research article

The impact of anaemia on treatment outcome in patients with squamous cell carcinoma of anal canal and anal margin

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Background. Radiochemotherapy is the main treatment for patients with squamous cell carcinoma of the anal canal. Anaemia is reported to have adverse effect on survival in cancer patients. The aim of the study was to evaluate the influence of anaemia on radiochemotherapy treatment outcome in patients with squamous cell carcinoma of the anal canal.

Patients and methods. One hundred consecutive patients with histologically confirmed squamous cell carcinoma of the anal canal were treated radically with 3-dimensional conformal or intensity-modulated radiation therapy followed by brachytherapy or external beam radiotherapy boost and with concurrent mitomycin C and 5-fluorouracil. The influence on survival of pre-treatment, mean on-treatment and end-of-treatment haemoglobin (Hb) concentrations was studied.

Results. The 5-year locoregional control, disease free survival, disease specific survival and overall survival rates for all patients were 72%, 71%, 77% and 62%, respectively. In univariate analysis, patients with pre-treatment and end-of-treatment Hb > 120 g/L survived statistically significantly better compared to patients with Hb ≤ 120 g/L. Patients with mean on-treatment Hb > 120 g/L only had statistically significant better locoregional control and overall survival than patients with Hb ≤ 120 g/L. In multivariate analysis, independent prognostic factors were pre-treatment Hb (> 120 g/L vs. ≤ 120 g/L) for overall survival (hazard ratio [HR] = 0.419, 95% confidence interval [CI] = 0.190–0.927, p = 0.032) and stage (I & II vs. III) for disease specific (HR = 3.523, 95% CI = 1.375–9.026, p = 0.009) and overall survival (HR = 2.230, 95% CI = 1.167–4.264, p = 0.015).

Conclusions. The pre-treatment, mean on-treatment and end-of-treatment Hb concentration > 120 g/L carried better prognosis for patients for with squamous cell carcinoma of the anal canal treated with radiochemotherapy. The pre-treatment Hb > 120 g/L was an independent prognostic factor for overall survival of patients with anal canal cancer.

Key words: anaemia; anal canal squamous cell carcinoma; radiochemotherapy

Introduction

Squamous cell anal cancer is a rare tumour which represents 1.5% of gastrointestinal cancers, but in Slovenia only 0.5%.[1-5] Despite its infrequent occurrence its incidence is increasing.[4] Women are more commonly affected than men.[3-6] Causal factors in the anal canal cancer are usually associated with human papilloma virus (HPV) infection (being the most important risk factor), human immuno-

deficiency virus (HIV) infection, anal intercourse, higher lifetime number of sexual partners, genital warts and cigarette smoking.[3,6-8]

Anal canal cancer is predominantly a loco-regional disease, because it metastasizes in less than 10% of patients, mainly to lungs and liver.[6]

The management of anal canal cancer has undergone an interesting transformation over the course of the past three decades. With the report by Nigro *et al*. in 1974 it shifted from abdominoperineal resection with or without inguinal lymph node dissection to radical radiochemotherapy.[9,10] Radiochemotherapy with 5-fluorouracil and mitomycin C, nowadays being the main treatment, results in complete tumour response in 70–90% and has a 5-year survival rate of 60–70%, leaving surgery only as a salvage treatment for tumours that do not respond to radiochemotherapy or recur.[4,7] Anal margin cancers are classified as skin tumours and small tumours can be treated by surgery, while tumours T2 or larger should be treated with definitive radiochemotherapy.[11]

Radiotherapy as well as chemotherapy is known to be more efficacious in the presence of oxygen than in hypoxic conditions.[12-15] Tumours are more hypoxic than the surrounding normal tissue.[13] Anaemia, present in 75% of cancer patients, could increase the proportion of hypoxic tumour cells.[13] Hypoxia is widely recognized as a major factor leading to the resistance of tumour cells to radiotherapy, but several mechanisms may also cause cells in the hypoxic region to be resistant to anticancer drugs.[16] The influence of anaemia on the outcome of treatment was first recognized in 1940s in cervical cancer patients and later in patients with other tumours such as head and neck squamous cell carcinoma, carcinoma of the lungs, bladder, prostate and anus.[7,17,18] The purpose of present study was to evaluate the influence of anaemia on radiochemotherapy treatment outcome in patients with squamous cell carcinoma of the anal canal.

Patients and methods

One hundred consecutive patients (60 females and 40 males) with histologically confirmed squamous cell carcinoma of the anal canal were included in the retrospective study. They were treated at the Institute of Oncology Ljubljana from January 2003 till June 2013.

For performance status (PS) the scoring system of the World Health Organization (WHO) was used[19], and for TNM staging the criteria of the Union for International Cancer Control (UICC).[20]

Pre-treatment evaluation

Pre-treatment evaluation consisted of physical and digital rectal examination, rectoscopy with biopsy and fine needle aspiration biopsy of enlarged inguinal lymph nodes, also ultrasound-guided, like in other cancer patients.[21] Imaging included chest X-ray or computer tomography (CT) of chest, abdominal ultrasound (US) or CT and magnetic resonance imaging (MRI) of the pelvis. Laboratory tests included serum chemistry and complete blood count in all patients, and testing for HIV infection in high-risk patients. A multidisciplinary team consisting of a surgeon, a radiation oncologist and a medical oncologist decided the treatment for each patient.

Radiotherapy

Clinical target volume (CTV) consisted of the tumour volume with a safety margin of 2–2.5 cm and the regional lymph node areas. An additional margin of 1 cm was applied to the CTV for the planning target volume (PTV). Initial tumour borders were marked with tattoo. Positron emission tomography with computed tomography (PET-CT) was used as an aid in treatment planning. The treatment schedule for external beam radiotherapy (EBRT) consisted of 3-dimensional (3-D) conformal photon beam radiotherapy or intensity modulated radiotherapy (IMRT) with individual field arrangement. The total dose was 45 Gy in 25 fractions, 5-times weekly with 15 MV photon beam linear accelerator, plus a boost 10–15 Gy with interstitial pulsed-dose rate brachytherapy if tumour size was less than 5 cm. Metal needles were homogeneously implanted through a perineal template according to the rules of the Paris system. In tumours larger than 5 cm or in N2–3 disease, the boost was delivered with EBRT. CTV (brachytherapy/EBRT) of the boost corresponded to the initial gross tumour extension. In cases with positive inguinal lymph nodes, inguinal areas were boosted with electrons to a total dose of 59.4 Gy. When IMRT technique was used, inguinal lymph nodes were involved in CTV and PTV and irradiated to the same total dose of 59.4 Gy. If the tumour involved or crossed the external anal sphincter, this area was covered with a 1 cm thick gelatinous bolus to raise the dose at the surface to at least 95% of the planned dose.

Chemotherapy

Chemotherapy protocol consisted of 2 cycles of 96-hour continuous infusion of 5-fluorouracil with a daily dose of 1000 mg/m^2 of body surface in the first and fifth week of radiotherapy. On day 1 the patients also received a bolus of mitomycin C in a dose of 10 mg/m^2. Since 2006, we administered peroral cytostatic capecitabine in a dose of 825 mg/m^2, twice daily, to cooperative patients with good performance status and without important comorbidities. First dose of capecitabine was administered one hour before the irradiation and the second dose 12 hours after. In cases of severe treatment toxicity according to common toxicity criteria[22] radiotherapy and/or chemotherapy was modified according to the patient's general condition and laboratory findings or was even temporarily interrupted.

Follow-up

During treatment, the patients were examined weekly to assess acute toxicity and compliance with radiochemotherapy, and complete blood count and serum biochemistry were performed as well.

The first post treatment examination was performed six weeks after the completion of radiochemotherapy, and then every 2–3 months for the first 2 years and every 6 months in the following 3 years.

When tumour response was incomplete, patients were examined every 6 weeks over a period of 4 months after the end of the treatment. In this period we performed all necessary investigations to prove tumour viability or its progression and in such cases surgery (abdomino-perineal resection) was recommended.

Tumour response was evaluated according to the WHO criteria.[19]

TABLE 1. Patients' and tumours' characteristics

Characteristics					No. of patients
Gender					
female					60
male					40
Mean age (range)					63 (34–87)
Performance status (WHO)					
0					76
1					20
2					3
3					1
Tumour type					
Carcinoma of the anal canal					72
Carcinoma of the anal margin					28
Tumour histology					
Basaloid					12
Squamous					88
TNM	N0	N1	N2	N3	
T1	9	0	1	0	
T2	36	6	1	0	
T3	19	10	3	1	
T4	1	1	7	5	
Tumour stage					
I					9
II					55
IIIA					17
IIIB					19

WHO = World Health Organization

Statistical analysis

The survival estimates were carried out by using the Kaplan-Meier method[23] and a log rank test[24] was used to test the differences in survival between subgroups.

The end points of survival analysis were defined as follows: loco-regional control (LRC) as the time interval from the beginning of the treatment to the appearance of local and/or regional progression; disease-free survival (DFS) as the time interval from the beginning of the treatment to the appearance of local and/or regional progression and/or appearance of distant metastases; disease-specific survival (DSS) as the time interval from the beginning of the treatment to the death because of cancer; and overall survival (OS) as the time interval from the beginning of the treatment to the death due to any cause.

For multivariate analysis, Cox proportional hazard model (with "Enter method") was used.[25]

All statistical tests were two-sided and a P-value of $p \leq 0.05$ was considered statistically significant. Statistical analyses were performed by using SPSS version 22 (Chicago, IL).

TABLE 2. Haemoglobin (Hb) values in subgroups of patients

Hb (g/L)	No. of patients	Median Hb (g/L)	Hb range (g/L)
Pre-treatment Hb		128	86–169
> 120 g/L	69	136	122–169
≤ 120 g/L	31	107	86–120
Mean on-treatment Hb		127	96–157
> 120 g/L	67	134	121–157
≤ 120 g/L	33	113	96–119
End-of-treatment Hb		121	77–159
> 120 g/L	46	134	121–159
≤ 120 g/L	54	114	77–120

Ethical consideration

The study was carried out according to the Helsinki Declaration (1964, with later amendments) and according to the European Council Convention on Protection of Human Rights in Bio-Medicine (Oviedo, 1997). It was approved by the Institutional Review Board Committee and by the National Committee for Medical Ethics, Ministry of Health, of the Republic of Slovenia.

Results

The study was closed on February 15, 2014. Median follow-up time of all patients was 52 months (range: 1–129 months) and 72 months (range: 6–129 months) for the survivors. On the day of analysis, 59 patients were alive, 22 patients died of anal canal cancer, 15 patients died of other causes and in 4 patients the cause of death was unknown.

Characteristics of patients and tumours are shown in Table 1.

Characteristics of Hb values in subgroup of patients are shown in Table 2.

Ninety-two patients (92%) completed their treatment according to the protocol. In 8 patients the treatment was modified: three did not receive chemotherapy due to significant comorbidities (ischemic heart disease or significant hepatopathy); in 1 patient chemotherapy was terminated due to acute side effects (chest pain due to a suspected ischemic event) and in 1 patient due to febrile neutropenia. One patient refused further treatment after 45 Gy and 1 patient refused chemotherapy. One patient received concurrent chemotherapy with cisplatin due to simultaneous treatment of the synchronous oropharyngeal cancer.

Median duration of radiochemotherapy was 1.9 months (range: 1–3.7 months). Fifty-six patients received brachytherapy boost with medial dose of 18.5 Gy (range: 10–25 Gy) or EBRT boost with medial dose of 14.4 Gy (range: 9–14.4 Gy). Capecitabine was used instead of 5-fluorouracil in 25 patients.

Tumour response to treatment

Complete clinical remission of the disease was achieved in 80 patients. The tumour disappeared within six weeks after the treatment completion in 73 patients, and within 4 months in 7 patients. One of them was operated on because of presumed persistent disease, yet the pathologist did not find disease residues. Of the remaining 20 patients, in 1 patient the disease progressed during treatment, 9 patients had APR performed and 2 patients had inguinal lymphadenectomy due to recurrence in inguinal lymph nodes; 8 patients had inoperable residual disease.

Survival

The 5-year LRC, DFS, DSS and OS rates for all patients were 72%, 71%, 77% and 62%, respectively.

Univariate analysis for survival according to the Hb level and other parameters is shown in Table 3.

In multivariate analysis, pre-treatment Hb (> 120 g/L *vs.* ≤ 120 g/L) was an independent prognostic factor only for OS (hazard ratio [HR]= 0.419, 95% confidence interval [CI] = 0.190–0.927, p = 0.032) and stage (I & II *vs.* III) for DSS (HR = 3.523, 95% CI = 1.375–9.026, p = 0.009) and OS (HR = 2.230, 95% CI = 1.167–4.264, p = 0.015).

Patients' age, gender, tumour site, type of radiotherapy boost (tele- or brachytherapy) and type of chemotherapy (5-fluorouracil or capecitabine) did not have an influence on survival.

Haemoglobin concentration during treatment

In the group of patients with Hb > 120 g/L the mean Hb concentration during the treatment slightly but not significantly decreased (mean pre-treatment Hb = 139 g/L, mean end-of-treatment Hb = 125 g/L). However in the group of patients with Hb ≤ 120 g/L it slightly increased (mean pre-treatment Hb = 106 g/L, mean end-of-treatment Hb = 113 g/L). One third of patients had low iron levels and received iron preparations. Nine patients received blood transfusion due to a drop in their Hb concentration below 100 g/L.

Acute side effects

None of the patients died because of acute side effects. Most grade 3 side effects were caused by radiodermatitis. Serious, life-threatening infections were observed in 3 patients: 2 patients experienced severe pneumonia that requested transfer to the intensive care unit and 1 patient developed febrile neutropenia which required termination of radiochemotherapy. One patient developed severe stomatitis and needed parenteral nutrition. In 1 patient, serious diarrhoea developed, which required hospitalization. Frequency and intensity of acute side effects are shown in Table 4.

Discussion

Survival rates of our patients and the profile and frequency of acute side effects are similar to the results of other researchers.[2,7,26-29] There was no difference in survival of anal canal and anal margin cancer patients. The survival rate of patients with higher pre-treatment and end-of treatment Hb concentrations was generally better, compared to those patients with lower Hb concentrations, yet only pre-treatment Hb concentration was an independent prognostic factor for OS. Patients with mean on-treatment Hb > 120 g/L only had statistically significant better LRC and OS than patients with Hb ≤ 120 g/L. Many authors found that anaemic patients respond worse to radiotherapy and/or chemotherapy and have worse survival rates.[2,8,12,13,15-18,30-41] There is convincing evidence of a correlation between Hb concentration and tumour oxygenation in various kinds of tumours.[42] Nordsmark's *et al*. comparison of pre-treatment Hb with pre-treatment tumour pO_2 measurements in head and neck cancer showed a quadratic regression correlation between Hb concentration and median pO_2.[43] Tumours of anaemic patients are consequently more hypoxic and more resistant to radiotherapy (and chemotherapy).[16] The *National Comprehensive Cancer Network* (NCCN) guidelines recommend the use of blood transfusion in symptomatic patients with Hb concentration <100 g/L to improve oxygen delivery to the tumour.[44] Nine patients in our study received blood transfusion. They had statistically significant worse OS than other patients. The conclusions about beneficial effect of transfusion in our study cannot be made because the patients who received transfusion were few. The contribution to low survival of other un-

TABLE 3. Univariate analysis of survival of patients at 5 years by Hb level, tumour-, patients-, and treatment characteristics

Characteristics	n	LRC	DFS	DSS	OS
Pre-treatment Hb					
> 120 g/L	69	79%	77%	85%	73%
≤ 120 g/L	31	57%	57%	56%	39%
		P = 0.011	**P = 0.017**	**P = 0.003**	**P = 0.000**
Mean on-treatment Hb					
> 120 g/L	67	78%	76%	82%	68%
≤ 120 g/L	33	60%	60%	67%	50%
		P = 0.037	**P = 0.054**	**P = 0.081**	**P = 0.007**
End-of-treatment Hb					
> 120 g/L	46	82%	80%	89%	75%
≤ 120 g/L	54	63%	63%	65%	49%
		P = 0.022	**P = 0.037**	**P = 0.011**	**P = 0.003**
Performance status					
PS 0	76	73%	73%	80%	72%
PS 1–3	24	69%	64%	66%	34%
		P = 0.480	P = 0.283	P = 0.231	**P = 0.000**
Tumour stage					
T1–3	86	75%	75%	84%	68%
T4	14	50%	44%	38%	25%
		P = 0.054	**P = 0.015**	**P = 0.000**	**P = 0.001**
Lymph node involvement					
no	65	79%	79%	87%	70%
yes	35	59%	56%	60%	48%
		P = 0.032	**P = 0.017**	**P = 0.000**	**P = 0.000**
Overall disease stage					
I / II	64	79%	79%	87%	70%
IIIA / IIIB	36	59%	57%	61%	49%
		P = 0.044	**P = 0.025**	**P = 0.000**	**P = 0.000**
Histologic tumour type					
basaloid	12	100%	100%	100%	100%
squamous	88	68%	67%	74%	57%
		P = 0.030	**P = 0.026**	P = 0.051	**P = 0.016**
Tumour site					
anal canal	72	69%	68%	78%	62%
anal margin	28	81%	81%	73%	61%
		P = 0.250	**P = 0.212**	**P = 0.994**	**P = 0.738**
Blood transfusion					
no	91	72%	71%	78%	64%
yes	9	0%	0%	0%	0%
		P = 0.993	P = 0.950	P = 0.333	**P = 0.044**
Overall radiation time					
≤ 1,08 months	29	89%	89%	93%	83%
> 1,08 months	71	64%	63%	69%	51%
		P = 0.015	**P = 0.011**	**P = 0.012**	**P = 0.012**
Operation					
no	73	89%	88%	88%	69%
yes	27	29%	29%	52%	45%
		P = 0.000	**P < 0.000**	**P = 0.001**	**P = 0.018**

DFS = disease-free survival; DSS = disease-specific survival; Hb = haemoglobin; LRC = loco-regional control; N = number of patients; OS = overall survival

TABLE 4. Acute treatment toxicities

Toxicity	Grade					
	0	1	2	3	4	Total
Stomatitis	68	12	10	9	1	**100**
Nausea, vomiting	79	9	9	3	0	**100**
Diarrhoea	57	17	12	13	1	**100**
Hand-foot syndrome*	22	0	1	2	0	**25**
Radiodermatitis	10	12	13	64	1	**100**
Infection	51	14	23	9	3	**100**
Leucocyte count	37	31	20	10	2	**100**
Haemoglobin level	43	44	11	2	0	**100**
Platelet count	58	36	3	3	0	**100**

* Only in patients treated with capecitabine

favourable factors, which are often combined with anaemia, was not possible to assess.

The reports in the literature of the influence of transfusions on the outcome are not consistent. Some authors found favourable effect[45], some found none[46,47] and some found unfavourable effect.[2,32] It is possible that a better oxygen delivery is not sufficient to improve oxygenation of a tumour with high oxygen consumption.[30,35] Moreover, anaemic patients are assumed to have a more aggressive disease from the start.[35,46] Immune suppression in patients could also play a part (7, 35).[7,35]

The use of erythropoetin is controversial due to the possible effect on tumour growth[14,33,48], however, only in the subpopulation of patients whose tumours expressed erythropoetin receptors.[49] Another potential mechanism by which erythropoetin therapy may result in negative outcomes in cancer patients is through promotion of thrombovascular events.[50] Therefore, it was not used in our patients. De Los Santos *et al*. believe the connection between anaemia and hypoxia is complex; therefore, it is not clear whether transfusion or erythropoetin do patients any favour.[51]

The Hb concentration during treatment progressively decreased, which is in agreement with other reports.[2,7,17,18,30-33,46] At the beginning of treatment, 31% of our patients were anaemic, and at the end 54%. That should cause more hypoxia in the tumour. It is possible that a decreased delivery of oxygen to the tumour due to of Hb drop during the treatment is partially counterbalanced by the reoxygenation due to shrinkage of the tumour and does not influence very much the outcome. In some patients with Hb ≤ 120 g/L it was possible to raise the mean Hb level by the blood transfusion or by iron preparations.

The significance of mean on-treatment Hb concentration and end-of-treatment Hb concentration is less clear. Some authors found a positive effect of higher mean on-treatment Hb concentration on treatment outcome[2,15,18,32,33,35] and some found a positive effect of higher end-of-treatment Hb concentration on treatment outcome[35,36], while others found no influence on outcome of either mean- or end- of-treatment Hb level.[31] In our patients, the mean- or end- of treatment Hb levels had less influence on survival compared to the pre-treatment values of Hb concentration.

Our study showed that pre-treatment Hb was an important independent prognostic factor for overall survival in patients with squamous cell carcinoma of the anal canal and anal margin treated with radiochemotherapy, which is in agreement with findings of most other authors. Mean on-treatment Hb and end-of-treatment Hb do not seem to have much influence on survival.

Because of a small number of patients who needed blood transfusion its influence on survival could not be assessed in our study.

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