate. If these results are confirmed in prospective studies, measurement of IMAT may emerge as a diagnostic tool indicating patients who are at increased risk of toxicities.

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Conflict of interest

All authors declare that they have no conflict of interest.

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