Blood markers of oxidative stress are strongly associated with poorer prognosis in colorectal cancer patients

Daniel Boakye1,2 | Lina Jansen1 | Ben Schöttker1,3 | Eugene H. J. M. Jansen4 | Martin Schneider5 | Niels Halama6 | Xin Gào1 | Jenny Chang-Claude7,8 | Michael Hoffmeister1 | Hermann Brenner1,9,10

1Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany
2Medical Faculty Heidelberg, Heidelberg University, Heidelberg, Germany
3Network of Aging Research, Heidelberg University, Heidelberg, Germany
4Centre for Health Protection, National Institute for Public Health and the Environment, Bilthoven, The Netherlands
5Department of General, Visceral and Transplantation Surgery, Heidelberg University Hospital, Heidelberg, Germany
6Division of Translational Immunotherapy, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg, Germany
7Unit of Genetic Epidemiology, Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany
8Cancer Epidemiology Group, University Cancer Center Hamburg (U.CCH), University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany
9Division of Preventive Oncology, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg, Germany
10German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany

Abstract

Oxidative stress has been implicated in the initiation of several cancers, including colorectal cancer (CRC). Whether it also plays a role in CRC prognosis is unclear. We assessed the associations of two oxidative stress biomarkers (Diacron’s reactive oxygen metabolites [d-ROMs] and total thiol level [TTL]) with CRC prognosis. CRC patients who were diagnosed in 2003 to 2012 and recruited into a population-based study in Germany (n = 3361) were followed for up to 6 years. Hazard ratios (HRs) and 95% confidence intervals (95% CIs) for the associations of d-ROMs and TTL (measured from blood samples collected shortly after CRC diagnosis) with overall survival (OS) and disease-specific survival (DSS) were estimated using multivariable Cox regression. Particularly pronounced associations of higher d-ROMs with lower survival were observed in stage IV patients, with patients in the highest (vs lowest) tertile having much lower OS (HR = 1.52, 95% CI = 1.14-2.04) and DSS (HR = 1.61, 95% CI = 1.20-2.17). For TTL, strong inverse associations of TTL with mortality were observed within all stages. In patients of all stages, those in the highest (vs lowest) quintile had substantially higher OS (HR = 0.48, 95% CI = 0.38-0.62) and DSS (HR = 0.52, 95% CI = 0.39-0.69). The addition of these biomarkers to models that...
included age, sex, tumor stage and subsite significantly improved the prediction of CRC prognosis. The observed strong associations of higher d-ROMs and lower TTL levels with poorer prognosis even in stage IV patients suggest that oxidative stress contributes significantly to premature mortality in CRC patients and demonstrate a large potential of these biomarkers in enhancing the prediction of CRC prognosis beyond tumor stage.

**KEYWORDS**
colorectal neoplasm, free radical, oxidative stress, prognosis, survival

# 1 | BACKGROUND

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths, accounting for about 900,000 deaths per year globally. A wide range of factors including personal and lifestyle factors (e.g., age, smoking) are associated with risk of several cancers, including CRC. These factors are also associated with oxidative stress, an imbalance in the formation and removal of reactive oxygen species (ROS), leading to damage of several biomolecules including DNA. ROS are by-products of cellular metabolism but can also be produced by immune cells (e.g., phagocytic cells) during infections. Because oxidative stress could continue to cause damage to the DNA of cancer cells after tumor initiation or could affect tumor characteristics such as aggressiveness, it could play a role in CRC prognosis. However, data on the association of oxidative stress with CRC prognosis are sparse, likely due to difficulties in measuring ROS in the human body because of their short half-life.

Recent development of more stable methods for quantifying oxidative stress such as Diacon’s reactive oxygen metabolites (d-ROMs, an indirect evaluation of oxidant status by hydroperoxides) and total thiol level (TTL, a proxy measure of antioxidant status by free thiol groups) has strongly enhanced possibilities to evaluate the association of oxidative stress with health outcomes. Epidemiological studies have shown associations of blood d-ROMs levels with lung, breast, colorectal cancer risk and with cancer mortality in lung cancer patients. Higher TTL were also found to be associated with lower lung and breast cancer risk and mortality in the general population.

Whether these biomarkers also predict CRC prognosis is unclear. One small-sized study (n = 89) from Poland has investigated the associations of different oxidative stress biomarkers (8-oxo-7,8-dihydro-2-deoxyguanosine and 8-oxo-7,8-dihydroguanine) with CRC prognosis and found lower survival in patients with higher levels of these biomarkers for oxidatively damaged DNA. However, the authors did not adjust for any of the known prognostic factors of CRC such as tumor stage. The aim of our study was to evaluate the association of oxidative stress, quantified by d-ROMs and TTL, with prognosis in a large cohort of CRC patients, paying particular attention to prognostic value beyond tumor stage and other known predictors of CRC prognosis.

# 2 | METHODS

## 2.1 | Study design and population

Our analysis is based on data from CRC patients who were diagnosed in 2003-2012 and recruited into the DACHS (Darmkrebs: Chancen der Verhütung durch Screening) study. The DACHS study is a population-based case-control study in the Rhine-Neckar region of Germany, with additional regular follow-up of cases. Patients with first time diagnosis of CRC (International Classification of Diseases, 10th Revision [ICD-10], codes C18-C20) and aged 30 years or older and physically able to participate in an interview lasting for about 1 hour were eligible. Patients were recruited from all 22 hospitals providing first-line treatment for CRC in the study region of about 2 million inhabitants. In the recruiting hospitals, eligible patients were informed about the study by their physicians and recruited either during or shortly after their hospital stay. Data from a population-based cancer registry indicate that about half of the eligible patients in the study region were recruited. There was also an age gradient, with higher rates of recruitment of younger patients, in whom comorbidities are often lower and chemo(radio)therapy administration and CRC survival rates are usually higher compared to older patients.
However, the DACHS study did not set an upper age limit, which has merits when aiming to assess the association of oxidative stress with CRC prognosis, as oxidative stress increases with age.\textsuperscript{15} Further details of the DACHS study have been described elsewhere.\textsuperscript{17,18} The DACHS study was approved by the ethics committees of the Medical Faculty of Heidelberg University and the state medical boards of Baden-Wuerttemberg and Rhineland-Palatinate. All participants gave written informed consent.

### 2.2 | Inclusion criteria

Our study population comprised all patients diagnosed in 2003-2012 who had data on either d-ROMs or TTL (n = 3730, 90%; Figure S1). Patients with poor hemolytic, icteric or lipemic serum samples and very low d-ROMs (<50 U.Carr) or TTL (<50 μmol/L) according to the manufacturer of the assays (n = 28), those who were not operated on for CRC (n = 50), those whose blood samples were taken within 3 days of CRC surgery or within 7 days of chemo(radio)therapy initiation (n = 141) and those with no information on tumor stage and other variables of interest (n = 150) were excluded. The cut-offs for CRC (n = 50), those whose blood samples were taken within 3 days of CRC surgery or within 7 days of chemo(radio)therapy initiation were chosen based on reasoning, as surgery and chemo(radio)therapy are likely to have an immediate effect on d-ROMs and TTL levels. A total of 3361 patients were included in the analysis.

### 2.3 | Data collection

At baseline (mostly during or shortly after hospital stay for CRC surgery), trained interviewers conducted personal interviews with the participants to collect information on lifestyle factors and medical history, using a standardized questionnaire. Tumor characteristics (eg, stage and subsite) and ICD-10 codes for comorbidities that were diagnosed either prior to or at the time of CRC diagnosis were abstracted from hospital records. We used the Charlson comorbidity index (CCI),\textsuperscript{19} adapted by Deyo et al,\textsuperscript{20} to quantify overall comorbidity as described elsewhere.\textsuperscript{15,21} Patients were grouped into four groups, namely, CCI scores 0 (no comorbidity), 1, 2 or 3+ (severe comorbidity). Vital status and cause of death were ascertained from population registries and public health authorities about 3, 5 and 10 years after CRC diagnosis. About 3 years after CRC diagnosis, detailed information on treatment was collected from medical records and from questionnaires sent to patients’ oncologists.

### 2.4 | Ascertainment of oxidative stress biomarkers

At baseline, blood samples were taken from patients (median time from diagnosis to blood sampling was 63 days, interquartile range [IQR], 23-270 days), from which serum aliquots were obtained and stored at −80 °C until analysis for various measures, including d-ROMs and TTL. Whenever possible, patient recruitment and sample collection was prior to or shortly after CRC surgery and prior to chemo(radio)therapy initiation. Of patients receiving chemo(radio)therapy, approximately 40% had their samples collected prior to initiation of this treatment. Median frozen time was 7.9 (IQR, 5.8-10) years. A previous study suggests that d-ROMs and TTL have a good long-term stability under storage conditions of −80 °C.\textsuperscript{22}

Samples were analyzed in two batches in the same laboratory and under similar conditions (70% in April, 2016 and 30% in June, 2016). Standardized assays that are used to measure d-ROMs and TTL (d-ROMs and SHp assay, respectively, both from Diacron, Grosseto, Italy) were adapted to an auto-analyzer (LX20-Pro, Beckman-Coulter, Woerden, The Netherlands) at the Laboratory for Health Protection Research (Bilthoven, The Netherlands). Of samples analyzed in the first 8 days, quality assessment showed comparable mean values, with no evidence of day-to-day variability. In brief, the d-ROMs assay evaluates the total hydroperoxide concentration in Carratelli Units (U.Carr). Each U.Carr is equivalent to 0.08 mg of hydrogen peroxide per 100 mL in the sample.\textsuperscript{23} The higher the U.Carr, the higher the level of oxidative stress. The SHp test is a spectrophotometric test which estimates the concentration of free thiol groups (eg, sulfhydryl groups) in serum in μmol/L using information on color intensity. Higher values indicate lower levels of oxidative stress.

### 2.5 | Statistical analysis

The mean values of the biomarkers, especially TTL, differed by year of blood sampling. To address this, we calculated a z-score for each patient by subtracting the mean of each year of blood sampling from the value recorded for each patient and dividing by the SD of that year.\textsuperscript{24} This standardized the data such that the mean value of each year’s sample was zero and the SD was one. We assessed the distributions of z scores of d-ROMs and TTL by baseline characteristics. ANOVA tests were used to test the mean differences, by adjusting for age and sex.

The associations of z scores of d-ROMs and TTL (in quintiles with overall survival (OS, mortality from any cause) and disease-specific survival (DSS, mortality from CRC) were investigated using Cox proportional hazards regression. d-ROMs and TTL violated the proportional hazards assumption, as their associations with survival outcomes became weaker after 6 years of follow-up. To address this, we censored patients at 6 years of follow-up (median follow-up time), as appropriate. Time was calculated from CRC diagnosis to the respective endpoints or end of follow-up, whichever occurred first. We also investigated the association of the TTL to d-ROMs ratio with OS and DSS. The TTL to d-ROMs ratio might reflect a balance between antioxidant and oxidant capacities and has recently been found to predict all-cause mortality more strongly than TTL or d-ROMs alone in patients with type II diabetes.\textsuperscript{25}

Two adjustment levels were applied: (a) adjustment for sex, age, tumor stage (Union for International Cancer Control, I-IV),\textsuperscript{26} tumor site, year of diagnosis, education level, smoking status, body mass index (BMI) at diagnosis, lifetime physical activity and alcohol...
| Baseline characteristics          | d-ROMs (n = 3237) z scores (−4.03 to 4.26) | Total thiol level (n = 3254) z scores (−3.08 to 3.54) |
|----------------------------------|--------------------------------------------|------------------------------------------------------|
|                                  | n  | Mean (SD) | P       | n   | Mean (SD) | P       |
| Sex                              |    |           |         |     |           |         |
| Women                            | 1290 | 0.24 (1.02) | .       | 1308 | −0.07 (0.99) | .       |
| Men                              | 1947 | −0.16 (0.95) | <.001  | 1946 | 0.05 (1.00)  | .001   |
| Age at diagnosis (years)         |    |           |         |     |           |         |
| 30-59                            | 660 | −0.02 (1.06) | .       | 658 | 0.39 (1.06)  | .       |
| 60-69                            | 1017 | 0.00 (1.00) | .       | 1026 | 0.08 (0.97)  | .       |
| 70-79                            | 1083 | −0.02 (0.98) | .       | 1083 | −0.10 (0.93) | .       |
| ≥80                              | 477 | 0.09 (0.95)  | .630   | 487 | −0.48 (0.87) <.001 |
| Years of school education        |    |           |         |     |           |         |
| <10                              | 2174 | 0.01 (1.00) | .       | 2181 | −0.06 (0.99) | .       |
| 10-11                            | 569 | 0.03 (1.00)  | .       | 577 | 0.08 (0.99)  | .       |
| >11                              | 494 | −0.06 (1.00) | .157   | 496 | 0.05 (1.04)  | <.001  |
| BMI at diagnosis (kg/m²)         |    |           |         |     |           |         |
| <18.5                            | 63  | 0.05 (0.96)  | .       | 64  | −0.07 (0.97) | .       |
| 18.5-24.9                        | 1176 | −0.02 (1.01) | .       | 1199 | 0.08 (1.04)  | .       |
| 25-29.9                          | 1388 | −0.04 (0.98) | .       | 1389 | 0.00 (0.99)  | .       |
| ≥30                              | 610 | 0.13 (1.00)  | .003   | 602 | −0.14 (0.93) | <.001  |
| Physical activitya (MET-h/wk)    |    |           |         |     |           |         |
| Q1 (3.3-115.0)                   | 652 | −0.06 (0.98) | .       | 650 | 0.02 (1.02)  | .       |
| Q2 (115.1-165.6)                 | 648 | −0.02 (0.97) | .       | 652 | 0.05 (1.01)  | .       |
| Q3 (165.7-215.9)                 | 646 | 0.00 (1.03)  | .       | 651 | −0.02 (0.99) | .       |
| Q4 (216.0-295.1)                 | 643 | 0.05 (1.02)  | .       | 654 | −0.01 (1.00) | .       |
| Q5 (295.2-788.2)                 | 648 | 0.03 (0.99)  | .244   | 647 | −0.05 (0.97) | .389   |
| Smoking status                   |    |           |         |     |           |         |
| Never                            | 1309 | 0.05 (1.03) | .       | 1322 | −0.08 (0.98) | .       |
| Former                           | 1422 | −0.09 (0.95) | .       | 1416 | 0.05 (0.99)  | .       |
| Current                          | 506 | 0.12 (1.02)  | <.001  | 516 | 0.08 (1.05)  | <.001  |
| Alcohol consumptiona (g of ethanol per day) |    |           |         |     |           |         |
| None                             | 562 | 0.20 (1.08)  | .       | 558 | −0.10 (1.02) | .       |
| T1 (0.1-6.6)                     | 885 | 0.06 (0.98)  | .       | 901 | −0.01 (1.01) | .       |
| T2 (6.7-19.9)                    | 890 | −0.08 (0.97) | .       | 897 | 0.02 (0.98)  | .       |
| T3 (20.0-381.9)                  | 900 | −0.11 (0.96) | <.001  | 898 | 0.05 (0.99)  | .030   |
| Vegetable intake                 |    |           |         |     |           |         |
| <Once per day                    | 481 | −0.01 (1.02) | .       | 476 | −0.09 (0.98) | .       |
| ≥Once per day                    | 2756 | 0.00 (0.99) | .790   | 2778 | 0.02 (1.00)  | .031   |
| Fruit intake                     |    |           |         |     |           |         |
| <Once per day                    | 1182 | −0.01 (1.03) | .       | 1180 | −0.04 (1.01) | .       |
| ≥Once per day                    | 2055 | 0.01 (0.98)  | .550   | 2074 | 0.02 (0.99)  | .108   |
| Charlson comorbidity score       |    |           |         |     |           |         |
| 0 (no comorbidity)               | 1861 | −0.03 (1.00) | .       | 1874 | 0.13 (0.99)  | .       |
| 1                                | 686 | 0.00 (0.98)  | .       | 694 | −0.07 (0.98) | .       |
| 2                                | 382 | 0.07 (1.07)  | .       | 385 | −0.19 (1.02) | .       |
| 3+ (severe comorbidity)          | 308 | 0.10 (0.94)  | .074   | 301 | −0.43 (0.89) <.001 |
consumption, vegetable and fruit consumption and time of blood
draw with respect to CRC surgery and chemo(radio)therapy initiation,
and (b) additional adjustment for comorbidity score. Results from
model (b) were reported as main results and were also used to assess
to what extent comorbidities explain the association between oxida-
tive stress and CRC prognosis. All covariates were added to the
models as categorical variables, as listed in Table 1 and Figures S2
andS3. We checked the proportional hazards assumption for all
covariates, by assessing whether their interaction with follow-up time
was statistically significant. Time-dependent covariates (interaction
terms of BMI, year of diagnosis, and tumor site with follow-up time)
were also added to the models because of violation of the propor-
tional hazards assumption. We also accounted for left truncation by
including “delayed entry time” in the models. In sensitivity analysis,
we used the Fine and Gray method27 to account for competing events
(mortality from causes other than CRC) in the association of the bio-
markers with DSS. Also, in a subsample of patients diagnosed in 2003
to 2010 who were selected for tumor characterization (n = 1876,
77%), we furthermore assessed whether and to what extent the asso-
ciation of oxidative stress is partly explained or modified by microsat-
etile instability (MSI), a prognostic factor for CRC.28
Subgroup analyses according to sex, age, comorbidity, smoking
status, tumor stage and subsite, MSI status, chemo(radio)therapy use,
time of blood sampling (tertiles of d-ROMs and TTL instead of quin-
tiles were used because of small numbers) were performed. Here, we
used backward selection to select covariates with \( P < .6 \) (defined a
priori) for the models, but age, sex and stage were forced into the
models. Potential variations of associations across subgroups defined
by these variables were assessed with statistical tests for interaction.
Stage-specific results were additionally illustrated with adjusted sur-
vival curves. We furthermore assessed potential linear associations of
the biomarkers with all-cause mortality according tumor stage, using
restricted cubic spline functions.29 Knots were placed at the 25th,
50th and 75th percentiles. Sensitivity analyses were also conducted
using different knot positions (eg, at 5th, 65th and 95th percentiles).
Finally, we assessed whether and to what extent adding d-ROMs
and TTL to models that included age, sex, tumor stage and tumor sub-
site improve the prediction of CRC prognosis, overall and by tumor
stage using the Harrell’s concordance index (C-index). Here, we cre-
ated 1000 bootstrapped samples and trained the models in 2/3 of
each of the samples (training set) and evaluated the predictive accu-
ricy of the regression coefficients in the remaining 1/3 of the samples
(validation set). The samples were split into training and validation sets
using random sampling.
The differences in the C-indexes of the two prediction models
were tested for statistical significance using the “compareC” package
in R (version 3.6.0). All other analyses were conducted with the SAS
software, version 9.4 (SAS Institute, Cary, North Carolina). Statistical
tests were two-sided, with a significance level of 5%.

### RESULTS

#### 3.1 Characteristics of the study population

A total of 3361 patients were included in the analysis (Figure S1). The
median age was 69 (IQR, 62-76) years. About 24%, 31%, 32% and
13% of the patients were in stage I, II, III and IV, respectively. Of the
analytic sample, 3237 and 3254 patients had data on d-ROMs and
TTL, respectively. There was a weak negative correlation between
d-ROMs and TTL \( (r = -.13; P < .001) \). Table 1 shows the distribution
of mean z-scores of d-ROMs and TTL by baseline characteristics.
d-ROMs were particularly high in women, obese patients, current smokers, those not consuming alcohol and stage IV patients. TTL was especially low in older and obese patients, those with severe comorbidities, and stage IV patients.

d-ROMs and TTL levels also differed by time of blood sampling (Figures S2 and S3). For d-ROMs, mean $z$-score was highest among patients whose blood samples were taken within 2 to 8 weeks of CRC surgery. Among patients who received chemo(radio)therapy, d-ROMs levels were higher when blood samples were taken prior to rather than after initiation of such therapy. For TTL, levels were lowest within 2 weeks and highest more than 8 weeks from CRC surgery (mean $z$ scores $< -0.46$ and $> 0.38$, respectively). TTL was lowest when samples were taken before chemo(radio)therapy initiation ($> 0.31$). Regarding time of blood sampling from CRC diagnosis, mean values of d-ROMs and TTL levels were similar to those reported for time of blood collection defined by CRC surgery (data not shown).

### TABLE 2

| Marker                  | Outcome | z score quintiles (range) | Cox model | Fine and Graya |
|-------------------------|---------|---------------------------|-----------|----------------|
|                         |         |                           | n_events/n_at_risk | HR^{c} (95% CI) | HR^{c} (95% CI) | sHR^{c} (95% CI) |
| d-ROMs (n = 3237)       | OS      | Q1 (−4.03; −0.79)         | 175/647   | 1.00           | 1.00           |                |
|                         |         | Q2 (−0.78; −0.24)         | 172/648   | 0.87 (0.70-1.07) | 0.86 (0.70-1.07) |                |
|                         |         | Q3 (−0.23; 0.23)          | 193/647   | 1.00 (0.81-1.23) | 0.95 (0.77-1.17) |                |
|                         |         | Q4 (0.24; 0.80)           | 231/648   | 1.08 (0.89-1.33) | 1.04 (0.85-1.28) |                |
|                         |         | Q5 (0.81; 4.26)           | 269/647   | 1.27 (1.03-1.55) | 1.21 (0.99-1.48) |                |
|                         | DSS     | Q1 (−4.03; −0.79)         | 119/647   | 1.00           | 1.00           | 1.00           |
|                         |         | Q2 (−0.78; −0.24)         | 116/648   | 0.83 (0.64-1.08) | 0.84 (0.64-1.08) | 0.84 (0.66-1.07) |
|                         |         | Q3 (−0.23; 0.23)          | 117/647   | 0.84 (0.65-1.09) | 0.83 (0.64-1.07) | 0.82 (0.64-1.05) |
|                         |         | Q4 (0.24; 0.80)           | 147/648   | 0.88 (0.68-1.13) | 0.87 (0.68-1.12) | 0.90 (0.71-1.15) |
|                         |         | Q5 (0.81; 4.26)           | 185/647   | 1.10 (0.86-1.40) | 1.08 (0.84-1.38) | 1.05 (0.83-1.33) |
| TTL (n = 3254)          | OS      | Q1 (−3.08; −0.92)         | 317/650   | 1.00           | 1.00           |                |
|                         |         | Q2 (−0.91; −0.29)         | 251/651   | 0.87 (0.73-1.03) | 0.92 (0.77-1.09) |                |
|                         |         | Q3 (−0.28; 0.26)          | 203/651   | 0.62 (0.51-0.74) | 0.66 (0.55-0.80) |                |
|                         |         | Q4 (0.27; 0.87)           | 161/651   | 0.54 (0.44-0.67) | 0.58 (0.47-0.71) |                |
|                         |         | Q5 (0.88; 3.54)           | 111/651   | 0.44 (0.35-0.56) | 0.48 (0.38-0.62) |                |
|                         | DSS     | Q1 (−3.08; −0.92)         | 211/650   | 1.00           | 1.00           | 1.00           |
|                         |         | Q2 (−0.91; −0.29)         | 159/651   | 0.85 (0.68-1.05) | 0.87 (0.70-1.07) | 0.90 (0.73-1.11) |
|                         |         | Q3 (−0.28; 0.26)          | 127/651   | 0.56 (0.45-0.71) | 0.58 (0.46-0.73) | 0.62 (0.50-0.78) |
|                         |         | Q4 (0.27; 0.87)           | 111/651   | 0.57 (0.45-0.73) | 0.58 (0.45-0.74) | 0.64 (0.51-0.82) |
|                         |         | Q5 (0.88; 3.54)           | 81/651    | 0.51 (0.38-0.68) | 0.52 (0.39-0.69) | 0.57 (0.43-0.75) |
| TTL to d-ROMs ratio (n = 3130) | OS | Q1 (−1.86; −0.70) | 313/626 | 1.00 | 1.00 |                |
|                         |         | Q2 (−0.69; −0.34)         | 245/626   | 0.76 (0.64-0.90) | 0.79 (0.66-0.94) |                |
|                         |         | Q3 (−0.33; 0.04)          | 184/626   | 0.56 (0.46-0.68) | 0.59 (0.49-0.72) |                |
|                         |         | Q4 (0.05; 0.58)           | 162/626   | 0.51 (0.41-0.63) | 0.55 (0.44-0.67) |                |
|                         |         | Q5 (0.59; 10.05)          | 101/626   | 0.38 (0.30-0.49) | 0.42 (0.32-0.54) |                |
|                         | DSS     | Q1 (−1.86; −0.70)         | 206/626   | 1.00           | 1.00           | 1.00           |
|                         |         | Q2 (−0.69; −0.34)         | 155/626   | 0.70 (0.57-0.87) | 0.72 (0.58-0.89) | 0.72 (0.59-0.89) |
|                         |         | Q3 (−0.33; 0.04)          | 118/626   | 0.54 (0.42-0.68) | 0.55 (0.43-0.70) | 0.60 (0.48-0.76) |
|                         |         | Q4 (0.05; 0.58)           | 113/626   | 0.55 (0.43-0.71) | 0.56 (0.44-0.72) | 0.59 (0.46-0.76) |
|                         |         | Q5 (0.59; 10.05)          | 73/626    | 0.47 (0.35-0.64) | 0.49 (0.36-0.66) | 0.54 (0.40-0.72) |

Note: Statistically significant results are highlighted in bold.

Abbreviations: BMI, body mass index; CI, confidence interval; DSS, disease-specific survival; d-ROMs, Diacron’s reactive oxygen metabolites; HR, hazard ratio; OS, overall survival; sHR, subdistribution hazard ratio; TTL, total thiol level.

aResults from the Fine and Gray model are accounted for competing events (number of events are different from those reported for the Cox models).

bAdjusted for age, sex, tumor stage, tumor site, year of diagnosis, years of school education, BMI, lifetime physical activity, smoking status, lifetime alcohol consumption, vegetable and fruit intake, time of blood draw according to chemo(radio)therapy initiation and according to CRC surgery, tumor site $\times \log$(time), year of diagnosis $\times \log$(time) and BMI $\times \log$(time).

cFurther adjustment for Charlson comorbidity score.
3.2 Associations of oxidative stress biomarkers with CRC prognosis

During a follow-up period of 6 years, 1078 (32%) deaths occurred, of which 708 (66%) were due to CRC. Table 2 shows hazard ratios (HRs) for the associations of d-ROMs, TTL and their ratio with CRC prognosis. In patients of all stages, those in the highest d-ROMs (vs lowest) quintile had 27% increased all-cause mortality, but this association was slightly attenuated and lost statistical significance after adjusting for comorbidities (HR = 1.21, 95% CI = 0.99-1.48). Higher TTL was associated with substantially lower mortality. HRs for the second, third, fourth, and fifth quintiles, compared to the lowest quintile, were 0.92, 0.66, 0.58 and 0.48 for OS (P < .001) and 0.87, 0.58, 0.58 and 0.52 for DSS (P < .001). Even stronger associations were seen for the TTL to d-ROMs ratio. HRs for the second, third, fourth and fifth quintiles, compared to the lowest quintile, were 0.79, 0.59, 0.55 and 0.42 for OS (P < .001) and 0.72, 0.55, 0.56 and 0.49 for DSS (P < .001). In the competing risk models, the associations of higher levels of TTL and the TTL to d-ROMs ratio with lower CRC mortality were only slightly attenuated. Further adjustment for MSI status did not affect the observed associations (Table S1).

In stage-specific analyses, we observed significant associations between higher d-ROMs and poorer survival in stages I-II and IV but not in stage III (Table 3 and Figure 1). Compared to the lowest tertile, stage I-II (HR = 1.38, 95% CI = 1.04-1.83) and IV patients (HR = 1.52, 95% CI = 1.14-2.04) in the highest tertile had poorer OS. In stage IV patients, those in the highest (vs lowest) tertile had much poorer DSS (HR = 1.61, 95% CI = 1.20-2.17). For TTL (Table 3 and Figure 2), strong inverse associations of TTL with both all-cause and CRC mortality were observed within all stages, but the associations were particularly pronounced for stages I-II and IV (Pinteraction = .070 for OS). Even slightly stronger associations of higher TTL to d-ROMs ratio with lower all-cause and CRC mortality were seen within all stages, with particularly pronounced associations in stage IV patients (Pinteraction = .013 for OS; Table 3 and Figure 3).

Figures 4 and 6 show stage-specific assessment of linear associations of d-ROMs and TTL with OS. For d-ROMs, nonlinear associations were observed in stages I-II and IV, with a steep increase in mortality for z-scores >0, which was especially strong for stage IV.

### TABLE 3 Associations of oxidative stress biomarkers with survival outcomes stratified by tumor stage

| Subgroup | Outcome | d-ROMs | TTL | TTL to d-ROMs ratio |
|----------|---------|--------|-----|---------------------|
|         |         | n_{event} | HR (95% CI) | n_{event} | HR (95% CI) | n_{event} | HR (95% CI) |
|         |         | n_{at risk} | Interaction (OS, 0.728) | n_{at risk} | Interaction (OS, 0.701) | n_{at risk} | Interaction (OS, 0.233) |
| Stages I-II | OS | Low | 943/594 | 1.00 | 165/590 | 1.00 | 158/574 | 1.00 |
|           |     | Moderate | 102/593 | 0.99 (0.74-1.31) | 109/593 | 0.70 (0.54-0.90) | 109/572 | 0.75 (0.57-0.98) |
|           |     | High | 114/597 | 1.38 (1.04-1.83) | 61/597 | 0.48 (0.35-0.67) | 54/571 | 0.42 (0.29-0.60) |
|           | DSS | Low | 39/594 | 1.00 | 33/593 | 0.55 (0.35-0.89) | 48/574 | 1.00 |
|           |     | Moderate | 36/593 | 0.83 (0.52-1.32) | 27/597 | 0.54 (0.32-0.92) | 35/572 | 0.83 (0.51-1.34) |
|           |     | High | 39/597 | 0.79 (0.49-1.29) | 27/597 | 0.54 (0.32-0.92) | 27/571 | 0.73 (0.41-1.28) |
| Stage III | OS | Low | 114/343 | 1.00 | 161/353 | 1.00 | 157/336 | 1.00 |
|           |     | Moderate | 109/349 | 0.86 (0.66-1.13) | 108/356 | 0.68 (0.52-0.88) | 99/338 | 0.62 (0.47-0.82) |
|           |     | High | 130/349 | 1.06 (0.80-1.40) | 88/354 | 0.65 (0.48-0.89) | 87/338 | 0.62 (0.45-0.84) |
|           |     | Moderate | 72/349 | 0.78 (0.56-1.08) | 74/356 | 0.71 (0.51-0.97) | 71/338 | 0.69 (0.50-0.96) |
|           |     | High | 88/349 | 0.90 (0.64-1.26) | 68/354 | 0.72 (0.50-1.03) | 64/338 | 0.69 (0.48-1.01) |
| Stage IV  | OS | Low | 105/136 | 1.00 | 132/137 | 1.00 | 127/133 | 1.00 |
|           |     | Moderate | 121/139 | 1.26 (0.96-1.66) | 116/137 | 0.74 (0.55-0.98) | 113/134 | 0.54 (0.40-0.73) |
|           |     | High | 124/137 | 1.52 (1.14-2.04) | 103/137 | 0.49 (0.37-0.66) | 101/134 | 0.46 (0.34-0.62) |
|           | DSS | Low | 100/136 | 1.00 | 124/137 | 1.00 | 122/133 | 1.00 |
|           |     | Moderate | 110/139 | 1.22 (0.92-1.63) | 110/137 | 0.74 (0.55-0.99) | 104/134 | 0.52 (0.38-0.70) |
|           |     | High | 119/137 | 1.61 (1.20-2.17) | 96/137 | 0.49 (0.36-0.66) | 95/134 | 0.45 (0.33-0.62) |

Note: Statistically significant results are highlighted in bold. Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; DSS, disease-specific survival; d-ROMs, Diacron’s reactive oxygen metabolites; OS, overall survival; TTL, total thiol level.

*Adjusted for age, sex, tumor site, tumor site × log(time), year of diagnosis, year of diagnosis × log(time), time of blood draw according to chemoradiotherapy initiation and according to CRC surgery, education level, BMI, BMI × log(time), lifetime physical activity, smoking status, lifetime alcohol consumption, vegetable and fruit intake and Charlson comorbidity score (backward selection of variables with P < .05; age and sex were forced into the models).
patients (Figure S4). A U-shaped association was observed in stage III, with a much higher mortality in patients having z-scores <−1.0 or >1.0. A strong monotonic association between higher TTL and lower mortality was seen in all stages (Figure S5). For the TTL to d-ROMs ratio, nonlinear associations with OS were observed, with a much lower survival in patients having z-scores <0 (Figure S6). No significant interactions between the oxidative stress biomarkers and tumor subsite for the investigated survival outcomes were observed (data not shown).

### 3.4 Subgroup analyses by sex, age, comorbidity, smoking status, MSI status and chemo(radio)therapy

There was a significant interaction between d-ROMs and comorbidity for OS (Table S2). For example, higher d-ROMs (highest vs lowest tertile) were associated with poorer OS in patients without comorbidities (HR = 1.45, 95% CI = 1.15-1.82), whereas no associations were seen in patients with comorbidities (P_interactions = .037). For TTL, similarly higher OS with increasing TTL was seen across genders.
comorbidity levels and smoking categories. The associations of TTL with survival were, however, restricted to patients aged <75 years ($P_{interaction} = .001$). Subgroup analyses by MSI status (Table S3) showed particularly pronounced associations of higher d-ROMs levels with lower survival among MSI-high patients, with less clear patterns of TTL levels with survival among MSI-high patients. No significant interactions between the oxidative stress biomarkers and chemo(radio)therapy use (yes/no) for the investigated survival outcomes were observed in stage II-IV or II-III patients (data not shown).

FIGURE 2  Adjusted survival curves for the association of TTL with all-cause mortality, overall and by tumor stage. Survival curves were adjusted for age, sex, tumor site, tumor site $\times \log$(time), year of diagnosis, year of diagnosis $\times \log$(time), time of blood draw according to chemo(radio)therapy initiation and according to colorectal cancer surgery, education level, BMI, BMI $\times \log$(time), lifetime physical activity, smoking status, lifetime alcohol consumption, vegetable and fruit intake and Charlson comorbidity score. BMI, body mass index; TTL, total thiol level [Color figure can be viewed at wileyonlinelibrary.com]

3.5 Subgroup analyses by time of blood sampling

Comparable associations of d-ROMs and TTL with OS were seen across strata defined by time of blood sampling with respect to CRC surgery and chemo(radio)therapy initiation (Table S4). However, the associations of TTL with OS seemed weaker in recipients of chemo(radio)therapy whose blood samples were taken within 5 months of initiation of such treatment ($P_{interaction} = .001$). Also, an inverse association between d-ROMs and all-cause mortality was seen in this patient group. But among patients receiving chemo(radio)therapy, there was no difference
in all-cause (HR = 0.99, 95% CI = 0.76-1.29) or CRC mortality (HR = 1.04, 95% CI = 0.78-1.39) between patients whose samples were taken after and before initiation of such treatment (data not shown).

3.6 Added value of d-ROMs and TTL in the prediction of CRC prognosis

Table 4 shows the C-indexes of models that included age, sex, tumor stage and site and those that additionally included d-ROMs and TTL. Results showed improvement in the prediction of CRC prognosis by the addition of d-ROMs and TTL in both the training and validation sets. Results from the validation set were very similar to those from the training set and the whole sample. In the whole study sample, the C-indexes of models with and without d-ROMs and TTL were 0.772 and 0.755 (P < .001) for OS and 0.832 and 0.823 (P < .001) for DSS, respectively. Stage-specific analyses similarly demonstrated superiority of the models with to those without d-ROMs and TTL in all stages and for both OS and DSS, with particularly pronounced improvements in stage IV patients. In stage IV patients, the C-indexes of models with

FIGURE 3 Adjusted survival curves for the association of TTL to d-ROMs ratio with all-cause mortality, overall and by tumor stage. Survival curves were adjusted for age, sex, tumor site, tumor site × log(time), year of diagnosis, year of diagnosis × log(time), time of blood draw according to chemo(radio)therapy initiation and according to colorectal cancer surgery, education level, BMI, BMI × log(time), lifetime physical activity, smoking status, lifetime alcohol consumption, vegetable and fruit intake, and Charlson comorbidity score. BMI, body mass index; d-ROMs, Diacron’s reactive oxygen metabolites; TTL, total thiol level [Color figure can be viewed at wileyonlinelibrary.com]
and without d-ROMs and TTL were 0.615 and 0.566 (P = .001) for OS and 0.608 and 0.557 (P = .002) for DSS, respectively.

### Discussion

Oxidative stress has been implicated in the initiation of several cancers, including CRC. Whether it also plays a role in CRC prognosis is unclear. We estimated the associations of two oxidative stress biomarkers (d-ROMs and TTL) with CRC prognosis. We found particularly poorer survival in stage IV patients with higher levels of d-ROMs. We also observed strong monotonic associations between TTL and survival within all stages, and the ratio of TTL and d-ROMs was even a stronger predictor of CRC prognosis than TTL alone. The addition of d-ROMs and TTL to prognostic models that included age, sex, tumor stage and subsite, moreover, significantly improved the prediction of CRC prognosis, overall and within all stages, with improvements being particularly pronounced in stage IV patients.

Several previous studies have reported associations of oxidative stress with poorer prognosis for patients with other types of cancer. For example, higher d-ROMs were found to be associated with poorer OS in patients with follicular lymphoma and lung cancer. Higher TTL was also found to be associated with higher OS in lung cancer patients. Whether these biomarkers also predict CRC prognosis has been unclear. To the best of our knowledge, only one small-sized study has assessed the associations of different oxidative stress biomarkers (8-oxo-7,8-dihydro-2-deoxyguanosine and 8-oxo-7, 8-dihydroguanine) with CRC prognosis. The authors found poorer OS in patients with higher levels of these biomarkers, but they did not adjust for any of the known predictors of CRC prognosis such as tumor stage, age and comorbidities.

Results from our large patient cohort, which were adjusted for a large number of relevant factors including tumor stage, comorbidities and time of blood sampling with respect to surgery and chemoradiotherapy initiation, showed poorer prognosis in patients with higher d-ROMs and lower TTL. Stage-specific analyses demonstrated particularly pronounced associations in stage IV patients. These findings suggest that an imbalanced redox state might play a major role in premature mortality in CRC patients. This hypothesis is further supported by the particularly pronounced association between the TTL to d-ROMs ratio, which reflects the balance between redox control and oxidative stress, and survival. To the best of our knowledge, our study was the first to assess this association in a cancer cohort.

There are several possible mechanisms through which oxidative stress might be related to CRC prognosis. First, besides tumor initiation, oxidative stress might also be involved in tumor promotion and progression. For example, cellular hypoxia is common in highly proliferating cells and induces mitochondrial ROS. Elevated ROS in turn stimulates chronic inflammation and maintains telomerase activity of tumor cells, leading to chemo(radio)resistance and increased survival of tumor cells. Higher d-ROMs and lower TTL might thus in part

### Table 4

Comparison of discriminatory ability of prediction models with and without d-ROMs and TTL

| Outcomes       | Whole study sample | Training set | Validation set | Training set | Validation set |
|----------------|--------------------|--------------|----------------|--------------|----------------|
|                | C index            |              |                | C index      |                |
|                | Model 1            | Model 2      | P              | Model 1      | Model 2        | diff           |
|                | Overall survival   | 0.755        | 0.772          | <.001        | 0.758          | 0.775          | 0.017          |
|                | Disease-specific   | 0.823        | 0.832          | <.001        | 0.825          | 0.835          | 0.010          |
| Stages I-III   | Overall survival   | 0.683        | 0.715          | .001         | 0.690          | 0.724          | 0.034          |
|                | Disease-specific   | 0.694        | 0.718          | .046         | 0.709          | 0.738          | 0.029          |
| Stage III      | Overall survival   | 0.638        | 0.669          | <.001        | 0.643          | 0.674          | 0.031          |
|                | Disease-specific   | 0.595        | 0.630          | <.001        | 0.607          | 0.644          | 0.037          |
| Stage IV       | Overall survival   | 0.566        | 0.615          | .001         | 0.580          | 0.625          | 0.045          |
|                | Disease-specific   | 0.557        | 0.608          | .002         | 0.573          | 0.620          | 0.047          |

Note: Model 1 included age, sex, tumor stage and subsite. Model 2: model 1 + d-ROMs and TTL (quintiles for patients of all stages and tertiles for stage-specific analyses).

Abbreviations: C index, Harrell's concordance index; diff, difference; d-ROMs, Diacron's reactive oxygen metabolites; TTL, total thiol level.

a1000 bootstrapped samples were created and the models were trained in 2/3 of each of the samples (training set) and the predictive accuracy of the regression coefficients were assessed in the remaining 1/3 of the samples (validation set). Samples were split into training and validation sets using random sampling.
reflect proliferative activity of the tumor. Second, oxidative stress has been implicated in the aging process, possibly through telomere erosion and cell senescence. For example, we found lower TTL and weak associations of TTL with survival in older patients, suggesting that the low TTL levels in this age group might primarily reflect the aging process. Third, it is possible that the association of oxidative stress with poorer prognosis in CRC patients is modified or partly explained by comorbidity, a prognostic factor of CRC. Although we adjusted for comorbidities in our analysis, we cannot exclude residual confounding by comorbidities or by unmeasured factors such as frailty. The observed strong associations persisting within tumor stages and across several patient subgroups are, however, unlikely to be due to just confounding. Another possible mechanism by which oxidative stress may impact survival is modification by DNA methylation, which has been suggested for other cancers, such as prostate cancer.

Because endogenous antioxidants (eg, glutathione) play important roles in detoxifying ROS, it has been hypothesized that exogenous supplements (eg, vitamin C) might also help scavenge free radicals. The potential for vitamin supplementation to correct redox imbalance has, however, not been demonstrated in clinical trials. Data from clinical trials suggest that regular consumption of vegetables and fruits might lower ROS. Given that an unhealthy lifestyle is associated with oxidative stress and worse health outcomes, eg, risk of long-term conditions and mortality, the adoption of a healthy lifestyle (eg, regular physical activity and smoking cessation) rather than just vitamin supplementation might help to achieve a balanced redox state and thereby enhance CRC prognosis.

Our study has limitations. First, we excluded patients lacking information on the measured biomarkers, which could have led to selection bias. However, it is worth mentioning that the excluded sample was small (n = 310, 8%) and was not different from the included sample in terms of age (mean 69.8 vs 68.3 years) but included a somewhat lower proportion of stage IV patients (13.6% vs 19%). Second, even though d-ROMs and TTL were quantified using standardized methods, their levels might be affected by surgery or chemo(radio)therapy administration. We made efforts to address this by excluding patients whose blood samples were taken <4 days after CRC surgery or <8 days after chemo(radio)therapy initiation and by also adjusting for time of blood sampling in our multivariable analysis. Even though no uniform timing of recruitment and blood sampling could be realized in this population-based study, our subgroup analysis of the associations of the biomarkers by time of blood sampling might provide useful information on the appropriate time for blood sampling for evaluation of oxidative stress biomarkers. Future studies should determine the most appropriate time point for blood sample collection for assessment of oxidative stress biomarkers. Third, it has been suggested that the d-ROMs assay also assesses non-ROMs such as ceruloplasmin and that some serum components (eg, thiols and albumin) might reduce lipid hydroperoxides levels, which might affect the accuracy of our d-ROMs measurement. However, regardless of uncertainties in the measurement of d-ROMs, previous studies using serum samples to estimate d-ROMs have similarly found associations between higher d-ROMs levels and increased mortality in other cancer types, including follicular lymphoma and lung cancer. Finally, our preliminary analyses suggested that the associations of d-ROMs and TTL with CRC prognosis might be weaker for patients with >6 years of follow-up. This suggests that it might not be possible for a one-time measurement of oxidative stress to be used for predicting CRC outcomes after longer follow-up (eg, 10+ years). We addressed this by censoring patients at 6 years of follow-up.

Major strengths of our study include recruitment of patients in a population-based setting, the use of prospective design and large sample size. Also, we were able to adjust for a large number of relevant factors such as tumor stage, age, comorbidities, and time of blood draw according to CRC surgery and chemo(radio)therapy initiation (which also takes into account whether or not a patient used CRC treatments). In particular, we were able to conduct subgroup analyses according to tumor stage, age, comorbidities, MSI status and chemo(radio)therapy use, which might be useful in understanding to what extent oxidative stress impacts CRC prognosis in specific patient groups. Also, results from the stage-specific analyses and from the assessment of the predictive accuracy of prognostic models with and without the measured oxidative stress biomarkers provide evidence on the potential for these biomarkers in enhancing risk stratification of CRC patients beyond tumor stage.

In conclusion, higher d-ROMs and lower TTL levels were associated with substantially poorer prognosis in CRC patients, including stage IV patients, in this large population-based study. These findings suggest that an imbalanced redox state might contribute significantly to premature mortality in CRC patients. Regardless of uncertainties on to what extent the observed associations reflect causality, the measured biomarkers may help to refine the prediction of prognosis beyond tumor stage at diagnosis, the by far most important prognostic factor in CRC patients. Future research should address if and to what extent such information could also be useful to inform treatment decisions or for monitoring treatment success in CRC patients.

ACKNOWLEDGEMENT

We would like to thank Ute Handte-Daub, Ansgar Brandhorst, Petra Bächer and Piet Beekhof for their excellent technical assistance. We are particularly grateful to the study participants, as well as the interviewers who assisted in the data collection. We also gratefully appreciate the cooperation of the below-listed clinics and institutions: Chirurgische Universitätsklinik Heidelberg, Klinik am Gesundbrunnen Heilbronn, St. Vincentiuskrankenhaus Speyer, St. Josefskrankenhaus Heidelberg, Chirurgische Universitätsklinik Mannheim, Diakonissenkrankenhaus Speyer, Krankenhaus Salem Heidelberg, Kreiskrankenhaus Schwetzingen, St. Marienkrankenhaus Ludwigshafen, Klinikum Ludwigshafen, Stadtklinik Frankenthal, Diakoniekrankenhaus Mannheim, Kreiskrankenhaus Sinsheim, Klinikum am Plattenwald Bad Friedrichshall, Kreiskrankenhaus Weinheim, Kreiskrankenhaus Eberbach, Kreiskrankenhaus Buchen, Kreiskrankenhaus Mosbach, Enddarmzentrum Mannheim, Kreiskrankenhaus Brackenheim and Cancer Registry of Rhineland-Palatinate, Mainz. This work was supported by grants from the German Research Council (BR 1704/6-1.
BR 1704/6-3, BR 1704/6-4, CH 117/1-1; German Federal Ministry of Education and Research (01KH0404, 01ER0814, 01ER0815, 01ER1505A, 01ER1505B); and the Ministry of Science, Research and Arts of Baden-Wuerttemberg. The funding bodies had no role in the design, performance of analysis or the decision to publish our study.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

DATA ACCESSIBILITY
The data supporting findings from our study are available from the corresponding author upon request.

ETHICS STATEMENT
The DACHS study was approved by the ethics committees of the Medical Faculty of Heidelberg University and the state medical boards of Baden-Wuerttemberg and Rhineland-Palatinate. All participants gave written informed consent.

ORCID
Daniel Boakye https://orcid.org/0000-0003-4684-0841
Lina Jansen https://orcid.org/0000-0001-8004-4940
Ben Schöttker https://orcid.org/0000-0002-1217-4521
Michael Hoffmeister https://orcid.org/0000-0002-8307-3197

REFERENCES
1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
2. Johnson CM, Wei C, Ensor JE, et al. Meta-analyses of colorectal cancer risk factors. Cancer Causes Control. 2013;24:1207-1222.
3. Block G, Dietrich M, Norkus EP, et al. Factors associated with oxidative stress in human populations. Am J Epidemiol. 2002;156:274-285.
4. Jones DP. Redefining oxidative stress. Antioxid Redox Signal. 2006;8:1865-1879.
5. Barzilai A, Yamamoto K. DNA damage responses to oxidative stress. DNA Repair. 2004;3:1109-1115.
6. Kryston TB, Georgiev AB, Pissis P, Georgakilas AG. Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res. 2011;711:193-201.
7. Perse M. Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence? Biomed Res Int. 2013;2013:9.
8. Griendling KK, Touyz RM, Zweier JL, et al. Measurement of reactive oxygen species, reactive nitrogen species, and redox-dependent signaling in the cardiovascular system: a scientific statement from the American Heart Association. Circ Res. 2016;119:e39-e75.
9. Leufkens AM, van Duijnhowen FI, Woudt SH, et al. Biomarkers of oxidative stress and risk of developing colorectal cancer: a cohort-nested case-control study in the European prospective investigation into cancer and nutrition. Am J Epidemiol. 2012;175:653-663.
10. Araki O, Matsumura Y, Inoue T, et al. Association of perioperative redox balance on long-term outcome in patients undergoing lung resection. Ann Thorac Cardiovasc Surg. 2018;24:13-18.
11. Schottker B, Brenner H, Jansen EH, et al. Evidence for the free radical/oxidative stress theory of ageing from the CHANCES consortium: a meta-analysis of individual participant data. BMC Med. 2015;13:300.
12. Gao X, Wilsgaard T, Jansen EH, et al. Pre-diagnostic derivatives of reactive oxygen metabolites and the occurrence of lung, colorectal, breast and prostate cancer: an individual participant data meta-analysis of two large population-based studies. Int J Cancer. 2019;145:49-57.
13. Gao X, Wilsgaard T, Jansen EHJM, et al. Serum total thiol levels and the risk of lung, colorectal, breast and prostate cancer: a prospective case–cohort study. Int J Cancer. 2019;146:1261-1267.
14. Dziaman T, Banaszekiewicz Z, Roszkowski K, et al. 8-Oxo-7, 8-dihydroguanine and uric acid as efficient predictors of survival in colon cancer patients. Int J Cancer. 2014;134:376-383.
15. Boakye D, Walter V, Jansen L, et al. Magnitude of the age-advancement effect of comorbidities in colorectal cancer prognosis. J Natl Compr Canc Netw. 2020;18:59-68.
16. Walter V, Boakye D, Weberpals J, et al. Decreasing use of chemotherapy in older patients with stage III colon cancer irrespective of comorbidities. J Natl Compr Canc Netw. 2019;17:1089-1099.
17. Brenner H, Chang-Claude J, Jansen L, Knebel P, Stock C, Hoffmeister M. Reduced risk of colorectal cancer up to 10 years after screening, surveillance, or diagnostic colonoscopy. Gastroenterology. 2014;166:707-717.
18. Hoffmeister M, Jansen L, Rudolph A, et al. Statin use and survival after colorectal cancer: the importance of comprehensive confounder adjustment. J Natl Cancer Inst. 2015;107:djv045.
19. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373-383.
20. Deyo RA, Cherkin DC, Ciol MA. Adapting a clinical comorbidity index for use with ICD-9-CM administrative databases. J Clin Epidemiol. 1992;45:613-619.
21. Boakye D, Walter V, Martens UM, et al. Treatment selection bias for chemotherapy persists in colorectal cancer patient cohort studies even in comprehensive propensity score analyses. Clin Epidemiol. 2019;11:821-832.
22. Jansen E, Beekhof PK, Viezelienie D, Muzakova V, Skalicky J. Long-term stability of oxidative stress biomarkers in human serum. Free Radic Res. 2017;51:970-977.
23. Verde V, Fogliano V, Ritieni A, Maiani G, Morisco F, Caporaso N. Use of N,N-dimethyl-p-phenylenediamine to evaluate the oxidative status of human plasma. Free Radic Res. 2002;36:869-873.
24. Luo J, Schumacher M, Scherer A, et al. A comparison of batch effect removal methods for enhancement of prediction performance using MAQC-II microarray gene expression data. Pharmacogenomics J. 2010;10:278-291.
25. Xuan Y, Gao X, Anusruti A, et al. Association of serum markers of oxidative stress with incident major cardiovascular events, cancer incidence and all-cause mortality in type 2 diabetes patients: pooled results from two cohort studies. Diabetes Care. 2019;42(8):1436-1445.
26. Sobin L, Gospodarowicz MK, Wittekind C. TNM Classification of Malignant Tumours. 7th ed. West Sussex, UK: John Willey & Sons Ltd; 2009.
27. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999;94:496-509.
28. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol. 2005;23:609-618.
29. Harrell FE. Regression Modeling Strategies: with applications to linear models, logistic regression, and survival analysis. New York, NY: Springer; 2010.
30. Arakaki H, Osada Y, Takanashi S, Ito C, Aisa Y, Nakazato T. Oxidative stress is associated with poor prognosis in patients with follicular lymphoma. Blood. 2016;128:1787.
32. Afanas’ev I. Reactive oxygen species signaling in cancer: comparison with aging. Aging Dis. 2011;2:219-230.
33. Li P, Wu M, Wang J, Sui Y, Liu S, Shi D. NAC selectively inhibit cancer telomerase activity: a higher redox homeostasis threshold exists in cancer cells. Redox Biol. 2016;8:91-97.
34. Rodic S, Vincent MD. Reactive oxygen species (ROS) are a key determinant of cancer’s metabolic phenotype. Int J Cancer. 2018;142:440-448.
35. Liguori I, Russo G, Curcio F, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018;13:757-772.
36. Boakye D, Rillmann B, Walter V, Jansen L, Hoffmeister M, Brenner H. Impact of comorbidity and frailty on prognosis in colorectal cancer patients: a systematic review and meta-analysis. Cancer Treat Rev. 2018;64:30-39.
37. Boakye D, Jansen L, Schneider M, Chang-Claude J, Hoffmeister M, Brenner H. Personalizing the prediction of colorectal cancer prognosis by incorporating comorbidities and functional status into prognostic nomograms. Cancer. 2019;11:1435.
38. Donkena KV, Young CYF, Tindall DJ. Oxidative stress and DNA methylation in prostate cancer. Obs Gynecol Int. 2010;2010:14.
39. Fumeron C, Nguyen-Khoa T, Saltiel C, et al. Effects of oral vitamin C supplementation on oxidative stress and inflammation status in haemodialysis patients. Nephrol Dial Transplant. 2005;20:1874-1879.
40. Huang H-Y, Helzlsouer KJ, Appel LJ. The effects of vitamin C and vitamin E on oxidative DNA damage: results from a randomized controlled trial. Cancer Epidemiol Biomarkers Prev. 2000;9:647-652.
41. Carraro E, Schillirò T, Biorci F, et al. Physical activity, lifestyle factors and oxidative stress in middle age healthy subjects. Int J Environ Res Public Health. 2018;15:1152.
42. Sakhi AK, Russnes KM, Thoresen M, et al. Pre-radiotherapy plasma carotenoids and markers of oxidative stress are associated with survival in head and neck squamous cell carcinoma patients: a prospective study. BMC Cancer. 2009;9:458.
43. Kilk K, Meitern R, Hármon O, Soomets U, Hõrak P. Assessment of oxidative stress in serum by d-ROMs test. Free Radic Res. 2014;48:883-889.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Boakye D, Jansen L, Schöttker B, et al. Blood markers of oxidative stress are strongly associated with poorer prognosis in colorectal cancer patients. Int. J. Cancer. 2020;147:2373–2386. https://doi.org/10.1002/ijc.33018