Telomere attrition and decreased Fetuin-A levels indicate accelerated biological ageing and are implicated in the pathogenesis of Colorectal Cancer

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Statement of Translational Relevance

Biological ageing, comprises ageing at the cellular and organ level and is affected by genetic, metabolic and environmental factors. We have demonstrated that patients with colorectal cancer display clear evidence of accelerated biological ageing, in the form of telomere attrition, when compared with control subjects. Furthermore, those patients with short telomeres had lower levels of fetuin-A, a circulating inhibitor of calcification. Our results suggest that manipulation of factors known to determine telomere length could alter the risk profile associated with colorectal cancer. We have also provided further evidence that colon and rectal cancers are distinct clinical entities that may be distinguished by fetuin-A levels. Further molecular characterisation of colon and rectal cancers could lead to the identification of novel targets for chemotherapeutic agents that could be used to enhance the neo-adjuvant management of rectal cancers.

Abstract

Purpose
Increasing chronological age is a risk factor for many types of cancer including colorectal. An understanding of the biology of ageing and factors which regulate it may provide insight into cancer pathogenesis. The role of telomere biology in both the cancer and ageing process could prove useful in this regard.

Experimental Design
Using quantitative-PCR we determined telomere length in the peripheral blood leucocytes of 64 colorectal cancer patients and 1348 controls. We also measured telomere length in 32 colorectal tumour samples and matched normal tissue. We aimed to assess if telomere
lengths were reflected in circulating mediators of inflammation and redox control factors, including fetuin-A a circulating modulator of calcium homeostasis.

Results

Colorectal cancer patients had shorter telomeres (adjusted mean RelT/S=0.61) compared with chronologically older controls (mean age 75, adjusted mean RelT/S=0.70) (ANCOVA, p=0.004). Telomere length in tumour tissue (median=0.43, IQR=0.40) was significantly shorter than adjacent normal tissue (median=0.65, IQR=0.28) (p=0.004). Patients with low fetuin-A levels were shown to have significantly shorter telomeres (p=0.041). Patients with rectal tumours had significantly higher levels of fetuin-A than those with colonic tumours (p=0.045).

Conclusions

We have observed that patients with colorectal cancer display clear evidence of telomere attrition compared with controls. This is congruent with accelerated biological ageing in the pathogenesis of colorectal cancer. An imbalance in redox control mechanisms and calcium homeostasis may be a contributing factor to telomere dynamics in our patients. Furthermore, fetuin-A levels can be used distinguish between colon and rectal cancers.
Introduction

Colorectal cancer (CRC) is the third most common cancer in the UK and is responsible for approximately 16,000 deaths every year. This is despite five-year survival rates having doubled over the last thirty years. Increasing chronological age is a risk factor for many types of cancer including colorectal, with eighty percent of CRC cases occurring in patients over the age of sixty (1). Consequently, an understanding of the biology of ageing may provide insight into cancer pathogenesis (2). Biological ageing comprises ageing at the cellular and organ level and is affected by genetic, metabolic and environmental factors. Fully delineating the key molecular mechanisms underpinning both the biological ageing and cancer processes could improve the understanding of the disease process and lead to the discovery of novel biomarkers or targets for therapeutic intervention, further improving survival rates.

A key manifestation of ageing at the cellular level is telomere attrition. Telomeres are nucleoprotein structures located at the ends of all eukaryotic chromosomes and are composed of a repetitive guanine-rich DNA sequence (TTAGGG)n (3). They possess a number of critical functions including maintenance of genomic integrity by protecting chromosomes from fusion events, repair of DNA damage and maintenance of cellular stability (2). Telomere attrition is associated with increasing chronological age and furthermore may act as a biomarker of replicative ageing, or mitotic clock (4). Once this progressive loss of telomeric DNA content reaches a critical level, cells are stimulated to either apoptose, or enter replicative senescence (5).
Telomeres not only potentially serve as biomarkers of senescence and biological ageing, but also form part of a damage sensing and signalling system, facilitating DNA repair or apoptosis (6). Potential determinants of telomere length are varied. Telomere length is highly heritable therefore a proportion is genetically determined (7). Alteration in the expression of telomerase, can significantly affect telomere dynamics. In mice, knock out of the telomerase coding sequence resulted in progressive loss of telomeric DNA and progeria (8). Reintroduction of telomerase reversed both these effects (9). A major influence on telomere length and hence telomere function is the control of redox state and potential damage induced by reactive oxygen species (10, 11). Correlation of telomere length with genes controlling redox state in a narrow age range cohort provides further evidence for a plausible mechanistic link between redox control and telomere biology (12). Pertinent in this respect, is the observation that fetuin-A, a mediator of redox homeostasis in the circulation, displays a dependent relationship with PBL telomere lengths (13).

Evidence is accumulating that telomere attrition and senescence are contributory factors in a range of age related disease processes including cancer (14), cardiovascular disease (15), chronic kidney disease (13) and pulmonary disease (16). Furthermore, critically short telomeres have been linked with life stress (17) and an overall increased likelihood of mortality (18). The body of work aimed at delineating the relationship between critical telomere attrition and the cancer process is rapidly expanding. Short telomeres in peripheral blood leucocytes (PBL) have been shown to be associated with an increased risk in a variety of solid tumours (19-22).
In the present study we aimed to test the hypothesis that patients with colorectal cancer display evidence of accelerated biological ageing in the form of telomere attrition when compared with healthy age matched controls. By determining telomere length in PBL, tumour tissue and normal colonic tissue we aimed to provide key information on telomere dynamics in each of these important cell compartments. Furthermore, we aimed to assess if telomere length in the PBLs of CRC patients were reflected in circulating mediators of inflammation and factors known to exert control over redox state including fetuin-A. Rationalising the determinants of telomere length in CRC cancer patients could provide valuable information used to enhance current management strategies in this important group of cancer patients.

**Materials and Methods**

**Patient Recruitment & Sample Collection**

For this study two patient groups were employed. The first group was comprised of patients recruited prospectively from the Department of Colorectal Surgery, Glasgow Royal Infirmary. All patients were admitted for assessment and management of histologically proven colorectal adenocarcinoma. For the first group blood samples (n=64) were collected for analysis during diagnostic workup and used to measure PBL telomere lengths in addition to potential correlates of telomere length including redox control factors, markers of systemic inflammation and fetuin-A (this group will be referred to as the CRC PBL group). The second group utilised colorectal cancer tissue (tumour and normal) collected under the auspices of the local biobank (NHS Greater Glasgow & Clyde). Patients were approached pre-operatively by biobank staff and full informed consent given for the collection and use of excess tissue for research purposes. All samples were snap frozen in liquid nitrogen and
stored at -80°C until use. All tissue samples were validated by a consultant pathologist and deemed representative of the pathological specimen. A total of 62 paired samples were obtained from the biobank, however DNA was available for analysis from 32 (this group will be referred to as the CRC tissue group). Where possible biochemical, haematological and pathological data were extracted from a prospectively maintained database. The Peterson Index (PI) was used as an additional measure to identify patients with pathologically more aggressive disease and hence poorer outcome. Pathologically determined vascular invasion, margin involvement or serosal breach was allocated a score of 1, with tumour perforation scoring 2. A cumulative score of 0-1 indicates a low risk and 2-5 a high risk PI. A high risk PI suggests aggressive disease and has been shown to correlate with a poorer outcome from colorectal cancer in lymph node negative patients (23). Table 1 presents details of the patient clinical and pathological variables for both groups. Full ethical approval from the local NHS ethics committee was gained prior to the commencement of any sample collection.

The control population was comprised of subjects from the West of Scotland Twenty-07 Cohort. Subjects used to form this cohort comprised of 1348 individuals aged either 57 (n=847) or 76 (n=501). This is a community-based cohort study designed to longitudinally investigate the social processes that produce or maintain inequalities in health and has been described in detail previously (24). The two age groups were specifically chosen to demonstrate that both the chronologically older group and an age adjusted combined group had longer telomeres than colorectal cancer patients.
DNA extraction

DNA was extracted from both blood and tissue using the Maxwell® automated purification system according to the manufacturer’s instructions (Promega, WI, USA). Briefly, whole blood samples were spun down into cellular and plasma components. The red cells and buffy coat were thoroughly mixed and 300µl aliquoted into the predispensed reagent cartridges. Tissue samples were thawed and 50mg added to the reagent cartridge. The DNA concentration and purity were quantified using the Nanodrop spectrophotometer (Thermo Fisher Scientific, MA, USA). All DNA samples were validated on 0.5% agarose gel.

Telomere length Determination

Telomere lengths were determined from DNA samples, from the PBLs of both the control and CRC population and from the colorectal normal and cancerous tissue, by Q-PCR (25). Telomere length determination was performed blindly using a Roche Light Cycler LC480. Briefly, telomere length analyses were performed in triplicate for each sample, using a single-copy gene amplicon primer set (acidic ribosomal phosphoprotein, 36B4) and a telomere-specific amplicon primer set (26). This method determines the ratio of telomere repeat copy number to single copy gene number (T/S) ratio in experimental samples relative to a control sample DNA. This normalised T/S ratio was used as the estimate of relative telomere length (Relative T/S). The co-efficient of variation (CV) for the telomere assay was 17%.
**Measurement of Plasma Fetuin-A**

Fetuin-A concentrations were measured from the plasma of blood samples of CRC PBL patients using a commercial Human Fetuin-A ELISA Kit (BioVendor R&D, Czech Republic). Fetuin-A was not measured in either the CRC tissue group or the control population. Samples were measured in triplicate. Absorbance of each sample was read by a microplate reader at dual wavelengths 405nm and 650nm, and sample concentrations were then calculated using the standard curve. All methodologies were carried out according to the manufacturer’s instructions.

**Measurement of Markers of Systemic Inflammation**

For the CRC PBL patients only, all routinely available indices of inflammation (CRP, albumin, white cell, neutrophil and lymphocyte count) were measured using routine methods in the Departments of Haematology and Biochemistry, Glasgow Royal Infirmary. Plasma concentrations of interleukin (IL)-6, IL-10 and vascular endothelial growth factor (VEGF) were measured using commercially available enzyme linked immunosorbent assay (ELISA) kits. The neutrophil to lymphocyte ratio (NLR) and modified Glasgow Prognostic Score (mGPS) was calculated for each patient thereby giving an estimation of systemic inflammation. mGPS is calculated by awarding a point for plasma albumin level under 35 mg/dl and C- Reactive Protein (CRP) under 10 mg/l thereby giving a score of 0,1 or 2. A score of 1 or 2 indicates higher levels of systemic inflammation and has been shown in numerous cancer types to correlate with poor cancer specific survival (27).
Measurement of Redox Control Factors

The separated plasma from each blood sample was used to create a redox profile for each CRC PBL patient. Concentrations of antioxidant vitamins A (retinol) and E (α-tocopherol) and the carotenoids (lutein, lycopene, α- and β-carotene) were determined using a high performance liquid chromatography (HPLC) based assay as previously described (28). Plasma was de-proteinised with alcohol containing internal standards and extraction of the analytes was performed using hexane. Analysis was carried out using reversed phase–HPLC (5 µm microbore, Phenomenex, Macclesfield, UK) and dual wavelength monitoring (Waters, MA). The 95% normal reference intervals for the above assays as established in our laboratory were as follows: retinol (1.0-2.8 µmol/l), α-tocopherol (14-39 µmol/l), lutein (82-202 µg/l), lycopene (100-300 µg/l), α-carotene (14-60 µg/l) and β-carotene (92-312 µg/l).

Statistical Analysis

All clinical, pathological and biochemical data were displayed either categorically or as median with inter-quartile range (IQR). Telomere length was analysed as both a continuous and categorical variable by sub-division into quartiles. Pearsons correlations were performed to establish any relationships between the various parameters. Comparison between groups of continuous variables was achieved using the Mann-Whitney or Wilcoxon Signed Rank test and categorical variables by chi-square analysis. Telomere length was corrected for age and sex using analysis of covariance analysis. All analyses were performed using SPSS version 15 (SPSS Inc, Chicago, Illinois).
Results

Telomere Length and Colorectal Cancer

Analysis of telomere lengths in peripheral blood leucocytes (PBLs) of colorectal cancer patients and healthy controls

Sixty-four (64) patients were available for analysis in the CRC PBL patient group (mean age = 68 ± 10.8). These were compared with one thousand three hundred and forty eight (1348) West of Scotland control subjects (no diagnosed cancer), aged approximately 57 (n=847, male 46%, female 54%) and 76 (n=501, male 42%, female 58%) years old (mean age = 64 ± 9.24). As expected there was a significant negative association between chronological age and telomere length in the healthy controls (Pearson r=-0.215, p<0.001). Likewise a significant negative relationship was observed between chronological age and PBL telomere length in the CRC group (Pearson r=-0.257, p=0.04), indicating that as patient age increased, telomere length decreased. This age-related telomere attrition demonstrates the association between chronological and biological ageing.

Patients had consistently shorter telomeres than the control population (p<0.001) (Figure 1), indicating that the colorectal cancer patients were of increased biological age. Since the median age of the control population was greater than that of the CRC group, analyses were performed correcting for both age and gender. After correction of telomere length for age and gender, colorectal cancer patients still had consistently shorter telomeres (adjusted mean RelT/S=0.66±0.02(se)) compared with those in the control group (adjusted mean RelT/S=0.75±0.005(se), p<0.001), indicating that the colorectal cancer patients were of increased biological age. To further validate that the cancer patients were more biologically
aged, we compared them to a sub-group of the control population, those individuals aged 76yrs. Analysis of covariance revealed that despite being on average chronologically younger, the cancer group had significantly shorter telomeres (adjusted mean RelT/S=0.61±0.03(se)) than the control population (adjusted mean RelT/S=0.70±0.01(se)) and hence were more biologically aged (p=0.004). The median duration of diagnosis in the CRC PBL group (defined as the date of positive tissue diagnosis until date of sample collection) was 42 (6-185) days. Analysis did not reveal any association between duration of diagnosis and telomere length.

Comparison of telomere lengths in colorectal tumour tissue and normal adjacent tissue

The relative T/S ratio of 32 matched colorectal tumour tissue and adjacent normal mucosa samples was compared to determine whether a difference in biological age was apparent between tissue types. Telomere length in the tumour tissue (median=0.43, IQR=0.40) was found to be significantly shorter than in the adjacent normal tissue (median=0.65, IQR=0.28) (p=0.004, Figure 2).

Clinical and Laboratory Correlates of Telomere Length in Colorectal Cancer Patients

Association between PBL telomere length and patient clinico-pathological characteristics

No associations were observed between PBL telomere length and the various clinico-pathological parameters when analysed using the continuous variable. Consequently, patients
were categorised into those with short telomeres (RelT/S < 0.55, shortest quartile) and those with long telomeres (RelT/S > 0.55). Short telomere length was significantly positively associated with high risk pathological features indicated by a high risk Peterson Index (Chi square, P=0.035). However, no significant relationship was identified between tumour site, Dukes stage, and any other clinico-pathological characteristic measured. No significant relationship was observed between telomere length and CRP, pro-inflammatory cytokines or mGPS. However, there was a significant relationship between patients with short telomere length and an elevated neutrophil:lymphocyte. (Mann-Whitney, p=0.047, Table 2). There was no significant association between telomere length and anti-oxidant status as determined by the measurement of antioxidant vitamins and micronutrients.

Plasma levels of Fetuin-A are associated with chronological and biological age in colorectal cancer

Fetuin-A concentration of plasma samples was measured to assess whether levels were associated with chronological and biological ageing within the CRC PBL group. A significant relationship was observed between fetuin-A concentration and the chronological age of subjects (Pearson r=-0.32, p=0.011, Table 2), increasing chronological age was associated with decreasing fetuin-A concentration. For analysis of the relationship between fetuin-A concentration and telomere length, patients were categorised into two groups around the median plasma level giving a low fetuin-A level group (fetuin-A < log median=1.47/median=29.6) and a high fetuin-A level group (fetuin-A > log median=1.47/median=29.6). Patients with low fetuin-A levels were shown to have significantly shorter telomeres (median RelT/S=0.6) than those patients with high fetuin-A levels (median RelT/S = 0.72) (Pearson r=0.3, p=0.019, Mann Whitney p=0.041, Table 2),
this relationship was maintained when the analysis was adjusted for age. Patients with low fetuin-A levels had significantly shorter telomeres (adjusted mean Rel T/S=0.59) in comparison to those patients with high fetuin-A levels (adjusted mean RelT/S =0.68, p=0.047). No difference in fetuin-A levels or telomere length was observed between males and females.

Associations between Fetuin-A plasma levels and patient clinico-pathological parameters

No association was apparent between fetuin-A concentration and tumour characteristics such as T-stage, lymph node involvement or Dukes stage, within the CRC PBL group. However, increasing concentrations of fetuin-A were significantly associated with increasing levels of albumin (Pearson r=0.28, p = 0.03) and calcium (Pearson r=0.30, p=0.022), but decreasing levels of interleukin 6 (Pearson r=-0.483, p=0.005) (Table 2). A trend also existed between fetuin-A concentration and interleukin 10 (Pearson r=-0.21, p=0.061) (Table 2), whereby increasing concentrations of fetuin-A in patients was associated with decreasing levels of interleukin 10.

Tumour site is distinguishable by Fetuin-A and White Cell Count

Patients in the CRC PBL group with rectal tumours (n=22) were associated with higher circulating concentrations of fetuin-A (log median=1.52/median=33.5), whereas those with colonic tumours (n=42) were associated with lower concentrations (log median=1.45/median=28.7) (p=0.045) (Figure 3a). Further comparison of the clinico-pathological differences between colonic and rectal tumours showed that colonic tumours
were significantly associated with an increased white cell count (median=7.9) compared with rectal tumours (median=6.8) (p=0.011, Figure 3b).

**Association between tumour tissue telomere length and patient clinico-pathological characteristics**

A significant relationship existed between tumour tissue telomere length and albumin concentration (Pearson r=0.36, p=0.009, n=32). As CRC tissue telomere length increased, albumin concentration also increased. No relationship was observed between the telomere length from either the cancer or normal tissue and any of the other clinico-pathological parameters.

**Discussion**

Our study demonstrates that patients with colorectal cancer display clear evidence of accelerated biological ageing in the form of telomere attrition when compared with healthy control subjects. The relationship between telomere length and cancer risk is proving to be a difficult one to fully delineate. Our demonstration of telomere attrition in PBLs of colorectal (CRC) cancer patients is similar to that reported by Pooley et al (2010) in both retro- and prospectively recruited patients (29). These data contradict two studies of both male and female CRC patients where no relationship between CRC and telomere length was identified (30, 31). However, prevalent studies investigating a number of other cancer entities including gastric (20), bladder (19), ovarian (21) and lung (22) are in concordance with our observations. Moreover, our findings are in keeping with those of Willeit *et al* (2010), who employed a longitudinal study to investigate cancer risk in 787 healthy participants followed-
up over a period of 10 years. They reported that both cancer risk and mortality from cancer was increased in patients in the shortest telomere group when compared with the longest (32). Our analysis suggests that the patients with the shortest telomeres have pathologically more severe disease as indicated by a higher Peterson Index. This observation raises the possibility of telomere biology not only affecting risk of cancer but also severity and thus outcome. Telomere length may therefore be a useful addition to the armamentarium of clinicians attempting to deliver improvements in current methods of prognosis.

Various investigators, using a number of experimental modalities, have identified damage induced by reactive oxygen species and oxidative stress as a key determinant of telomere erosion rates (11, 33). The relationship between redox state and telomere dynamics is likely to be a complex one involving interaction between a wide array of genetic and environmental factors. We investigated the potential role of disordered redox state by determining levels of anti-oxidant vitamins, micro-nutrients and fetuin-A. Whilst we did not observe a significant relationship between telomere length and anti-oxidant levels, patients with lower fetuin-A levels had shorter telomeres. These findings are consistent with those in CKD patients where there is an established link between low fetuin-A levels and short telomere length (13). This relationship demonstrates that biological ageing is associated with reduced redox capacity within the blood of CRC patients. Interleukin 6 (IL-6), a pro-inflammatory cytokine, alters the gene expression and synthesis of fetuin-A by hepatocytes, similar to its action on albumin (34). Our study showed that decreasing fetuin-A levels correlates with decreasing albumin concentration and increasing IL-6 and IL-10 levels, supporting the hypothesis of inflammation-dependent down-regulation of fetuin-A expression. These observations concur with those described in patients with renal failure on dialysis (35) and also with results from a rodent model of lethal systemic inflammation where fetuin-A exerted a protective role (36).
However, the relationship between fetuin-A is not a straightforward one given the finding of elevated levels in patients with previous myocardial infarction and obesity (37).

In patients with glioblastoma, the most commonly occurring brain tumour low serum fetuin-A levels predicted poor survival (38). Moreover, Rho et al (2009) report differential expression of fetuin-A between lung adenocarcinoma and adjacent normal tissue with both total protein and mRNA abundance reduced in cancer samples (39). These findings, in conjunction with our own observations, lead to the intriguing possibility of utilising fetuin-A as a prognostic/predictive marker for tumours from a histologically varied origin. Work is required to determine whether it is by virtue of its role in calcium homeostasis and hence redox state that fetuin-A contributes to the determination of telomere length.

Cellular senescence is a permanent state of growth arrest and hence a potent mechanism of tumour suppression. The triggers of senescence in vivo include critical telomere attrition, activation of oncogenes, oxidative stress, genotoxic stress and some therapeutic interventions for example irradiation and chemotherapy. Recent evidence indicates that senescent cells secrete a multitude of signalling factors, termed the “senescence associated secretory phenotype” (SASP) (40). These signals are mostly pro-inflammatory and include factors such as IL-1α and β, IL-6 and IL-8. The SAPS provides an intuitive explanation of our observed association between short telomere length and systemic inflammation, indicated in our patients by a raised neutrophil:lymphocyte ratio. Interestingly, patients with ulcerative colitis an inflammatory condition of the colon who exhibit an increased risk of CRC display evidence of telomere attrition in leucocytes (41). Even minute quantities of pro-inflammatory cytokines released by populations of senescent cells in biologically aged individuals could stimulate a more chronic systemic inflammation by virtue of positive feedback loops. At the
peri-tumoural level these factors could also act in a paracrine fashion to create an environment where tumour cells can flourish by stimulating hyperproliferation, de-differentiation, immune evasion, migration and invasion (42).

In concordance with previous studies we have confirmed that telomere length in colorectal cancer tissue is significantly shorter than normal adjacent colorectal mucosa (43). One might expect that given telomerase activity in neoplastic cells, including those of a colorectal origin, telomere length would be elongated in representative neoplastic tissue. However, our data suggest the opposite, meaning telomerase must maintain telomeric DNA content at a level consistent with a high rate of cell proliferation. This avoids the initiation of senescence or apoptosis which would otherwise mean exit from the cell cycle, and prohibit the rapid proliferation of neoplastic cells. These cells can therefore continue to divide but do so with requisite maintenance of telomere length. The adenoma-carcinoma sequence of colorectal neoplastic initiation still holds in the face of these observations. This is further reinforced by work demonstrating that telomere length in epithelial cells at the earliest morphological definable stage of carcinoma (high grade dysplasia with minimal invasive growth) was shorter compared with surrounding adenoma (44).

Rampazzo et al (2010) have also demonstrated that colorectal tumour telomere length is shorter than adjacent mucosa. Furthermore they have identified right-sided tumours as having shorter telomere length than left-sided and rectal cancers, which may result from an alteration in mismatch repair pathways (45). The molecular and clinical characteristics of right and left colon cancers are well established (46). Our observation that fetuin-A levels vary with the
anatomical site of the primary tumour is pertinent. The pre-operative management of colonic and rectal cancers in the context of chemo-radiotherapy differs, hence, further molecular differentiation of colon and rectal cancers could lead to the discovery of new therapeutic targets thereby improving the outcome of rectal cancer. Obviously, further work in a larger patient group is required to substantiate these preliminary findings.

We have observed that patients with CRC have significantly shorter telomeres than control subjects, congruent with accelerated biological ageing in the pathogenesis of CRC. These observations are in keeping with the hypothesis of telomere attrition predisposing to disease. Furthermore, patients with shorter telomeres display evidence of systemic inflammation and pathologically more advanced disease. An imbalance in redox control mechanisms and calcium homeostasis may be a contributing factor to telomere dynamics in our group of patients. We believe further work is merited to fully delineate the factors determining telomere length and whether the risk profile of colorectal cancer could be altered by manipulating these factors.

Acknowledgements

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Table 1: Patient clinical and pathological characteristics.

Table 2: Table displaying indices of inflammation and redox control factors as a correlate of telomere length in the CRC PBL group.
**Figure 1:** Measurement of PBL telomeres from a control and colorectal cancer (CRC) population revealed that the CRC patients had significantly shorter telomeres (Mean telomere length ± standard error (Rel T/S 0.66 ± 0.02) than the control population (0.75 ± 0.05) p<0.001).

**Figure 2:** Boxplot highlighting the difference in telomere lengths between thirty two matched tumour and adjacent normal tissue samples of colorectal cancer patients. Tumour tissue (median Rel T/S = 0.43) displayed significantly shorter telomeres than normal tissue samples (median Rel T/S = 0.65, Wilcoxon signed rank test, p=0.004).

**Figure 3:** Differentiation of tumour site in the CRC PBL group using plasma fetuin-A (A) and while cell count (B). Patients with rectal cancers had significantly higher levels of fetuin-A (p=0.045) and a significantly lower white cell count (p=0.011) when compared with colon cancer patients.

**References**


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† Includes patients with in-operable disease and patients who underwent synchronous resection of their primary cancer and liver metastasis.
‡ Synchronous resection of liver metastasis.
‡ This compares with 58% of the control population who had ‘ever’ smoked.
<table>
<thead>
<tr>
<th>Systemic Inflammation</th>
<th>Median (range)/No Patients (%)</th>
<th>Difference between short &amp; long telomere group (p-value)</th>
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<td>C-Reactive Protein</td>
<td>7.5 (0.40-95.0) mg/l</td>
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<td>IL-6</td>
<td>6.5 (1.75-39.8) pg/ml</td>
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<td>IL-10</td>
<td>11.2 (5.68-34.82) pg/ml</td>
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<td>VEGF</td>
<td>84.75 (7.28-952.96) pg/ml</td>
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<td>Neutrophil:Lymphocyte (NLR)</td>
<td>0.32 (0.04-0.94)</td>
<td><strong>0.047</strong>†</td>
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<td>Redox Control</td>
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<td>Retinol</td>
<td>1.80 (0.7-3.70) µmol/l</td>
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<td>α-tocopherol</td>
<td>26.5 (12.0-40.0) µmol/l</td>
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<td>Lutein</td>
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<td>Lycopene</td>
<td>83.0 (10.0-373.0) µg/l</td>
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<td>Fetuin-A</td>
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<td>Fetuin-A</td>
<td>27.16 (14.71-67.27) ng/ml</td>
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<td>*Age</td>
<td>r = -0.32</td>
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<td>*Albumin</td>
<td>r = 0.28</td>
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<td>*Calcium</td>
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<td>*IL-10</td>
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<td>*IL-6</td>
<td>r = -0.483</td>
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</table>

NS – Not significant

†Significant difference in NLR between short (Rel T/S <0.55, NLR 0.39 (IQR 0.90)) and long (Rel T/S >0.55, NLR 0.28 (IQR 0.71)) telomere group (Mann-Whitney).

‡Patients with low fetuin-A (fetuin-A < log median=1.47/median=29.6) had significantly shorter telomeres (median T/S = 0.6 Vs 0.72, Mann-Whitney).
*Relationship with plasma log Fetuin-A concentration, displayed as Pearson correlation.
Figure 1

Telomere Length (Rel T/S)

Control Population

CRC Population

Group

0.65
0.70
0.75
0.80

P < 0.001
Figure 3
Telomere attrition and decreased Fetuin A levels indicate accelerated biological ageing and are implicated in the pathogenesis of Colorectal Cancer

Fraser Maxwell, Liane M McGlynn, Hannah C Muir, et al.

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