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DNA base oxidation in relation to TNM stages and chemotherapy treatment in colorectal cancer patients 2–9 months post-surgery

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A R T I C L E I N F O

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A B S T R A C T

Accumulation of DNA damage is a critical feature of genomic instability, which is a hallmark of various cancers. The enzyme-modified comet assay is a recognized method to detect specific DNA lesions at the level of individual cells. In this cross-sectional investigation, we explore possible links between clinicopathological and treatment related factors, nutritional status, physical activity and function, and DNA damage in a cohort of colorectal cancer (CRC) patients with non-metastatic disease. Levels of DNA damage in peripheral mononuclear blood cells (PBMCs) assessed 2–9 months post-surgery, were compared across tumour stage (localized (stage I-II) *vs.* regional (stage III) disease), localization (colon *vs.* rectosigmoid/rectum cancer), and adjuvant chemotherapy usage, with the last dosage administrated 2–191 days prior to sampling. Associations between DNA damage and indicators of nutritional status, physical activity and function were also explored. In PBMCs, DNA base oxidation was higher in patients diagnosed with regional compared with localized tumours ($P = 0.03$), but no difference was seen for DNA strand breaks ($P > 0.05$). Number of days since last chemotherapy dosage was negatively associated with DNA base oxidation ($P < 0.01$), and patients recently receiving chemotherapy (<15 days before blood collection) had higher levels of DNA base oxidation than those not receiving chemotherapy ($P = 0.03$). In the chemotherapy group, higher fat mass (in kg and %) as well as lower physical activity were associated with greater DNA base oxidation ($P < 0.05$). In conclusion, DNA base oxidation measured with the enzyme-modified comet assay varies according to tumour and lifestyle related factors in CRC patients treated for non-metastatic disease.

1. Introduction

Colorectal cancer (CRC) is one of the most common causes of cancer-related deaths worldwide [1]. Even though screening programs are running in several countries, the mortality rate is high, mainly due to late diagnosis and high number of relapses after primary therapy [2]. CRC is a heterogeneous disease with different molecular and clinico-pathological features. Treatment strategies include complete primary tumour resection and, frequently, radiotherapy (rectal carcinoma) and chemotherapy, primarily determined by tumour location and staging, including a histopathological examination [3,4]. At present, the tumour-node-metastasis (TNM) system is regarded as the standard approach for staging colorectal tumours [5].

Accumulation of DNA damage is a critical feature of genomic instability, which is a hallmark of various cancers [6]. Although cancer is a multi-factorial disease, a common consequence of exposure to

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several of the known cancer risk factors is oxidative stress, resulting from the presence of free radicals (reactive oxygen). In general, a certain level of reactive oxygen is essential for normal cellular function. However, an imbalance between reactive oxygen and antioxidant defence mechanisms may lead to DNA oxidation damage and genomic instability, thus increasing the chance of mutations in oncogenes and tumour suppressor genes [7]. Such damage to DNA, includes single- and double-strand breaks, abasic sites, oxidized DNA bases, and inter- and intrastrand cross-links [8]. DNA bases are sensitive to oxidation, particularly guanine due to its low redox potential. Consequently, a typical lesion formed under oxidative conditions is 8-oxoguanine (8-oxoG), which tends to mis-pair with adenine during replication, causing a G > T transversion and representing the most pro-mutagenic consequence of oxidative stress [9].

Oxidative stress and inflammation are closely related pathophysiological processes in the initiation and progression of CRC [5]. Additionally, interactions between tumour and host are important regulators of tumour progression [6,10,11]. Therefore, cancer progression is determined not only by factors intrinsic to the tumour, but also by multifaceted systemic processes [12]. Oxidative stress contributes to the maintenance of pro-tumorigenic signalling, where pathways related to avoidance of cell death, proliferation, angiogenesis, invasion, and metastasis are activated [13,14]. It appears that oxidative stress and inflammation associated with CRC progression cause increased 8-oxoG, but also affect repair enzyme activities, in both tumour and normal tissue, in complex ways [15,16].

Most chemotherapeutics generate reactive oxygen in target malignant cells, causing DNA damage and triggering apoptosis. These anticancer drugs also induce DNA damage in non-malignant cells (i.e., non-targeted cells), such as those of the gastrointestinal, haematological and neurological system, causing negative side effects. There is a large inter-individual variation in tolerance to chemotherapeutic agents [17] and growing evidence suggests that intrinsic biological factors such as drug metabolism, antioxidant, and DNA repair capacity are important [18]. In addition, it is likely that exogenous factors such as diet, nutritional status, and physical activity can have an impact on the biological processes that underpin both treatment response and tolerance [19–21].

The comet assay is a widely used method in human biomonitoring to measure DNA damage in isolated leucocytes or peripheral mononuclear blood cells (PBMCs) [22]. In the standard comet assay, DNA strand breaks and alkali-labile sites can be quantified, whereas employing an enzyme-modified version enables the measurements of oxidized DNA bases as well. In the past decade, the comet assay has emerged as an important tool in cancer research, including the evaluation of treatment response and adverse effects accompanying chemotherapy [23]. However, the majority of studies so far have investigated the association between DNA damage and cancer at the time of diagnosis, as well as before and immediately after chemotherapy treatment [24–28]. Additionally, only a few studies have included repair enzymes to identify specific DNA lesions such as oxidized bases [29–31]. To date, no studies have employed the enzyme-modified comet assay to investigate the long-term host response after curative treatment in CRC patients.

In the present explorative study, we aimed to assess whether DNA damage in PBMCs assessed 2–9 months post-surgery is associated with patient characteristics, clinicopathological- and treatment related factors or lifestyle.

2. Materials and methods

2.1. Patients and eligibility

The present cross-sectional study included CRC patients recruited from the ongoing randomized clinical trial, The Norwegian Dietary Guidelines and Colorectal Cancer Survival (CRC-NORDIET) study [32]. The baseline visit formed the basis for all analyses conducted in the present study. Eligible patients were women and men aged 50–80 years diagnosed with primary CRC (ICD-10 codes 18–20) and staged I-III according to the TNM staging system [5]. Patients with metastases were excluded. Patients had undergone surgery at Oslo University Hospital or Akershus University Hospital, 2–9 months prior to the baseline visit. Some patients were receiving adjuvant chemotherapy at baseline. Patients assigned to chemotherapy were either given monotherapy (5-fluorouracil (5-FU) plus folinic acid or capecitabine) or combination therapy (5-FU plus folinic acid and oxaliplatin, capecitabine plus oxaliplatin, or 5-FU plus irinotecan) according to national guidelines [33].

Eligible participants for the current study included patients from the CRC-NORDIET study who had available PBMCs prepared for comet assay analyses, collected during the baseline visit in the period from 2012 to 2020.

2.2. Patient information

Information about comorbidity, smoking status, and dietary supplement use at the baseline visit was self-reported. Details regarding the registration methods can be found elsewhere [32].

2.2.1. Clinicopathological and treatment related factors

Clinicopathological data (tumour stage and localization) as well as information on cancer treatment (time of colorectal surgery and chemotherapy usage) were obtained from electronic patient records. Stomy status (stoma/no-stoma) was recorded by the research staff at the baseline visit. For patients scheduled for chemotherapy, the following information was collected: type of chemotherapeutic drugs prescribed and administrated for each treatment cycle, duration of chemotherapy before blood sampling (i.e., start of the clinical trial), total days on chemotherapy, number of treatment cycles completed, and time from last chemotherapy dosage to blood sampling. Based on compliance to the chemotherapy protocol, patients were classified into those experiencing and those not experiencing dose-limiting toxicity (DLT), where DLT was defined as presence of either “administration of ≤80 % of total planned dose due to toxicity”, “treatment delay due to toxicity”, “withdrawal of oxaliplatin at any time”, or “premature cancelation of treatment”.

2.3. Nutritional status

Nutritional status was assessed by use of the Patient-Generated Subjective Global Assessment (PGS-GA), anthropometric measurements, and Dual-energy X-ray Absorptiometry (DXA).

2.3.1. Patient-Generated Subjective Global Assessment (PG-SGA)

The PG-SGA is widely used in clinical practice and academic research as a reference method for nutritional screening, assessment, monitoring, and triaging for interventions in cancer patients [34]. The assessment tool includes four patient-generated historical components (weight history, food intake, nutrition impact symptoms, and activity and function) and three professional components (age and diagnosis, metabolic stress, and physical examination). Based on an overall evaluation of these components, the patients are categorized as either well-nourished (PG-SGA A), moderately malnourished (PG-SGA B) or severely malnourished (PG-SGA C). Patients also receive a global numeric score. Although the PG-SGA category and numeric score are related, they are independent assessment and triage systems.

A validated Norwegian version of the PG-SGA (15-004 v10.13.16) was used in the present study [35]. The PG-SGA assessment was carried out by registered clinical dietitians or trained personnel.

2.3.2. Anthropometric measures

Body weight (kg) was measured by use of a non-slip Marsden M – 420 Digital Portable Floor Scale (Marsden, Rotherham, South Yorkshire, United Kingdom) or a digital wireless measuring station for height and weight, Seca 285 (Seca, Birmingham, United Kingdom). Height (cm)

was measured using either a mechanical height rod (Kern MSF-200) or a digital wireless stadiometer (Seca 285). Body mass index (BMI) (kg/m^2) was calculated using recorded weight and height, and patients were subsequently categorized into BMI groups based on age-specific cut-off values in accordance with national guidelines [36]. Abdominal obesity was assessed by measuring waist circumference (WC) at the midpoint between the top of the iliac crest and the lower margin of the last palpable rib. The cut-offs for being categorized with abdominal obesity were ≥88 cm for women and ≥102 cm for men [37].

2.3.3. Dual-energy X-ray absorptiometry (DXA)

Body composition was assessed using Lunar iDXA (GE Healthcare Lunar, Buckinghamshire, UK) with enCORE v18, which has previously demonstrated high validity and precision for CRC patients in the CRC-NORDIET study [38]. The following body compartments were used from the whole-body scan: body fat mass (FM), percentage of body fat (FM %), fat-free mass (FFM), and visceral adipose tissue (VAT). FFM-index was defined as FFM divided by the square of height in meters (kg/m^2). Patients were then categorized with "low FFM-index" (<15 kg/m^2 for women and <17 kg^2 for men) or "normal FFM-index" (≥15 kg/m^2 for women and ≥17 kg/m^2 for men), in accordance with the European Society for Clinical Nutrition and Metabolism (ESPEN) Consensus Statement [39]. Sex-specific cut-offs for increased percentage of body fat (FM %) were used to categorize patients as obese or non-obese. The cut-offs for being categorized as obese were ≥35 % for women and ≥25 % for men [37]. VAT values were used to calculate VAT-index (VAT (g)/height (m^2)).

2.4. Physical activity and function

2.4.1. Recording of daily physical activity

The physical activity monitor SenseWear Armband Mini (SWAM) (BodyMedia, Pittsburgh, Pennsylvania, USA) [40] was used to record daily physical activity and sedentary time during seven consecutive days. The SWAM has been validated for estimating total energy expenditure and demonstrated accuracy in measuring daily expenditure under free-living conditions [41]and has previously been tested in cancer population [42]. Moreover, shorter monitoring periods have been shown to provide reliable estimates of physical activity levels in cancer survivors when monitored continuously with wearable device [43]. Metabolic equivalents (METs) were calculated based on the accelerometer and temperature sensors through algorithms in the SWAM software. Total physical activity was calculated as the sum of light- (1.5–3 METs), moderate- (3–6 METs), and vigorous (≥6 METs) intensity level, while sedentary time was defined as all daily activity ≤1.5 METs. The armband was placed around the non-dominant arm and pre-programmed with co-predictors such as study ID number, age, sex, weight, height, and smoking status (smoker/non-smoker). Data recorded from the armbands were analyzed using the manufacturer's software (SenseWear Professional Software Version 7.0).

2.4.2. Physical performance tests

Handgrip strength. Handgrip strength of both hands were measured using a digital handheld dynamometer (KERN & SOHN GmbH, Ballingen, Germany). The maximal handgrip strength of three measurements was registered from each hand. Absolute handgrip strength was defined as the maximal handgrip strength, regardless of dominant or non-dominant hand. Low absolute handgrip strength was defined as < 16 kg and <27 kg in women and men, respectively [44]. Relative handgrip strength was defined as the absolute handgrip strength (kg)/total body mass (kg).

Sit-to-stand test. A 30 s sit-to-stand test was performed using a straight back chair with a solid seat at the height of 44 cm. From a sitting position, the patients were instructed to stand up and sit down as quickly and frequently as possible, keeping both arms folded across the chest. The number of stands for 30 seconds were counted.

6-min *walking test.* The patients were invited to an indoor 6-min walk test conducted in a long, flat, straight enclosed corridor with a hard surface (30 m). The total distance walked (in meters) during 6 min of time was recorded. The test is described in detail elsewhere [32].

2.5. Chemicals and materials

Lymphoprep was purchased from Axis-Shield Poc AS, dimethyl sulfone (DMSO) and phosphate buffered saline (PBS) from Sigma-Aldrich. Roswell Park Memorial Institute (RPMI) 1640 medium and fetal bovine serum (FBS) was purchased from Gibco™. Normal and low melting point (NMP and LMP) agarose as well as SYBR™ Gold Nucleic Acid Gel stain were purchased from Invitrogen. The photosensitizer Ro 19–8022 was provided by Hoffman-La Roche, and the lesion-specific enzyme formamidopyrimidine DNA glycosylase (Fpg) was produced by Norgenotech AS, Norway.

2.6. Blood collection, processing, and storage

All samples used in the study were collected in the fasting state. Venous blood was collected into Vacutainer® tubes containing citrate as anticoagulant. Citrate tubes were centrifuged for 15 min at 2500×*g*, and two buffy coats from each patient were resuspended in 3 ml PBS. The mixture of buffy coats and PBS was carefully layered onto 3 ml Lymphoprep (Axis-Shield) in a 15 ml centrifuge tube. The tube was then centrifuged for 25 min at 400×*g* with no brake. PBMCs were isolated by transferring the cloudy band above the Lymphoprep into a 15 ml centrifuge tube and diluted with PBS to 10 ml. The tubes were centrifuged for 7 min at 400×*g* with normal brake. The supernatant was decanted, and the cells suspended in 14 ml PBS. The cells were from that point kept on ice. A sample was taken for a cell count before centrifuging again for 7 min at 400×*g* (4 °C). The pelleted PBMCs were resuspended in 2 ml freezing medium (RPMI with 10 % FBS and 10 % DMSO) at 10^6 cells/ml and divided into three aliquots, which were frozen slowly to – 80 °C.

2.7. DNA damage

The standard comet assay, modified with a lesion-specific endonuclease, was performed according to Azqueta et al. [45], with slight modifications. Recommended assay controls were prepared from a single batch of PBMCs, either untreated (negative controls) or treated with photosensitizer Ro 19-8022 plus light to induce 8-oxoguanine (positive controls) [46]. The assay controls were slowly frozen in a large number of aliquots in freezing medium and stored at - 80 °C; controls were included in each experiment.

2.7.1. DNA strand breaks

DNA strand breaks and alkali-labile sites were measured in PBMCs using the standard comet assay protocol. Briefly, frozen PBMCs from buffy coats were thawed and added to 5 ml PBS in a centrifuge tube and centrifuged for 7 min at 250×*g*, 4 °C. After the pellet was suspended, 30 μl of the suspension (at 1×10^6 cells/ml) was mixed with 140 μl of 0.7 % LMP agarose at 37 °C. Gels of 70 μl were set on NMP agarose-precoated slides (two gels per slide). Slides were immersed in cold lysis solution (2.5 M NaCl, 0.1 M Na_2EDTA, 10 mM Tris, 1 % Triton X-100, pH 10.0) at 4 °C for 1 h. After lysis, slides were incubated in alkaline electrophoresis solution (0.3 M NaOH, 1 mM EDTA, pH > 12) at 4 °C for 20 min; electrophoresis was then carried out for 20 min at 0.8 V/cm across the platform. Slides were neutralized in PBS, rinsed in distilled water, and left to dry overnight. For scoring, slides were stained with SYBR Gold at 10,000 x dilution in Tris-EDTA buffer for 30 min in the dark.

2.7.2. Fpg-sensitive sites

To determine formation of oxidized DNA bases, slides for enzyme treatment were after the lysis step exposed to formamidopyrimidine

DNA glycosylase (Fpg), which recognizes 8-oxoG and ring-opened purines. Slides were incubated (37 °C) for 1 h before being placed together with untreated slides in the electrophoresis tank. To ensure that the lesion of interest was quantitatively detected, the optimal reaction condition for the enzyme was determined by titration prior to the experiments. In parallel with the enzyme treatment of the slides, other slides were incubated in buffer without enzyme, and the resulting comet score was subtracted from the slides incubated in the enzyme. To increase the number of samples that could be handled simultaneously, slides incubated in buffer without enzyme were carried out only as a control every two weeks. Therefore, slides without enzyme (untreated slides to measure strand breaks) were used to calculate net Fpg-sensitive sites.

2.7.3. Quantification of DNA damage

Images of the comets were acquired using an Olympus fluorescence microscope (BX51 Fluorescent Motorized Microscope) with a digital camera (BASLER scA 1300 - 321 m). Semi-automated image analysis software (Comet Assay IV; Perceptive Instruments) was used to score 50 randomly selected comets per gel, and a total of 100 comets per sample were evaluated to calculate the median value. All scoring was performed blindly. The level of DNA damage was expressed as percentage DNA in the tail (% tail DNA), which is linearly related to break frequency over the range of damage levels expected [47]. Net Fpg-sensitive sites were calculated by subtracting the % DNA in tail for untreated samples (strand breaks) from % tail DNA for the respective enzyme incubation. Highly damaged cells were not excluded from the analysis. All comet assay analyses were conducted by the same investigator (ALN).

2.8. Statistical analysis

Continues and discrete variables were tested for normality by visual inspection of histograms and Q-Q-plots, as well as by using the Shapiro-Wilk test. Descriptive statistics are given as median and interquartile range (median (Q1-Q3)) for continuous variables, as most variables violated the assumptions of normality. Categorical variables are presented as number and proportion (n, %). To compare study groups with regard to baseline characteristics, Brunner-Munzel test (also known as Generalized Mann-Whitney test) were used for continuous and discrete, whereas Fisher's exact tests were used for categorical variables.

In order to evaluate whether DNA damage was related to age, sex, clinicopathological factors (TNM stage and cancer location), and treatment characteristics, including modality (chemotherapy/no chemotherapy) and various indicators of treatment status at blood sampling: days on chemotherapy (continuous), rounds of chemotherapy completed (continuous), days since last dosage (continuous), and dose-limiting toxicity (categorical), a Brunner-Munzel test and generalized linear models (GLM) with the Gamma family and identity link function were applied. The Gamma GLM approach was chosen because the residuals from linear regression, as well as for log-transformed data, did not meet the assumption of normality. Gamma was found to be the best fit for the DNA damage data. Adjustments for age, sex, treatment modality, and TNM status were performed in separate models using multiple Gamma GLM. Gamma GLM was also used to investigate whether DNA damage was related to various aspects of nutritional status: overall PG-SGA category (PG-SGA A/PG-SGA B) and the global numeric score (continuous), patient-generated and professional components of the PG-SGA (dichotomization of variables described in the table legend), age-specific low and high BMI, WC (continuous) and sex-specific cut-offs for abdominal obesity, FM (continuous) and sex-specific cut-offs for obesity, FM % (continuous), VAT and VAT-index (both continuous), FFM (continuous) and sex-specific cut-offs for low FFM-index – as well as to explore whether DNA damage was related to physical activity and function: daily recorded physical activity (continuous), handgrip strength (continuous) and sex-specific cut-offs for low handgrip strength, relative handgrip strength (continuous), sit-to-stand (continuous), and 6 min walking test (continuous). Adjustments for age, sex, treatment modality, and TNM status were performed in separate models using multiple Gamma GLM. The results of the are presented as crude estimates and adjusted estimates (only for associations between DNA damage and clinicopathological factors).

For some patients, DXA scanning was not performed, and a multiple model-based imputation procedure [48] to impute missing body characteristics such as FM, FM %, FFM, and VAT was used. All statistical analyses were performed using R software [49], except for characterization of the study population, which was performed using STATA, version 17.0. A significance level of $P < 0.05$ was used.

3. Results

3.1. Characteristics of the study population

Out of the 503 patients participating at baseline in the CRC-NORDIET study, PBMCs prepared for comet assay were available from 255 patients. Characteristics of the study population ($n = 255$) are described in Table 1/Supplementary Table S1. The median (Q1-Q3) age was 68 (61-72) years, and nearly half (49 %) were women. At the time of diagnosis, 72 % of the cases were staged with TNM I-II (localized tumour), while 28 % were staged with TNM III (regional tumour). Of the 255 patients, 56 % had colon cancer and 44 % had rectosigmoid or rectum cancer. A stoma was present in 28 % of the patients. Median (Q1-Q3) time from surgery to time of assessment was 160 (118–203) days. Forty-nine patients (19 %) had received at least one cycle of chemotherapy at time of assessment (referred to as the chemotherapy group). The remaining 206 patients had either not yet started on chemotherapy or were not intended for chemotherapy treatment (referred to as the non-chemotherapy group).

Regarding nutritional status, 14 % of the patients were classified as moderately malnourished according to the PG-SGA, and none as severely malnourished. The median (Q1-Q3) BMI and FM % were 26.3 (23.3–29.3) kg/m^2 and 33.2 (29.0–38.5) %, respectively. Only 4 % reported losing weight in the last month. Recorded median (Q1-Q3) daily physical activity was 4.7 (3.5–5.8) hours/day. Further details regarding nutritional status, physical activity, and function can be found in Supplementary Tables S2 and S3.

As expected, there was a close relationship between clinicopathological features and treatment given. Specifically, chemotherapy was more common among those with a stage III disease, as well as in those with a proximally located tumour ($P < 0.001$ for both). Further, the use of stoma was more prevalent among those with a locally advanced disease ($P < 0.01$) and for cancers localized in the rectum ($P < 0.001$). With regard to the other characteristics, nutritional status was found to be significantly associated with both TNM status and treatment modality. Specifically, being moderately malnourished was more common in those with a locally advanced disease ($P = 0.035$) and among those receiving chemotherapy ($P = 0.038$). No significant differences were found for the other characteristics studied ($P > 0.05$).

Of the 49 patients receiving chemotherapy, 39 % were scheduled for monotherapy (5-FU plus folinic acid or capecitabine), and 61 % were scheduled for combination therapy (5-FU plus folinic acid and oxaliplatin, capecitabine plus oxaliplatin, or 5-FU plus irinotecan). Median (Q1-Q3) time on chemotherapy at the time of blood sampling was 144 (100–160) days, corresponding to approximately 9 (6-12) of the intended 12 treatment cycles. The time since last dosage of chemotherapy to blood draw showed a wide variation, with a median (Q1-Q3) of 15 (8-61) days. Out of the 46 patients for whom information on dose-limiting toxicity was available, 33 % had experienced some kind of dose-limiting toxicity.

3.2. DNA damage in relation to clinicopathological factors

To explore whether clinicopathological factors were related to DNA

Table 1
Characteristics of the study population by TNM stages, cancer location, and treatment modality.

Variables	n	Overall (n = 255)	TNM I-II (n = 183)	TNM III (n = 72)	P[a]	C18 (n = 142)	C19 – C20 (n = 110)	P[a]	Chemotherapy (n = 49)	Non-chemotherapy (n = 206)	P[a]
Age, years	255	67.7 (60.6, 72.1)	67.9 (60.7, 72,3)	66.9 (59.5, 72.05)	0.547	68.4 (60.8, 72.3)	66.4 (60.4, 71.7)	0.278	63.5 (59.0, 72.0)	68 (60.8, 72.3)	0.340
Sex, n (%)	255										
Men		130 (51.0)	93 (50.8)	37 (51.4)	1.000	66 (46.5)	63 (57.3)	0.099	24 (49.0)	106 (51.5)	0.874
Women		125 (49.1)	90 (49.2)	35 (48.6)		76 (53.5)	47 (42.7)		25 (51.0)	100 (48.5)	
Smoking status, n (%)	255										
Current smoker		23 (9.0)	14 (7.7)	9 (12.5)	0.231	12 (8.5)	11 (10.0)	0.667	6 (12.2)	17 (8.3)	0.406
Dietary supplements[b], n (%)	249										
Users		175 (70.3)	129 (72.1)	46 (65.7)	0.356	103 (75.2)	70 (64.2)	0.069	33 (68.75)	142 (70.65)	0.861
Number of comorbidities, n (%)	253										
0		89 (35.2)	66 (36.5)	23 (31.9)	0.582	47 (33.3)	41 (37.6)	0.585	15 (30.6)	74 (36.3)	0.748
1		89 (35.2)	60 (33.1)	29 (40.3)		48 (34.5)	39 (35.8)		19 (38.8)	70 (34.3)	
≥2		75 (29.6)	55 (30.4)	20 (27.8)		46 (32.2)	29 (26.6)		15 (30.6)	60 (29.4)	
Clinicopathological information											
TNM stage, n (%)	255										
I-II (localized tumour)		183 (71.8)	–	–	–	102 (71.8)	78 (70.9)	0.889	8 (16.3)	175 (85.0)	<0.001
III (regional tumour)		72 (28.2)	–	–	–	40 (28.2)	32 (29.1)		41 (83.7)	31 (15.0)	
Cancer location	252										
C 18 (colon)		142 (56.3)	102 (56.7)	40 (55.6)	0.889	–	–	–	39 (79.6)	103 (50.7)	<0.001
C19 – C20 (rectosigmoid/rectum)		110 (43.7)	78 (43.3)	32 (44.4)		–	–	–	10 (20.4)	100 (49.6)	
Treatment-specific information											
Ostomy, n (%)	255										
Yes		72 (28.2)	43 (23.5)	29 (40.3)	0.009	13 (9.2)	58 (52.7)	<0.001	12 (24.5)	60 (29.1)	0.598
No		183 (71.8)	140 (76.5)	43 (59.7)		129 (90.8)	52 (47.3)		37 (75.5)	146 (70.9)	
Days since surgery, n	255	160 (118, 203)	152 (114, 203)	163 (125, 211)	0.261	160 (118, 200)	155 (120, 210)	0.971	166 (121, 211)	154 (118, 202)	0.278
Treatment modality	255										
Chemotherapy		49 (19.2)	8 (4.4)	41 (56.9)		39 (27.5)	10 (9.1)		–	–	–
Non-chemotherapy		206 (80.8)	175 (95.6)	31 (43.1)	<0.001	103 (72.5)	100 (90.9)	<0.001	–	–	–
Days on chemotherapy, n	46	144 (100, 160)	154 (1, 169)	141 (104, 158)	0.963	135 (69, 160)	154 (123, 173)	0.414	144 (100, 160)	–	–
Treatment rounds completed, n	46	9 (6, 12)	12 (1, 12)	9 (7,12)	0.990	9 (4, 12)	12 (8, 12)	0.292	9 (6, 12)	–	–
Days since last dosage, n	46	15 (8, 61)	13 (8, 129)	16 (8, 54)	0.836	14 (8, 66)	16 (13, 31)	0.825	15 (8, 61)	–	–
Type of chemotherapy initiated, n (%)	49										
Monotherapy		19 (39)	5 (62.5)	14 (34.1)	0.269	15 (38.5)	4 (40.0)	1.000	19 (39)	–	–
Combination therapy[c]		30 (61)	3 (37.5)	27 (65.9)		24 (61.5)	6 (60.0)		30 (61)	–	–
DLT[d], n (%)	46										
Yes		33 (71.7)	3 (9.1)	30 (90.9)	0.878	26 (68.4)	7 (87.5)	0.409	33 (71.7)	–	–
No		13 (28.3)	2 (15.4)	11 (84.6)		12 (31.6)	1 (12.5)		–	–	–
Nutritional status											
PG-SGA global rating, n (%)	253										
PG-SGA A		217 (85.8)	162 (89.0)	55 (77.5)	0.035	124 (87.3)	90 (83.3)	0.467	37 (75.5)	180 (88.2)	0.038
PG-SGA B		36 (14.2)	20 (11.0)	16 (22.5)		18 (12.3)	18 (16.7)		12 (24.5)	24 (11.8)	
PG-SGA numeric score, n	253	3 (2, 4)	3 (2, 4)	3 (2, 5)	0.005	3 (2, 4)	3 (2, 4.5)	0.776	3 (2, 6)	3 (2, 4)	0.027
BMI, kg/m^2	255	26.3 (23.3, 29.3)	26.4 (23.3, 29.3)	26.0 (23.1, 29.3)	0.710	26.4 (23.1, 29.5)	26.2 (23.4, 29.2)	0.722	25.4 (22.9, 28.5)	26.4 (23.4, 29.5)	0.293
Waist circumference, cm	255	94.0 (84.0, 104.3)	94.3 (84.0, 105.0)	92.6 (83.5, 100,4)	0.510	94.2 (83.0, 104,1)	93.4 (85.2, 105.0)	0.983	91.3 (82.3, 98.9)	94.0 (85.0, 105.0)	0.151
Presence of weight loss[e], n (%)	253										
Yes		10 (4.0)	7 (3.9)	3 (4.2)	1.000	4 (2.8)	6 (5.6)	0.336	2 (4.1)	8 (3.9)	1.000
No		243 (96.0)	175 (96.1)	68 (95.8)		138 (97.2)	102 (94.4)		47 (95.9)	196 (96.1)	

Values are medians (Q1-Q3) or n (%).
[a]Brunner-Munzel test or Fisher's exact test, significance level $P < 0.05$.
[b] Includes all type of dietary supplements.
[c] Patients receiving at least one dosage of oxaliplatin or irinotecan before blood sampling were categorized in the combination therapy group.
[d] DLT was present if a patient experienced any of the following incidents: "received ≤80 % of total planned dose due to toxicity", "withdrawal of Oxaliplatin", "treatment delay due to toxicity" or "discontinuation of chemotherapy due to toxicity before last planned toxicity".
[e] Defined as any weight loss ≥2 % last month or ≥2 % last 6 months (if data from last month were missing) or are weight losing at recording (indicated by weight loss last two weeks).

damage, associations between TNM stages and cancer location, and DNA damage levels were evaluated (Table 2A). Patients diagnosed with localized tumours (TNM stage I-II) were compared to those with regional involvement (TNM stage III). A significant difference in level of Fpg-sensitive sites was observed (P = 0.034), indicating a higher level of DNA base oxidation in patients with advanced disease. However, no difference was seen for DNA strand breaks (P > 0.05). No significant associations were observed between DNA damage and cancer location (P > 0.05). Since patients with regional tumours more often receive chemotherapy (83.7 % compared to 15 % in the non-chemotherapy group), adjustments for treatment modality as well as age, sex, and cancer location were conducted in separate models (data not shown). After the adjustment for treatment modality, there was no longer a significant difference in the levels of Fpg-sensitive sites between the TNM stages (P = 0.093). Given the few patients diagnosed with localized tumours in the chemotherapy group (n = 8), a sensitivity analysis using the Brunner-Munzel test was conducted to examine whether this could have had an impact on the results. The sensitivity analysis showed a significant difference in the level of Fpg-sensitive sites between the TNM stages (I-II vs III) overall (P = 0.01) as well as in the non-chemotherapy group separately (P = 0.023), indicating a higher level of DNA base oxidation in relation to advanced disease independent of chemotherapy (Table 2B).

3.3. DNA damage in relation to treatment factors

To investigate how chemotherapy affected genomic instability in our study population, levels of DNA damage were evaluated in relation to treatment modality and treatment status, including type of chemotherapy initiated (mono-/combination therapy), duration of chemotherapy before blood sampling (days on chemotherapy, number of treatment cycles completed), time since last dosage, and dose-limiting toxicity (Table 3). Somewhat unexpectedly, the level of DNA damage did not differ between patients exposed and not exposed to chemotherapy (P > 0.05). However, in the subgroup undergoing chemotherapy (2–191 days since last dosage), a significant negative association was found between time since last dosage and levels of Fpg-sensitive sites (P = 0.005), while no association was observed for strand breaks (P > 0.05). Dividing the chemotherapy group by number of days since last dosage (<15 days, n = 23; ≥15 days, n = 23), a notable difference of time was detected (those recently receiving chemotherapy having 1.4 higher % tail DNA than those with longer time since last dosage, P = 0.028). The differences remained significant after separate adjustment for age, sex, TNM status, and cancer location (data not shown). Since 28 (11 %) of the patients had received chemoradiotherapy before surgery, a sensitivity analysis was conducted to examined whether this influenced the result. However, no change in effect estimates were observed when excluding these patients from the analyses (data not shown). Comparing those with a recent dosage (<15 days) to those not receiving chemotherapy, a significant difference in the levels of Fpg-sensitive sites was observed (median values of 5.66 % and 3.26 % tail DNA, respectively, P = 0.033) (Fig. 1). No significant associations were found between DNA damage and any of the other treatment status measures explored (P > 0.05).

Table 2B
Sensitivity analysis to examine the potential influence of chemotherapy usage on the relationship between TNM stage(s) and DNA damage.

TNM stage	n	Strand breaks Median	P[a]	Fpg-sensitive sites Median	P[a]
Overall					
I-II	183	3.09	0.687	3.12	0.010
III	72	2.89		4.42	
Non-chemotherapy					
I-II	175	3.05	0.858	3.20	0.023
III	31	2.34		4.58	
Chemotherapy					
I-II	8	3.50	0.842	2.52	0.579
III	41	2.90		4.21	

[a]Brunner-Munzel Test, significance level P < 0.05.

3.4. DNA damage in relation to nutritional status

To evaluate whether DNA damage was related to nutritional status, associations between DNA damage markers and data collected from the PG-SGA, anthropometric measurements, and DXA-scan were explored (Supplementary Table S2). Overall, the indicators of body composition (FM, FM %, VAT, and FFM), abdominal obesity (WC) and nutritional status, as well as the different obesity phenotypes, were not associated with the level of DNA damage (P > 0.05). Interestingly, within a sub-analysis in the chemotherapy group (n = 49), patients with elevated FM (in kg and %) exhibited higher levels of Fpg-sensitive sites (P < 0.01), suggesting that fat tissue may have an impact on DNA base oxidation in patients undergoing chemotherapy (Fig. 2A and B).

3.5. DNA damage related to physical activity and function

To explore whether DNA damage was related to physical activity, function, and sedentary behaviour we evaluated associations between daily recorded physical activity and physical performance tests, and DNA damage. (Supplementary Table S3). Overall, no associations were observed between DNA damage and any of the variables examined (P > 0.05). However, within a sub-analysis in the chemotherapy group (n = 45), associations between the level of Fpg-sensitive sites and sedentary behaviour (positively) as well as physical activity (negatively) were observed (P < 0.05) (Fig. 3A and B).

Table 2A
Associations between DNA damage (% tail DNA) and clinicopathological factors.

Variables	n	Crude estimates[b] (95 % CI) Strand breaks	P[a]	Crude estimates[b] (95 % CI) Fpg-sensitive sites	P[a]	Adjusted estimates[c] (95 % CI) Strand breaks	P[a]	Adjusted estimates[c] (95 % CI) Fpg-sensitive sites	P[a]
Cancer location	252								
C18 (colon)	142								
C19–C20 (rectum)	110	−0.18 (−0.82, 0.47)	0.579	−0.04 (−0.75, 0.69)	0.910	−0.23 (−0.90, 0.46)	0.504	0.097 (−0.63, 0.84)	0.795
TNM stage	255								
I-II	183								
III	72	−0.00 (−0.48, 0.53)	0.988	0.67 (0.09, 1.34)	0.034	0.05 (−0.52, 0.69)	0.883	0.66 (−0.05, 1.46)	0.093

Estimates are derived from gamma GLM with identity link.
[a]Significance level P < 0.05.
[b] Crude estimates.
[c] Adjusted for treatment modality (chemotherapy/non-chemotherapy).

Table 3
Associations between DNA damage (% tail DNA) and treatment factors.

Variables	n	Crude estimates (95 % CI) Strand breaks	P[a]	Crude estimates (95 % CI) Fpg-sensitive sites	P[a]
Treatment modality	255				
Non-chemotherapy	206				
Chemotherapy	49	−0.11 (−0.83, 0.77)	0.794	0.66 (−0.24, 1.77)	0.193
Treatment type	49				
Monotherapy	19				
Combination therapy	30	−1.01 (−2.47, 0.45)	0.157	−0.37 (−2.08, 1.5)	0.674
Treatment length, days	46	−0.01 (−0.02, 0.01)	0.392	0.00 (−0.01, 0.02)	0.537
Treatment cycles completed, n	46	−0.09 (−0.33, 0.09)	0.360	0.04 (−0.23, 0.26)	0.727
Time since last dosage, days	46	0.01 (0, 0.03)	0.255	−0.02 (−0.03, 0)	0.005
<15 days	23				
≥15 days	23	0.32 (−0.75, 1.45)	0.550	−1.41 (−2.79, −0.25)	0.028
Dose-limiting toxicity	46				
No	13				
Yes	33	−0.61 (−2.74, 0.92)	0.487	−1.43 (−4.18, 0.52)	0.213

Estimates are derived from gamma GLM with identity link. [a]Significance level $P < 0.05$. Adjustments for age, sex, TNM status, and cancer location in separate models were also performed, but is not presented in the table as the adjustments did not alter the interpretation of the results.

3.6. DNA damage in relation to age, sex, and smoking status

To evaluate whether DNA damage (strand breaks and Fpg-sensitive sites) was related to age, sex, and smoking status, biomarker levels were compared between patients under and over 70 years of age, across sexes, and between smokers and non-smokers. No significant differences were observed for any of the comparisons tested ($P > 0.05$) (Supplementary Table S4).

4. Discussion

We have explored possible links of clinicopathological factors, treatment factors, nutritional status, physical activity, and function with DNA damage in PBMCs of CRC patients recovering from curative surgery (post 2–9 months). To measure DNA damage, we used the enzyme-modified comet assay to detect oxidized purines, as well as DNA strand breaks and alkali-labile sites. Interestingly, a higher level of DNA base oxidation (but not DNA strand breaks) was observed in those diagnosed with regional disease (TNM stage III) compared to patients with localized disease (stage I-II). DNA damage was not different between those receiving *vs.* not receiving chemotherapy. However, when analysing the chemotherapy group separately, a higher degree of DNA base oxidation was observed in patients undergoing recent chemotherapy administration (<15 days from blood sampling) compared with those that had a longer chemotherapy-free period (≥15 days from blood sampling). In those who had received chemotherapy, high absolute and relative amounts of fat tissue as well as sedentary behaviour were positively associated with levels of DNA base oxidation.

4.1. DNA damage in relation to clinicopathological factors

To the best of our knowledge, no previous study applying the enzyme-modified comet assay has investigated the relationship between DNA base oxidation and TNM stage. However, in human biomonitoring studies, various biomarkers in blood and urine have been used to reflect the level of oxidative stress and DNA oxidation. Our findings are in line with previous studies demonstrating that the levels of blood markers related to oxidative stress increase with tumour stage in CRC patients who underwent surgery [50–53]. However, a one-year follow-up study among CRC patients observed that the increased levels of oxidative stress tended to progressively recover to control levels after treatment [54]. Accordingly, a decrease in the level of urinary 8-oxoG in cancer patients after surgery has been observed [55]. Importantly, the base excision repair (BER) pathway is the primary mechanism for repairing oxidized DNA base lesions such as 8-oxoG [56], and in PBMCs, this pathway has been shown to be downregulated in the presence of active disease, but returns to normal levels one year after the diagnosis and successful treatment [57].

In the present study, blood samples were collected at a single time point – 160 (median) days post-surgery – which limits our ability to track the course of recovery from pre-surgery. Cancer cells are consistently under oxidative stress, due to increased metabolism driven by aberrant cell growth. Therefore, it is reasonable to expect an association between the level of DNA base oxidation and disease severity when the cancer is still present. However, it is interesting that even long after curative surgery, the level of oxidized bases still reflects the tumour stage. Inflammation has been closely associated with all stages in cancer pathogenesis, as well as with the efficacy of anti-cancer treatments [58–60]. Moreover, surgical stress-induced inflammation may exert a significant influence on long-term survival. Proinflammatory cytokines are locally produced by the injured tissue as a direct consequence of trauma, which leads to both local and systemic consequences [61]. Thus, we can speculate whether curative resection of regional tumours promotes DNA base oxidation to a greater extent compared to resection of local tumours.

Surgical resection of primary CRC effectively cures most patients diagnosed with locoregional diseases. However, around 5 % of patients with stage I, 15 % with stage II and 40 % with stage III will develop metastases in the following years [62]. In this context, our result may reflect this estimation expressed as DNA base oxidation. Since inflammation is a major cause of oxidative stress, which again is a major cause of genomic instability, our results may indicate the disease status post-treatment. It should be mentioned that no association between DNA damage and days since surgery was observed.

In our study, we could not observe that DNA strand breaks were related to TNM stages, which is in line with some [24,26,63], but not all previous studies [25,64–66]. A higher nuclear expression of DNA damage-inducible transcript 4 (DDIT4), which is induced in various

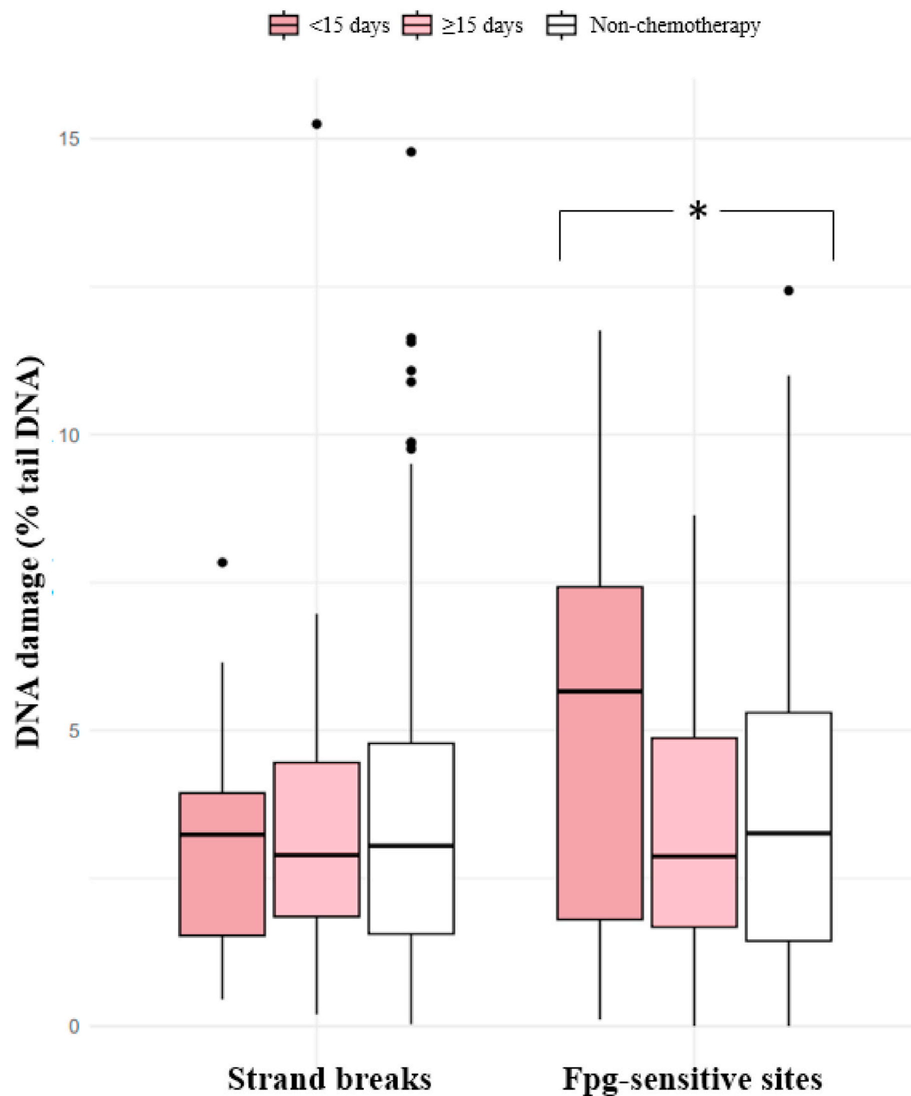

Fig. 1. DNA damage levels in the chemotherapy group (<15 days and ≥15 days since last dosage) and the non-chemotherapy group. Median levels of Fpg-sensitive sites; chemotherapy group (<15 days; n = 23): 5.66 % tail DNA, non-chemotherapy group (n = 206): 3.26 % tail DNA, P = 0.033.

cellular stress conditions, has also been found associated with more aggressive tumour and more advanced stage in CRC patients [67]. However, the majority of the studies conducted addressed the association of the levels of DNA strand breaks with cancer at the time of diagnosis, which may explain the inconsistency with our findings. In addition, although interaction between tumour and host is an important regulator of the disease progression [6,10,11], it may be significant whether DNA damage is measured in tumour or host tissue [23].

TNM stage remains the most important prognostic factor in predicting recurrence and survival among CRC patients. However, several studies demonstrated that host characteristics such as inflammatory response, circulating tumour DNA as well as blood markers for nutritional status (e.g., the prognostic nutritional index (PNI)) are associated with outcome, independent of stage [68–72]. Since DNA base oxidation is one of the main contributors to mutations and genomic instability implicated in chronic diseases, including cancers, the enzyme-modified comet assay could be a tool to capture the host characteristics in their entirety. Moreover, findings from the hComet cohort study revealed that DNA damage in peripheral lymphocytes may serve as a predictive indicator for overall mortality, especially related to diseases of the circulatory system [73].

4.2. DNA damage in relation to treatment factors

The present study was unable to observe differences in the level of any of the DNA damage markers between those receiving and those not receiving chemotherapy. In contrast, several studies across various cancer populations have observed an increase in the level of DNA damage in response to chemotherapy [26,74–77]. However, the majority of previous studies have measured DNA damage prior to and immediately after chemotherapy administration. Since in our study there was a great variation in time since last chemotherapy exposure (2–191 days), we additionally explored the effect of time on DNA damage (both as a continuous measure and by dividing the exposed according to number of days since last dosage (<15 *vs.* ≥ 15 days from blood draw). By doing this, we observed that recent exposure to chemotherapy was an important predictor of DNA base oxidation. Since the chemotherapy regimens used to treat non-metastatic CRC are assumed to predominantly cause single- and double strand breaks as well as crosslinking of DNA [78,79], we speculate that the associations observed are most likely not a direct effect of the chemotherapy *per se*. Instead, increased DNA base oxidation could be a secondary effect in response to chemotherapy-induced inflammation. This is in line with studies demonstrating increased local and systemic inflammation following the administration of chemotherapy [80,81].

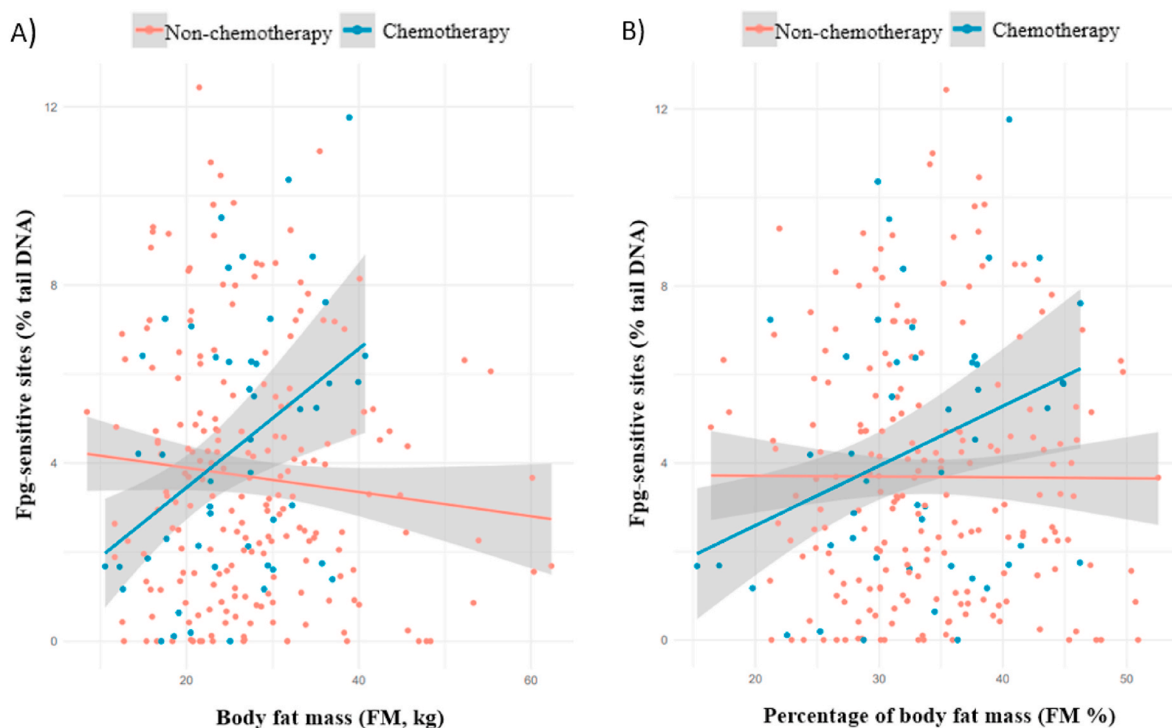

Fig. 2. Scatter plots illustrating the relationship between **A)** body fat mass (kg) and Fpg-sensitive sites (% tail DNA), and **B)** body fat mass percentage (FM %) and Fpg-sensitive sites (% tail DNA) in the non-chemotherapy group (n = 206) and the chemotherapy group (n = 49) (P < 0.01).

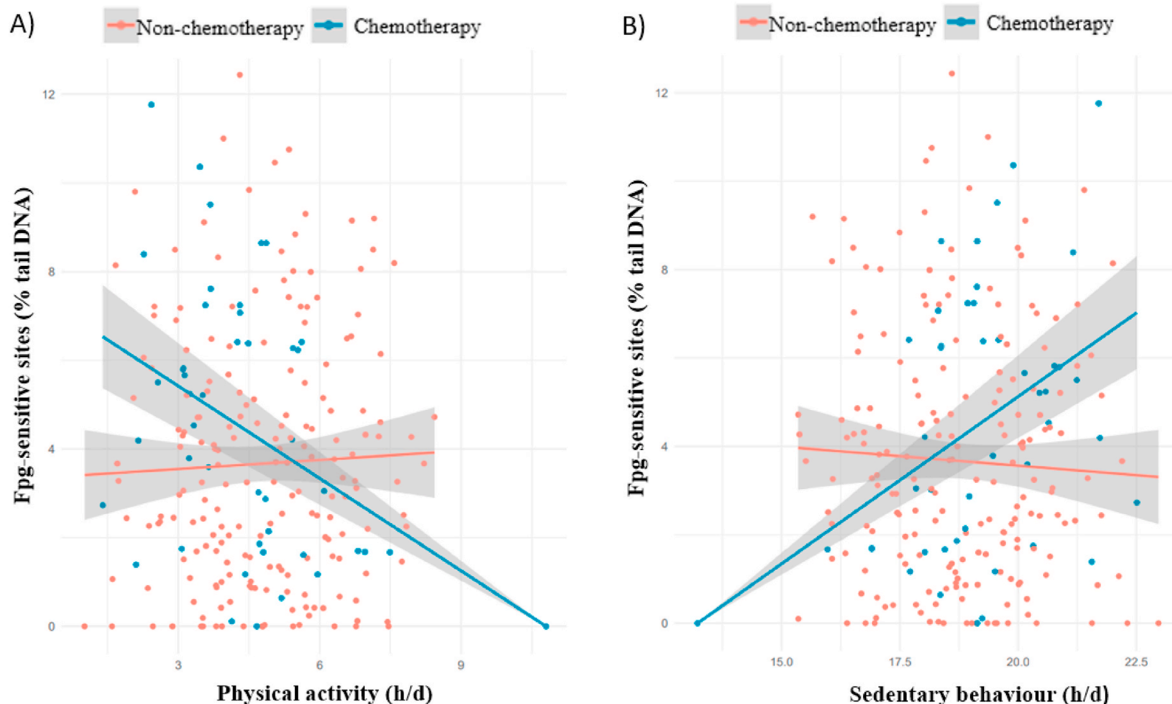

Fig. 3. Scatter plots illustrating the relationship between **A)** daily physical activity and Fpg-sensitive sites (% tail DNA), and **B)** sedentary behaviour and Fpg-sensitive sites (% tail DNA) in the non-chemotherapy group (n = 206) and the chemotherapy group (n = 49) (P < 0.01).

In the current study, the levels of DNA strand breaks did not differ between patients receiving chemotherapy and those not receiving chemotherapy (irrespective of time since last dosage). In another subpopulation of the CRC-NORDIET study, selected based on other inclusion criteria, we found a significant difference in DNA strand breaks between those exposed to chemotherapy and those not exposed [74]. The discrepancy in results may be due to different sampling method for measuring DNA damage, where Kværner et al. [74] used whole blood, rather than isolated PBMCs for the comet assay analyses. The use of whole blood *vs.* PBMCs for DNA damage assessment has been identified as one of the key sources of variation in technique that can account for variability in the results [82]. A likely explanation for this variation is

the difference in cell populations; whereas the nucleated cells of whole blood mainly are represented by neutrophils (60–75%), the predominant cells of PBMCs are lymphocytes (95–98%) [82]. Moreover, the discrepancy between the two sub-populations may also be due to differences in procedures for freezing and thawing, sample size, individual variability (including intrinsic biological factors such as antioxidant status and DNA repair capacity), lifestyle behaviours, as well as gut microbes [18,19,83].

4.3. DNA damage in relation to nutritional status, physical activity, and function

In patients exposed to chemotherapy, we observed that greater FM was associated with a higher level of DNA base oxidation. It is well known that obesity is linked to chronic inflammation and oxidative stress. Further, obesity is recognized as an indicator of poor prognosis as well as a predictor of cancer recurrence after adjuvant chemotherapy [84,85]. Body fatness might affect pharmacokinetics of antineoplastics agents, by altering tissue distribution and drug elimination [86], which in turn may promote chemoresistance and reduce response to chemotherapy [87]. Since we could not observe an association between DNA damage (strand breaks or oxidized bases) and body fatness in the study population as a whole, the current study may suggest that body fatness might cause greater oxidatively damaged DNA in the context of genotoxic exposures such as chemotherapy.

Furthermore, within the chemotherapy group, we also observed that sedentary behaviour was associated with a higher level of DNA base oxidation. Physical activity has emerged as an important factor to reduce the risk of recurrence and mortality in CRC patients [88,89], with strong evidence supporting its diverse benefits related to fatigue, depression, quality of life, physical function, body composition, and cardiorespiratory fitness [90]. There is a growing body of evidence that regular exercise may up-regulate the antioxidant defence system and enhance DNA repair processes [91]. Moreover, physiological and biological processes during and after exercise may also affect the tumour rate and boost the immune system, which in turn could affect therapeutic toxicity and tolerance [19]. In our study, metabolic health seems to be an important determinant of oxidatively damaged DNA in patients receiving chemotherapy.

In contrast to our previous study [74], we could not demonstrate an association between DNA damage and nutritional status as measured by the PG-SGA; DNA damage was neither associated with the overall PG-SGA categorization, nor with the patient-generated historical components or professional components of the form. Possible explanations for this discrepancy are outlined above. To our knowledge, our studies are the only ones that have investigated the associations between DNA damage and nutritional status in term of the PG-SGA. Thus, further studies on this relationship are warranted in the future.

4.4. Limitations and strengths

The major limitation of our study is the cross-sectional design, which prevents us from tracing the disease process from pre-surgery to blood samples collection, as well as from making causal inference. Additionally, inclusion of markers such as inflammation, antioxidant profile, and DNA repair might have provided additional information in relation to DNA damage, but unfortunately, these were not available at the time this study was conducted. A substantial strength, distinguishing our study from most previous studies examining DNA damage in cancer patients [23], was the large number of patients included as well as the level of detail encompassing measures of clinicopathological and treatment related factors, nutritional status, and physical activity and function. Moreover, since most studies address the association of DNA damage with cancer at time of diagnosis, a unique aspect of our study is the investigation of the host response 2–9 months after curative surgery. Finally, the strength of our study is increased by the use of enzyme-modified comet assay to detect specific DNA lesions; the standard comet assay for strand breaks, alkali-labile sites and repair intermediates is, in contrast, a general, non-specific indicator of DNA damage.

5. Conclusion

In the present explorative study, CRC patients diagnosed with regional disease (stage III) exhibited higher levels of DNA base oxidation compared with patients diagnosed with localized disease (stage I-II) in blood samples taken 2–9 months from surgery. Further, recent exposure to chemotherapy was linked to increased DNA base oxidation. Among patients exposed to chemotherapy, body fatness and sedentary behaviour were positively associated with levels of oxidized bases. High levels of DNA base oxidation potentially affect prognosis and the risk of long-term side effects. Consequently, characterizing host effects using the enzyme-modified comet assay may serve as a valuable biomarker for monitoring of CRC patients. However, prospective trials are essential to fully understand the clinical significance of PBMC DNA base oxidation in cancer patients related to long-term outcome.

Ethical approval

The CRC NORDIET study is carried out in accordance with the Helsinki Declaration. The study was approved by the Regional Committees for Medical and Health Research Ethics (REC Protocol Approval 2011/836) and by the data protection officials at Oslo University Hospital and Akershus University Hospital. Informed consent was obtained from all participants. The study is registered on the National Institutes of Health Clinical Trials (www.ClinicalTrials.gov; Identifier: NCT01570010).

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Author contributions

ALN – Conceptualization, comet analysis, formal analysis, investigation, methodology, visualization, writing – original draft, writing – review and editing. ASK – Conceptualization, methodology, supervision, writing – review and editing. AK – Formal analysis, methodology, writing – review and editing. DTA – Conceptualization, methodology, writing – review and editing. HBH – Conceptualization, methodology, writing – review and editing. CH – Conceptualization, methodology, writing – review and editing. TR – Conceptualization, methodology, writing – review and editing. SS – Conceptualization, methodology, writing – review and editing. SKB – Conceptualization, methodology, writing – review and editing. SS – Conceptualization, funding acquisition, methodology, writing – review and editing. ARC – Conceptualization, methodology, supervision, writing – review and editing. RB – Conceptualization, funding acquisition, investigation methodology, project administrator, resources, supervision, writing – review and editing.

Declaration of generative AI technologies

In the process of preparing this work, the main author used ChatGTP in order to improve language and readability. Following the utilization of this tool/service, the main author subsequently reviewed and edited the content as required, thereby assuming full responsibility for the entirety of contents of the publication.

Data availability

All necessary data for evaluating the conclusions in this study are present in the main text and/or in the Supplementary Material. Additional data generated during this study are available upon request to the corresponding author.

Declaration of competing interest

ALN received industrial PhD scheme for a doctoral project in industry from The Research Council of Norway. ARC is a shareholder of Norgenotech AS, Oslo, Norway. SS is a shareholder and CEO of Norgenotech AS, Oslo, Norway. RB is a shareholder in the company Vitas AS, Oslo, Norway. The other authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.freeradbiomed.2023.12.016.

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