

BIOCHEMICAL SIGNS OF IMPAIRED COBALAMIN STATUS DURING AND AFTER RADIOTHERAPY FOR RECTAL CANCER

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Purpose: The aim of the study was to investigate whether pelvic radiotherapy for rectal cancer had a negative impact on cobalamin status.

Methods and Materials: Consecutive patients receiving pelvic radiotherapy (50 Gy) for rectal cancer were evaluated prospectively ($n = 54$). Serum cobalamin, holotranscobalamin (holoTC), methylmalonic acid (MMA), and total homocysteine (tHcy) were measured at start and end of radiotherapy, at follow-up 4–6 weeks and 1 year ($n = 23$) after radiotherapy.

Results: Mean serum cobalamin decreased from 306 pmol/L before treatment to 267 pmol/L at the end of radiotherapy ($p < 0.001$), 247 pmol/L 4–6 weeks after radiotherapy ($p < 0.001$), and 249 pmol/L 1 year after radiotherapy ($p = 0.02$). Mean serum MMA was 0.16 $\mu\text{mol/L}$ pretreatment, 0.17 $\mu\text{mol/L}$ at the end of radiotherapy (n.s.), and increased to 0.19 $\mu\text{mol/L}$ after 4–6 weeks ($p = 0.007$), and to 0.21 $\mu\text{mol/L}$ after 1 year ($p < 0.001$). There was no change in serum tHcy. Mean serum holoTC was reduced from 111 pmol/L pretreatment to 93 pmol/L 4–6 weeks after radiotherapy ($p = 0.002$).

Conclusions: The data suggest rapid and persistent decrease in cobalamin status after radiotherapy for rectal cancer, as reflected by reduced serum cobalamin combined with increased serum MMA. This observation, though modest, may motivate routine monitoring of cobalamin status at follow-up after radiotherapy.
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INTRODUCTION

Abdominal and pelvic radiotherapy may result in injury of the small intestine. Radiation causes damage to the mucosa, decreasing the intestinal surface area, increasing permeability, and impairing absorption (1, 2). These acute effects usually present with diarrhea (3). Late signs of intestinal damage may occur after several months. These are characterized by submucosal lesions with inflammation, vascular endarteritis and fibrosis, and may cause strictures, fistulas, changes in bacterial flora, and diarrhea (1, 4, 5). As a consequence, the absorptive area and capacity of the mucosa are reduced, resulting in malabsorption. The terminal ileum may be particularly susceptible to damage because the position of the ileocecal valve is fixed, and part of the terminal ileum is located within the radiation field when treating rectal cancer or other pelvic malignancies.

Cobalamin bound to intrinsic factor is absorbed by a receptor-mediated process in a relatively short segment (50–70 cm) of the terminal ileum; thus mucosal damage or loss of ileal absorptive area may lead to cellular cobalamin deficiency. In the enterocytes, cobalamin is transferred from intrinsic factor to transcobalamin (total TC) and released into the blood (6). Cobalamin bound to TC, holotranscobalamin (holoTC), is the bioavailable fraction of total cobalamin in blood, and undergoes receptor-mediated uptake into peripheral cells (7).

Development of cobalamin deficiency has been observed in several gastrointestinal diseases affecting the terminal ileum, especially after resection or by diseases affecting more than 50 cm of the terminal ileum (8). However, since the liver has large stores of cobalamin, which are effectively preserved through the enterohepatic recirculation, defi-

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ciency due to malabsorption normally develops gradually over many years (9).

Diagnosis of cobalamin deficiency is often based on measurement of serum cobalamin. However, even patients with serum cobalamin values within the lower reference range may be deficient. In these borderline cases, measurement of methylmalonic acid (MMA) and total homocysteine (tHcy) may be useful (10–12). MMA and tHcy in serum/plasma are indicators of functional cobalamin status, and increase in cobalamin-deficient subjects. Whereas MMA is a relatively specific indicator of cobalamin deficiency, tHcy may also be increased in folate deficiency (12). In addition, tHcy concentration is influenced by a diversity of genetic and lifestyle factors and disease states (13). HoloTC has been proposed as an early marker of cobalamin deficiency (7), but the test is, as yet, not available for clinical routine use.

Previous studies have indicated that pelvic radiotherapy may lead to cobalamin deficiency. Decreased absorption of vitamin B₁₂ has been found in connection with pelvic radiotherapy for cancer (14). Cobalamin deficiency (serum cobalamin <150 pmol/L) was observed in 13–38% of subjects 1–16 years after radiotherapy for bladder cancer and gynecologic cancer (15–17). However, similar radiation effects have not been studied in patients treated for rectal cancer, and cobalamin status is not routinely monitored in the follow-up of these patients.

The aim of this study was to investigate whether pelvic irradiation for rectal cancer results in decreased cobalamin status. Cobalamin status was evaluated by combined measurement of serum cobalamin, serum MMA, and tHcy in addition to determination of total TC and holoTC.

METHODS AND MATERIALS

Patients and treatment

Patients with rectal cancer receiving pelvic radiotherapy were included consecutively in a study evaluating quality of life and symptoms during radiotherapy (18). Patients with primary inoperable rectal cancer or recurrent tumor received preoperative radiotherapy, and patients with high risk of developing recurrence received postoperative chemoradiotherapy.

Radiotherapy was given in 2 Gy daily fractions, 5 treatments per week, during 5 weeks. For preoperative radiotherapy, the clinical target volume received 46 Gy, and the gross tumor volume received an additional 4 Gy boost. Most patients were treated by a 3-field technique with one posterior and two lateral portals. The upper border of the treatment volume was the L5-S1 disc, and the lower border was the perineum. Lateral borders of the posterior-anterior (PA) field were 1 cm outside the true bony pelvis. The posterior and anterior borders of the lateral fields were 1 cm behind the sacrum and the center of the femoral heads, respectively. Patients were treated in the prone position, preferably with a full bladder. In patients with extensive anterior infiltration, a 2-field technique (opposing AP-PA fields) was used, with patients in a supine position.

Postoperative radiotherapy was applied by a 3-field tech-

nique. The clinical target volume received 50 Gy, and concomitant chemotherapy was given to patients <75 years of age (5-fluorouracil [5-Fu] 400 mg/m² intravenous bolus followed by leucovorin 100 mg intravenous bolus, before fractions 1, 2, 11, 12, 21, and 22), slightly modified from the regimen used in a previous Norwegian adjuvant trial (19).

Blood samples were collected at the start and end of radiation therapy, at follow-up 4–6 weeks after completed therapy, and 1 year after therapy. Patients also completed quality-of-life questionnaires, and a 5-day diary including frequency of defecation, at the same follow-up times. Only patients aged <80 years, in good performance status (Eastern Cooperative Oncology Group [ECOG] ≤1, that is, fully active, or ambulatory and able to carry out light work), and without previous major gastrointestinal disease or surgery were eligible for the study. The regional ethical committee approved the study.

Sixty patients fulfilled the inclusion criteria. Four withdrew from the study after the first assessment, and two lacked baseline serum samples; thus, a total of 54 patients were included in the study. Four patients received vitamin B₁₂ supplementation by the treating physician during the study period, and serum cobalamin, MMA, tHcy, total TC, and holoTC values after the B₁₂ supplementation were excluded from analysis. Likewise, 2 patients received folate supplementation during the study period, and serum folate and tHcy after the supplementation were excluded from analyses. Furthermore, in patients receiving chemotherapy with 5-Fu and leucovorin, folate and tHcy values were excluded from data analysis ($n = 17$), as it has been shown that leucovorin results in markedly increased serum folate and reduced tHcy level (20). One year after radiotherapy, 39 patients were alive. Serum samples were obtained from 23 patients. Among these, 8 had recurrent or metastatic disease, and folate and tHcy were excluded from the analyses because they probably received chemotherapy including leucovorin.

Biochemical analyses

Cobalamin, MMA, tHcy, total TC, holoTC, and folate (s-folate) were analyzed in serum samples stored at -70°C . Serum cobalamin was determined by a *Lactobacillus leichmannii* microbiological assay (21), and serum folate by a *Lactobacillus casei* microbiological assay (22). Both cobalamin and s-folate assays were adapted to a microtiter plate format (23), and carried out by a robotic workstation (MicroLab AT plus 2; Hamilton Bonaduz AG, Bonaduz, Switzerland). Hemoglobin, albumin, and erythrocyte folate were determined in fresh samples by the routine methods of the hospital laboratory. The method used for determining erythrocyte folate by the hospital laboratory was changed during the study period, as was the reference levels; mean values are therefore not reported.

Methylmalonic acid and tHcy were determined by gas chromatography–mass spectrometry after derivatization with methylchloroformate (24). Briefly, sample (100 μL) was treated with dithiothreitol, and then deproteinized by adding ethanol. The supernatant was derivatized with meth-

Table 1. Patients and treatment

	<i>n</i>	%
Gender		
Male	32	59
Female	22	41
Radiation therapy		
Preoperative primary	21	39
Preoperative recurrence	13	24
Postoperative	20	37
Chemotherapy	17	32

ylchloroformate. The derivatives were extracted into toluene, and separated on a DB-5 capillary column and detected in positive ion mode (*m/z* 174 for MMA; *m/z* 233 for homocysteine) by mass spectrometry.

Creatinine in serum was determined by a modification of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) described previously (25). Creatinine was detected in the multiple-reaction monitoring mode of the tandem mass spectrometer with the transition *m/z* 114 to 44.2.

Serum total TC and holoTC were measured by enzyme-linked immunosorbent assay (ELISA) as recently described (26, 27), but modified to allow the use of an automated ELISA analyzer (BEP-2000, Dade Behring, Germany).

We defined cobalamin deficiency as "certain" if a serum cobalamin <150 pmol/L was combined with MMA >0.26 $\mu\text{mol/L}$ and/or tHcy >15.0 $\mu\text{mol/L}$, and as "borderline" if serum cobalamin was between 150–250 pmol/L combined with MMA >0.26 $\mu\text{mol/L}$ and/or tHcy >15.0 $\mu\text{mol/L}$. Furthermore, we defined folate deficiency as serum folate <6.0 nmol/L, erythrocyte folate below lower reference level, and tHcy >15.0 $\mu\text{mol/L}$.

Statistics

Most of the laboratory data showed a positively skewed distribution. Values that were not normally distributed were

log-transformed, and geometric means are presented. Changes from pretreatment levels were analyzed by paired samples *t* tests on log-transformed data. Simple correlations were analyzed with Spearman's tests. Analyses of the percentages of cases with deficiency were performed with McNemar's test for paired analyses. Two-sided *p* values <0.05 were considered statistically significant. Data were analyzed using SPSS, 11.0 (SPSS Inc., Chicago, IL).

RESULTS

Patient characteristics

The characteristics of the 54 patients are shown in Table 1. The median age was 66 years (range, 38–78 years), and 59% were males. Preoperative radiotherapy was given to 34 patients, of whom 21 had a primary inoperable rectal cancer and 13 a recurrent rectal cancer. Postoperative radiotherapy was given to 20 patients, 17 of whom received concomitant chemotherapy (5-FU/leucovorin). The patients were in a good general condition, with mean body mass index of 25.3 kg/m^2 (range, 16.5–40.4), and mean albumin of 38 g/L (range, 27–46 g/L).

Blood indices at baseline

The blood analyses at the start of radiation therapy are shown in Table 2. Eight men and three women (20%) had hemoglobin (Hgb) values at baseline below the reference range (12.5 g/dL in males and 11.5 g/dL in females), and among these two had Hgb <10 g/dL. There were no significant differences in blood indices between patients receiving preoperative and postoperative radiotherapy.

Three patients had folate deficiency according to our definition with reduced serum folate (<6 nmol/L), reduced erythrocyte folate, and increased tHcy (>15.0 $\mu\text{mol/L}$). In two of these patients, the serum and erythrocyte folate levels remained low, and tHcy high, and after the 1-month follow-up, they received folic acid supplementation. No patients

Table 2. Blood indices at the start of radiotherapy (RT), at the end of RT, and at follow-up 4–6 weeks after RT. Geometric mean (GM) and 10–90 percentiles (10–90 perc) shown

	Start of RT		End of RT			Follow-up (4–6 weeks)			Reference level
	GM	(10–90 perc)	GM	(10–90 perc)	<i>p</i> *	GM	(10–90 perc)	<i>p</i> *	
Hgb (g/L)	13.1	(11.2–15.1) [†]	12.7	(11.1–14.7)	0.01	12.7	(11.1–14.6)	n.s.	♂ 12.5–16.5 ♀ 11.5–15.5
Cobalamin (pmol/L)	306	(185–538) [†]	267	(157–451)	<0.001	247	(160–381)	<0.001	>150
Total TC (pmol/L)	1106	(803–1420) [†]	1121	(832–1499)	n.s.	1161	(921–1548)	n.s.	580–1500
HoloTC (pmol/L)	111	(71–179) [‡]	102	(56–165)	n.s.	93	(56–163)	0.002	40–150
Serum folate (nmol/L)	10.6	(5.1–24.3) [§]	10.1	(6.3–19.3)	n.s.	8.4	(4.2–13.4)	0.006	≥6
MMA ($\mu\text{mol/L}$)	0.16	(0.10–0.25) [†]	0.17	(0.10–0.29)	n.s.	0.19	(0.08–0.31)	0.007	≤0.26
tHcy ($\mu\text{mol/L}$)	14.2	(7.8–20.8) [§]	15.0	(8.7–28.6)	n.s.	14.1	(9.1–25.4)	n.s.	5–15
Creatinine ($\mu\text{mol/L}$)	64.8	(45.4–92.1) [†]	59.9	(40.9–99.5)	0.03	62.7	(34.1–99.3)	n.s.	
Albumin (g/L)	37.7	(33.5–43.0) [†]	35.3	(29.8–41.0)	<0.001	36.2	(29.2–42.0)	0.03	35–50

Abbreviations: GM = geometric mean; Hgb = hemoglobin; HoloTC = holotranscobalamin; MMA = methylmalonic acid; n.s. = not significant; perc = percentiles; tHcy = total homocysteine; total TC = transcobalamin.

* Compared with values at baseline, by paired *t* test.

[†] *n* = 54; [‡] *n* = 45; [§] *n* = 37.

Table 3. Blood indices in 23 patients in whom serum samples were available both at start of radiotherapy (RT) and at follow-up 1 year after RT. Geometric means (GM); 10–90 percentiles (10–90 perc)

	Start of RT GM (10–90 perc)	1 year after RT GM (10–90 perc)	<i>p</i> *
Hgb (g/dL)	13.4 (11.1–15.3)	13.5 (10.9–15.3)	n.s.
Cobalamin (pmol/L)	299 (179–544)	249 (148–396)	0.02
Total TC (pmol/L) [†]	1118 (772–1453)	1171 (868–1578)	n.s.
HoloTC (pmol/L) [‡]	112 (69–269)	91 (48–169)	n.s.
MMA (μmol/L)	0.15 (0.10–0.24)	0.21 (0.14–0.34)	<0.001

Abbreviations as in Table 2

* Compared with values at baseline, by paired *t* test; [†] *n* = 21; [‡] *n* = 15.

were identified with “certain” cobalamin deficiency. Seven had “borderline” cobalamin deficiency with cobalamin <250 pmol/L and either MMA >0.26 μmol/L or tHcy >15.0 μmol/L.

Bivariate analyses

Serum cobalamin levels were positively correlated to holoTC, and inversely correlated to MMA and tHcy. The Spearman’s correlation coefficients (*r*) were 0.55 (cobalamin vs. holoTC), −0.29 (cobalamin vs. MMA), and −0.33 (cobalamin vs. tHcy). Serum folate was negatively related to tHcy (*r* = −0.42). All correlations were highly significant (*p* < 0.001). Serum holoTC was correlated to total TC (*r* = 0.45, *p* < 0.001), inversely correlated to MMA (*r* = −0.26, *p* = 0.001), but not correlated to tHcy. Serum total TC was not significantly correlated to cobalamin, MMA, or tHcy. Serum creatinine was correlated with MMA (*r* = 0.21, *p* = 0.005) and tHcy (*r* = 0.44, *p* < 0.001), but not with cobalamin, folate, total TC, or holoTC.

Blood indices during and shortly after radiotherapy

Table 2 shows the blood indices at start of radiotherapy, at the end of radiotherapy, and at follow-up 4–6 weeks after radiotherapy. At the end of therapy, mean serum cobalamin was significantly decreased as compared to baseline (*p* < 0.001), whereas holoTC, MMA, tHcy, and total TC were unchanged. Hemoglobin levels (*p* = 0.01), creatinine (*p* = 0.03), and albumin (*p* < 0.001) were decreased compared to baseline. Comparison of the values at follow-up 4–6 weeks later with those at baseline showed a significant reduction in serum cobalamin (*p* < 0.001), serum holoTC (*p* = 0.002), serum folate (*p* = 0.006), and albumin (*p* = 0.03), and a significant increase in MMA (*p* = 0.007), whereas total TC and tHcy remained unchanged. The blood indices showed no significant difference between patients treated with 2-field versus 3-field technique, or between patients receiving chemotherapy or not (data not shown).

Blood indices 1 year after radiotherapy

Table 3 shows the cobalamin, total TC, holoTC, and MMA levels 1 year after radiotherapy in the 23 patients in whom we had serum samples both at baseline and 1 year after radiotherapy. Mean serum cobalamin was significantly decreased (*p* =

0.02) and MMA was significantly increased (*p* < 0.001), as compared with baseline levels (23 sample pairs). The serum levels of cobalamin and MMA in these patients are depicted in Fig. 1. There were no significant changes in total TC (in 21 sample pairs). HoloTC showed a decline from 112 to 91, but this reduction was not significant, possibly because sufficient serum for analysis of holoTC at the two time points was only available in 15 cases.

Eight patients had recurrent and/or metastatic disease and possibly received chemotherapy combined with leucovorin, whereas 15 had no evidence of disease (NED). In these 15 patients with NED, we compared serum folate and tHcy after 1 year with the values measured at baseline. No significant changes were observed (data not shown).

Cobalamin deficiency

Table 4 shows the frequency of subjects with “certain” (cobalamin <150 and MMA >0.26 and/or tHcy >15) or “borderline” (cobalamin 150–250, MMA >0.26, and/or tHcy >15) cobalamin deficiency. At start of radiotherapy, none of the patients fulfilled the criteria of cobalamin deficiency, and 6 of 54 patients (11%) had borderline cobalamin deficiency. At the end of radiation therapy, 10 of 53 patients (19%) were borderline cobalamin-deficient (n.s.). One month after radiation therapy, 12 of 45 (27%) were borderline cobalamin-deficient (*p* = 0.04, as compared with baseline). One year after radiotherapy, 2 of 23 (9%) had certain cobalamin deficiency, while 3 of 23 (13%) had possible cobalamin deficiency; the increase from baseline was not significant. Only one patient had holoTC <40 pmol/L; this occurred 1 year after radiotherapy, at which time the patient fulfilled the criteria of “certain” cobalamin deficiency.

Cobalamin status and diarrhea

The patients included in this study reported increased diarrhea during radiotherapy for rectal cancer (18), both when reported in quality-of-life questionnaires (European Organization for Research and Treatment of Cancer [EORTC] QLQ-C30) and when using the Common Toxicity Criteria. Because diarrhea may be associated with malabsorption, we investigated the possible relationship between diarrhea during radiotherapy, and serum cobalamin levels. We only found a relationship between diarrhea and serum levels of cobalamin at

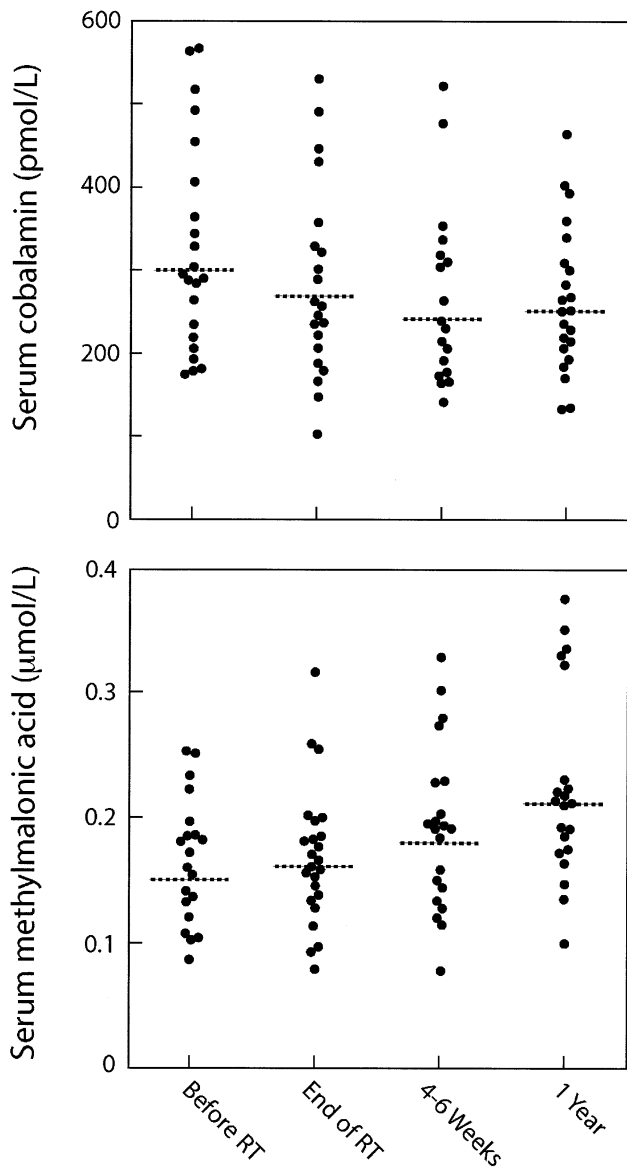


Fig. 1. Serum cobalamin and methylmalonic acid in the patients for whom serum samples were available both at baseline and at 1-year follow-up. Individual values are plotted, and geometric means are indicated with a horizontal line. RT = radiotherapy.

follow-up 4–6 weeks after radiotherapy ($r = -0.39$, $p = 0.02$), not at other time points. There was no correlation between diarrhea and MMA, or between changes in diarrhea and changes in serum levels of cobalamin or MMA (data not shown).

DISCUSSION

The present study demonstrates a gradual and persistent reduction in serum cobalamin in patients treated with radiotherapy for rectal cancer, during a follow-up of 1 year. These changes were accompanied by a concurrent, gradual increase of MMA; however, only a few cases of certain cobalamin deficiency were identified.

Study design

This was a prospective study of changes in cobalamin status during and after radiotherapy. The longitudinal design of the study with repeated measurements provides sufficient statistical power to detect even moderate changes in cobalamin status over time.

Reduced total plasma cobalamin combined with elevated MMA represents complementary indicators of impaired cobalamin status, and indicates functional cobalamin deficiency. Plasma tHcy is another marker of cobalamin status; however, plasma tHcy is influenced by various disease states, including cancer (28), and is also related to folate status (13). Furthermore, a rapid and pronounced reduction in tHcy occurs in patients receiving chemotherapy including leucovorin (20). Serum holoTC has been advocated as a sensitive marker of cobalamin malabsorption (7, 29), and has been proposed to be an early sign of negative vitamin B₁₂ balance (29, 30). However it is not in current routine use and its role is not yet established.

Understandably, the number of patients was reduced 1 year after radiotherapy, as locally advanced rectal cancer has a high mortality rate; in addition, 9 patients withdrew from the study or were lost to follow-up, which may have caused some bias.

Cobalamin status and radiotherapy

In the present study of patients with rectal cancer, we found biochemical evidence of decreased cobalamin status already at the end of a 5-week course of radiotherapy, with a further deterioration at follow-up after 4–6 weeks. Previous longitudinal studies have demonstrated early effects of abdominal/pelvic radiotherapy on serum cobalamin concentrations in cancer patients (14, 29, 31). In one study, cobalamin malabsorption was shown during pelvic irradiation (14). Our findings confirm these reports of early impairment of cobalamin status in patients receiving radiotherapy. In addition, concurrent increase in MMA concentration indicates that reduction in circulating cobalamin is accompanied by impaired cobalamin status in tissues.

We found signs of decreased cobalamin status in a significant number of patients 1 year after completed radiotherapy. Late intestinal radiation injury in patients treated for pelvic or abdominal neoplasms may cause chronic malabsorption (4). Impaired cobalamin absorption and long-term reduction of cobalamin status have been observed after pelvic radiotherapy years after the end of therapy (15–17, 32), but have not previously been shown after treatment for rectal cancer.

Possible mechanisms

Poor nutritional status may be a cause of cobalamin deficiency, and may be seen in cancer patients. However, the patients included in the present study were in generally good condition (ECOG status 0 or 1) and had good nutritional status with mean body mass index of 25 kg/m² and mean albumin of 38 g/L at the start of radiotherapy. Furthermore, the patients had a mean weight loss of only 0.9 kg during the 5 weeks of radiotherapy (data not shown). This

Table 4. Number of patients with metabolic indicators of decreased cobalamin status at the start of radiotherapy (RT), at the end of RT, and at follow-up 4–6 weeks and 1 year after RT

	Start RT	End RT	4–6 Weeks	1 Year
Total <i>n</i>	54	53	45	23
Cobalamin <150 pmol/L	1	4	3	2
Cobalamin 150–250 pmol/L	17	20	20	10
MMA >0.26 μ mol/L	4	6	11	5
tHcy >15 μ mol/L	13	17	11	3
“Certain” cobalamin deficiency, <i>n</i> (%) [*]	0	1 (2) [‡]	0	2 (9)
“Borderline” cobalamin deficiency, <i>n</i> (%) [†]	6 (11)	9 (17)	12 (27)	3 (13)
“Certain” or “borderline” deficiency, <i>n</i> (%)	6 (11)	10 (19)	12 (27)	5 (22)

Abbreviations as in Table 2

* “Certain” cobalamin deficiency defined as cobalamin <150 pmol/L and MMA >0.26 μ mol/L and/or tHcy >15 μ mol/L.

† “Borderline” cobalamin deficiency defined as cobalamin 150–250 pmol/L and MMA >0.26 μ mol/L and/or tHcy >15 μ mol/L.

‡ One person had low serum cobalamin at the first three assessment times (147–181), but normal MMA (0.11–0.13), low s-folate (2.7–3.3), and elevated tHcy (47–61). It was considered as secondary to folate deficiency; after receiving folate substitution, cobalamin and MMA 1 year after RT were normal (cobalamin 266, MMA 0.13).

suggests that a poor nutritional status during radiotherapy is not a likely explanation for changes in cobalamin status in these patients.

The early reduction in serum cobalamin combined with an increase in MMA suggests a rapid change in overall cobalamin homeostasis. This can hardly be explained by reduced cobalamin absorption, because of the slow turnover of cobalamin body stores. The daily recommended cobalamin intake of 2–3 μ g is low compared with the total body stores of cobalamin of 2–5 mg, which are largely preserved by the enterohepatic circulation (9, 33). After ileal resection, which perturbs the enterohepatic circulation, the daily cobalamin demands may increase to 10–15 μ g, and it normally takes 2–5 years before deficiency is manifest.

The observed short-term effects on cobalamin status may result from a direct effect of radiation on the cobalamin molecule and/or acute changes in the concentrations of its binding proteins. Ionizing radiation may either damage the cobalamin molecule directly or indirectly through radiolytic generation of peroxy radicals. Radiation may induce acute alterations in cobalamin transport proteins. However, we observed no change in total TC during radiotherapy in the present study. We found that holoTC concentrations decreased during radiotherapy. The observation that holoTC is reduced 4–6 weeks after radiotherapy, while total TC is unchanged, may suggest radiation induced degradation of cobalamin, or that less cobalamin is absorbed.

Radiation effects on the cobalamin molecule or its binding proteins would be expected to be transient, with the associated negative cobalamin balance to return to normal within months. The observation of persistently reduced serum cobalamin after radiation therapy could be explained by

cobalamin malabsorption due to progressive, chronic radiation enteropathy, leading to enterocyte damage, impaired bowel motility, impaired bile acid absorption, and intestinal bacterial overgrowth (4, 34, 35). The terminal ileum is the site of receptor-mediated uptake of the cobalamin-intrinsic factor complex (6), and this segment may be particularly vulnerable to radiation injury because of the fixed position of the ileocecal valve. Involvement of the ileocecal valve seems to be more important than the total length of the ileum segment exposed to radiation (36).

Implications and conclusion

Radiotherapy for rectal cancer is given with curative intent in order to reduce the risk of local recurrence and increase the rate of survival. Hence, early identification and treatment of long-term side effects are therefore important. Symptoms of cobalamin deficiency may be subtle and diffuse, but once acknowledged, supplementation treatment is simple and effective.

This study demonstrated a significant reduction of serum cobalamin already within weeks after start of radiotherapy, accompanied by a moderate, but significant increase in MMA. Even though the changes in some cobalamin indices reached statistical significance, the extent of cobalamin depletion and its long-term clinical consequences are uncertain. The present data suggest that some patients can develop cobalamin deficiency or early signs of imbalance within 1 year after radiotherapy, but long-term follow-up and intervention studies are necessary to confirm these preliminary findings. In the meantime, our data may justify routine monitoring of cobalamin status after radiation therapy even in patients without overt evidence of enteropathy and/or malabsorption.

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