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Dosimetric predictors of acute hematologic toxicity during concurrent chemoradiation for anal cancer

Ana Rita Ribeiro Pinho

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“For what it's worth... it's never too late (…) to be whoever you want to be.
There's no time limit. Start whenever you want. You can change or stay the same.
There are no rules to this thing.
We can make the best or the worst of it.
I hope you make the best of it.
I hope you see things that startle you.
I hope you feel things you've never felt before.
I hope you meet people who have a different point of view.
I hope you live a life you're proud of, and if you're not,
I hope you have the strength to start over again.”

– *F. Scott Fitzgerald*

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Resumo

O cancro do canal anal é uma doença rara do sistema gastrointestinal cujo tratamento *standard* é quimio-radioterapia concomitantes. Este tratamento está associado a elevadas taxas de toxicidade aguda, que pode resultar em interrupções imprevistas no tratamento, devido ao esquema de QT/RT com 5-FU/MMC. A toxicidade hematológica é um efeito secundário *major* do tratamento ao cancro do canal anal, resultante da irradiação de medula óssea altamente radiossensível durante a RT pélvico-inguinal.

Dados de outras patologias pélvicas sugerem que alguns parâmetros de dose da medula óssea pélvica estão associados a toxicidade hematológica, a principal causa de interrupções de tratamento. Desta forma, o principal objectivo deste estudo é identificar factores dosimétricos preditivos de toxicidade hematológica aguda em doentes com carcinoma espinocelular do canal anal, tratados com QT/RT radical.

Trinta doentes com doença locoregional, tratados com QT/RT, foram analisados retrospectivamente. Para cada doente, foi delineado o contorno externo de todos os ossos pélvicos na TC de planeamento, em representação da medula óssea pélvica (PBM), sendo posteriormente dividida em três *subsites*: medula óssea lombo-sagrada (LSBM), medula óssea ilíaca (IBM) e medula óssea da pélvis inferior (LPBM). A toxicidade hematológica foi avaliada através dos hemogramas realizados semanalmente (hemoglobina, leucócitos, neutrófilos e plaquetas) e tendo em conta qualquer evento hematológico grau ≥3 (HT3+).

Foi encontrada associação significativa entre PBM-Dméd e nadir dos neutrófilos e HT3+, e entre PBM-V30 e V40 e dose máxima e HT3+. Na LSBM, Dméd e V40 foram estatisticamente significativos para o nadir dos neutrófilos e HT3+; HT3+ foi correlacionada com V10-V30. Foi encontrada associação entre HT3+ e IBM-V5-30 e IBM-Dméd. Em relação à LPBM, V30 e Dméd foram associados ao nadir dos neutrófilos e HT3+, com a Dmáx também correlacionada com HT3+.

Vários factores dosimétricos preditivos de toxicidade hematológica revelaram significância estatística: PBM-Dméd ≥28 Gy, PBM-Dmáx ≥51 Gy, PBM-V30 ≥54% e PBM-V40 ≥33%, LSBM-Dméd ≥27 Gy, LSBM-V10 ≥65%,

LSBM-V20 ≥58%, LSBM-V30 ≥53%, LSBM-V40 ≥45%, IBM-Dméd ≥23Gy, IBM-V5 ≥75%, IBM-V10 ≥65%, IBM-V20 ≥53%, IBM-V30 ≥39%, LPBM-Dméd ≥33 Gy, LPBM-Dmáx ≥53 Gy e LPBM-V30 ≥59%.

Sugere-se que passem a ser incluídos na prática diária *constraints* dosimétricos direcionados à PBM e a cada *subsite*, por forma a reduzir as taxas de toxicidade hematológica aguda e o seu impacto nas interrupções no tratamento e aumento do tempo total de tratamento (TTT).

Summary

Anal cancer is a rare malignant disease of the GI tract for which the standard treatment is definitive chemoradiation. This treatment is associated with a high rate of acute toxicity that may lead to unintended treatment interruptions, due to the CRT scheme with 5-FU/MMC. Hematologic toxicity is a major side effect of anal cancer treatment as a result of the irradiation of highly sensitive bone marrow (BM) during pelvic-inguinal RT.

Data from other pelvic malignancies suggest that some pelvic bone marrow (PBM) dose parameters are associated with hematologic toxicity, which is the main cause of treatment interruptions. Therefore, the main purpose of this study was to identify dosimetric predictors of acute hematologic toxicity in patients with squamous cell carcinoma of the anal canal treated with definitive chemoradiotherapy.

Thirty patients treated with CRT to locoregional disease were retrospectively analysed. For each patient, the external contour of all bones within the pelvis was delineated on the planning CT scan, as a representation of the pelvic bone marrow (PBM), and was then divided into three subsites: lumbosacral bone marrow (LSBM), iliac bone marrow (IBM), and lower pelvis bone marrow (LPBM). Hematologic toxicity was assessed by weekly blood counts (hemoglobin [Hg], white blood cell [WBC], absolute neutrophil [ANC] and platelets [Plts] counts) and any hematologic event grade ≥3 (HT3+).

PBM-mean dose was found to be associated with ANC nadir and HT3+. HT3+ was also correlated with maximum dose and V30 and V40 of the PBM. In the lumbosacral BM (LSBM) subsite, V40 and mean dose were found to be associated with ANC nadir and HT3+. HT3+ was also correlated with V10, V20, and V30. An association was found between HT3+ and IBM-V5-30 and IBM-mean dose. Concerning the lower pelvis subsite of BM (LPBM), V30 and mean dose were found to be correlated with both ANC nadir and HT3+, and LPBM-maximum dose was also found to be associated with HT3+.

Several significant dosimetric predictors for acute hematologic toxicity were found: PBM-mean dose ≥28 Gy, PBM-maximum dose ≥51 Gy, PBM-V30 ≥54% and PBM-V40 ≥33%, LSBM-mean dose ≥27 Gy, LSBM-V10 ≥65%, LSBM-V20

≥58%, LSBM-V30 ≥53%, LSBM-V40 ≥45%, IBM-mean dose ≥23Gy, IBM-V5 ≥75%, IBM-V10 ≥65%, IBM-V20 ≥53%, IBM-V30 ≥39%, LPBM-mean dose ≥33 Gy, LPBM-maximum dose ≥53 Gy and LPBM-V30 ≥59%.

The results suggest that dosimetric constraints to PBM and each subsite should be included to routine practice, in order to reduce acute hematologic toxicity rates and its impact in treatment interruptions and overall treatment time (OTT) increase.

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List of abbreviations

AIN	Anal Intraepithelial Neoplasia
AJCC	American Joint Committee on Cancer
AP/PA	Antero-Posterior/Postero-Anterior
ANC	Absolute Neutrophil Count
ATZ	Anal Transition Zone
BCC	Basal Cell Carcinoma
BM	Bone Marrow
BT	Brachytherapy
CBCT	Cone-Beam CT
CRT	Chemoradiation
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTV	Clinical Target Volume
DVH	Dose-Volume Histograms
GI	Gastrointestinal
GTV	Gross Tumor Volume
Gy	Gray
Hg	Hemoglobin
HIV	Human Immunodeficiency Virus
HPV	Human Papilloma Virus
HSIL	High-grade Squamous Intraepithelial Lesions
HT	Hematologic Toxicity
HT3+	Hematologic Events grade 3 or 4
HU	Hounsfield Unit
IBD	Inflammatory Bowel Disease
IBM	Iliac Bone Marrow
IGRT	Image-Guided Radiotherapy
kV	Kilovoltage
IMRT	Intensity Modulated Radiation Therapy
LINAC	Linear Accelerator
LKB	Lyman-Kutcher-Burman
LPBM	Lower Pelvis Bone Marrow

LSBM	Lumbosacral Bone Marrow
LRC	Locoregional Control
LSIL	Low-grade Squamous Intraepithelial Lesions
LSS	Lumbosacral Spine
MMC	Mitomycin-C
MSM	Men who have Sex with Men
MV	Megavolts
NTCP	Normal Tissue Complication Probability
OAR	Organ At Risk
OBI	On-Board Imaging
OTT	Overall Treatment Time
PET	Positron Emission Tomography
PBM	Pelvic Bone Marrow
Plts	Platelets
PTV	Planning Target Volume
RT	Radiotherapy
SCC	Squamous Cell Carcinoma
TCP	Tumor Control Probability
VMAT	Volumetric Modulated Radiotherapy
V*x*	Volume receiving at least *x* Gy
WBC	White Blood Cell Count
2D	Two-Dimensional
3D	Three-Dimensional
3D-CRT	3D-Conformal RT
5-FU	5-Fluorouracil

Introduction

1. Anal Canal

1.1 Anatomy and Histology

The anal canal is the most terminal part of the lower gastrointestinal (GI) tract, measuring about 2.5–4 cm in length and is formed by a complex anatomic and histological structure. The anal canal corresponds to the segment invested by the internal anal sphincter and it extends from the inferior margin of the rectum, the anorectal ring, to the proximal margin of the anal verge or anal margin (Figure 1). The dentate or pectinate line is located at the midpoint. The boundaries of the anal canal are the coccyx posteriorly, with interposed adipose and muscular tissues, and the ischiorectal fossa laterally. [1–5]

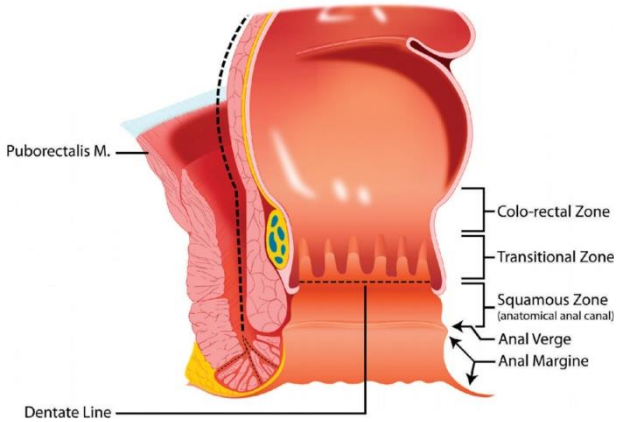

Figure 1 – Schematic drawing of the anatomy of the anal canal. Adapted from [4].

Histologically, the anal canal is divided into 3 parts, proximal to distally, according to its lining epithelium: the colorectal zone, the anal transition zone (ATZ), and the squamous mucosa-lined lower part. The lower border of the anal canal is often evident, delimitated by the junction between squamous mucosa and anal margin epidermis. The upper border, however, is difficult to delineate because of the lack of any discernible landmarks either macro or microscopically. [4] Attempting standardization, the 6th edition of the American Joint Committee on Cancer (AJCC) staging manual in 2002 subjectively defined the anal canal as beginning 1–2 cm above the dentate line. [6]

1.2 Anal Cancer

The anal canal consists of various epithelial elements that can give rise to a variety of tumour types. According to the histologic classification, two categories of tumours arise in this region. Tumours that develop from the mucosa are termed anal canal tumours and those arising within the skin at or distal to the mucocutaneous junction are termed anal margin or perianal tumours. [7–9]

About 80% of all anal canal tumours are squamous cell carcinoma (SCC). A small minority consists of adenocarcinoma or skin cancer variants. [10] The term "anal cancer" commonly refers to SCC, and given its predominance, the two terms will be used interchangeably in this Thesis.

Tumours of the anal margin include anal SCC and its precursor anal intraepithelial neoplasia (AIN). AIN 3 is a carcinoma *in situ*, formerly known as Bowen's disease. Other anal margin tumours include giant condyloma (also described as verrucous carcinoma or Buschke–Lowenstein tumours), basal cell carcinoma (BCC) and Paget's disease. Tumours of the perianal skin are most often SCC but other types of cutaneous malignancies, such as BCC and melanoma, can arise within this region more rarely. [7–9, 11]

Lymphatic drainage of anal tumours is dependent upon the anatomic site of origin. Proximally, lymphatic drainage occurs to perirectal nodes along the inferior mesenteric artery, while immediately above the dentate line drainage occurs to internal pudendal nodes, and to the internal iliac system. Infra-dentate and perianal skin drains to the inguinal, femoral and external iliac nodes. [3, 9, 12]

1.3 Epidemiology and Risk Factors

Anal cancer is a rare malignant disease of the GI tract, accounting for an estimated 0.35–0.5% of all cancers in the United States (US), with about 5,290 new cases of anal SCC reported in 2009. In 2017, it is estimated that there will be 8,200 new cases of anal cancer and that 1,100 people will die of this disease in

the US. Anal cancer is most frequently diagnosed among people aged 55–64 (median age at diagnosis: 61). [13–15]

Throughout the years, anal cancer has been slightly more common in women than men overall. However, in African Americans anal cancer is more common in men than in women. [13] Based on 2012–2014 SEER Cancer Statistics data, approximately 0.2% of men and women will be diagnosed with anal cancer at some point during their lifetime. [15] Although the worldwide rates of anal cancer incidence have not varied significantly in the last years, the annual incidence in western countries has been steadily increasing, nearly doubling in the last 25 years (Figure 2). Most increases in this incidence seem to be attributable to increases in SCC incidence. Among high-risk populations, as Human Immunodeficiency Virus (HIV) infected and men who have sex with men (MSM), the incidence of anal cancer may even exceed the rates of colon cancer. [7, 13, 15, 16]

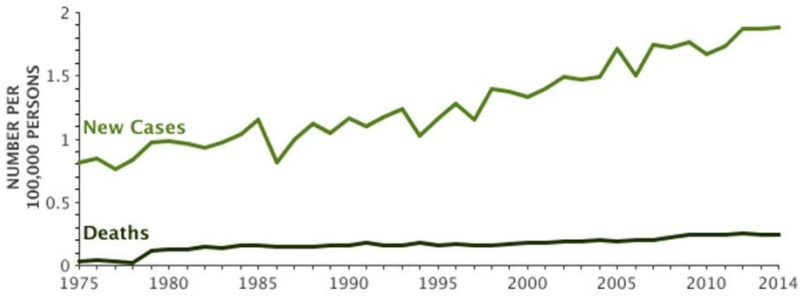

Figure 2 – Anal cancer incidence and mortality, 1975-2014.
All races, both sexes. Adapted from SEER Cancer Statistics [15].

In the past, anal cancer was thought to develop in areas of chronic inflammation or irritation associated with anal or perianal benign conditions such as haemorrhoids, fissures and fistulae. However, subsequent case-control studies found little to no impact on the development of anal cancer, and in a large Danish series, none of the 1160 patients with inflammatory bowel disease (IBD) developed anal cancer. [17–23]

Anal intraepithelial neoplasia (AIN), also known as anal squamous intraepithelial lesion (SIL), seems to explain tumour progression. AIN is assumed to be the precursor lesion to anal cancer, in which cases of low grade squamous intraepithelial lesions (LSIL) progress to high grade squamous intraepithelial lesions (HSIL), and then to SCC. In the general population AIN incidence is low. [7] However, well-defined high-risk groups, particularly those with HIV infection and

men who have sex with men (MSM), have been identified and subsequently studied. Table 1 summarizes the estimated risks for anal cancer among various populations.

Table 1 - Estimated risks for anal cancer among various populations. Adapted from [7, 24].

Anal cancer rates in selected populations, per 100000 person-years	
General population	2
General population, female	0.55–2.4
Solid organ transplant	10–15
Prior HPV-related malignancy	0.8–63.8
HIV-positive women	3.9–30
HIV-negative MSM	5.1
HIV-positive MSM	49.5–135
HIV-positive non-MSM	45
Colon cancer in general population	41

*HIV: Human Immunodeficiency Virus; MSM: Men who have Sex with Men; HPV: Human Papilloma Virus.

Risk factors for anal cancer, and AIN indirectly, consist mostly of clinical factors and behaviours associated with the acquisition and persistence of Human Papilloma Virus (HPV) infection, whose subtype most likely to cause anal cancer is HPV-16. Up to 93% of anal SCC seem to be related to HPV infection. A close association between HPV infection and many premalignant and malignant lesions of the genital tract, anus and rectum has been described. Women with a history of HPV-related cervical cancers (or pre-cancerous lesions) have also an increased risk of anal cancer. [7, 12, 15, 23, 25–27]

The strongest HPV-associated risk factors are HIV infection and high-risk sexual behaviour. People infected with HIV are much more likely to develop anal cancer than those not infected, and the risk appears to be especially high in MSM populations. [9, 15, 28–36]. High-risk sexual behaviour (MSM, receptive anal intercourse or history of multiple sexual partners) has also been shown to be associated with higher rates of HSIL. [8, 37–40]

Cigarette smoking has been implicated as a risk factor for the development of anal cancer in several case-control studies, despite a lack of clear understanding of the mechanism involved. [8, 41, 42] A recent study by Gautier *et al*. found that AIN regression after therapy failed to occur in any smoking patient, while AIN reverted in more than 60% non-smoking patients [43]

Higher rates of anal cancer also occur more frequently among people with reduced immunity. Besides HIV-related immunosuppression, other causes of chronic immunosuppression, such as solid organ transplantation, are associated with the development of HSIL and invasive anal carcinoma, a risk that is associated with persistent HPV infection. Similarly, chronic glucocorticoid therapy for the treatment of autoimmune diseases may predispose to both HPV infection and HPV-related invasive anal cancer. [8, 31, 44-48]

1.4 Anal Cancer Staging

The most used staging system is the TNM classification, detailed on Table 2. TNM staging system is based on accurate assessment of size (T-stage), regional lymph node involvement (N-stage) and metastatic spread (M-stage). Nodal status is based on the distance from the primary site rather than the number of nodes involved. Lymph node involvement at diagnosis is observed in 30-40% of cases, while only 5-8% present distant extrapelvic metastasis at diagnosis. [12]

Table 2 - TNM classification, AJCC/UICC, 7th edition. Adapted from [49].

Primary tumour (T)			
TX	Primary tumour cannot be assessed		
T0	No evidence of primary tumour		
Tis	Carcinoma in situ (i.e., Bowen disease, high-grade squamous intraepithelial lesion, and anal intraepithelial neoplasia II-III)		
T1	Tumour ⩽2 cm in greatest dimension		
T2	Tumour >2 cm but ⩽5 cm in greatest dimension		
T3	Tumour >5 cm in greatest dimension		
T4	Tumour of any size invades adjacent organ(s), e.g., vagina, urethra, and bladder.		
Regional lymph nodes (N)			
NX	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastasis		
N1	Metastases in perirectal lymph node(s)		
N2	Metastases in unilateral internal iliac and/or inguinal lymph node(s)		
N3	Metastases in perirectal and inguinal lymph nodes and/or bilateral internal iliac and/or inguinal lymph nodes		
Distant metastasis (M)			
M0	No distant metastasis		
M1	Distant metastasis		
Anatomic stage/prognostic groups			
Stage	**T**	**N**	**M**
0	Tis	N0	M0
I	T1	N0	M0
II	T2	N0	M0
	T3	N0	M0
IIIA	T1	N1	M0
	T2	N1	M0
	T3	N1	M0
	T4	N0	M0
IIIB	T4	N1	M0
	Any T	N2	M0
	Any T	N3	M0
IV	Any T	Any N	M1

1.5 Anal Cancer Therapies

Despite its growing incidence, anal cancers are relatively infrequent tumours, and factors influencing outcome and long-term survival are still not completely defined. The primary aim of treatment is to achieve cure with locoregional control (LRC) and preservation of anal function, with the best possible quality of life. The standard of care for anal SCC has therefore changed throughout the past 40 years. Radical abdominoperineal resection, which was the cornerstone of anal cancer treatment, even though it resulted in a lifelong colostomy and a subsequent immediate and prolonged loss of quality of life, has been broadly abandoned as first-line treatment to give rise to combined modality therapy. [50]

Organ preservation therapy was first reported by Nigro *et al.,* in 1974 [51] with favourable disease-related outcomes, changing the paradigm in the management of anal SCC to chemoradiation (CRT) with 5-FU (5-fluorouracil) and MMC (mitomycin-C) as standard of care. [16, 52] Evidence supporting the effectiveness of CRT as a radical treatment and the definition of the best chemotherapy regimen were based on the results of phase II and randomized phase III trials – EORTC 22861, UKCCCR ACT I, RTOG 87-04, RTOG 98-11, ACCORD-03 and CRUK ACT II. [53-58]

In the past two decades, trials have refined radiotherapy (RT) techniques and proved the efficacy of relatively low total radiation doses. Doses of at least 45-50 Gy without treatment gaps are recommended for T1-2 N0. Higher doses may be required for more advanced tumours, particularly if there are treatment interruptions. It remains unclear whether increasing the radiation dose to >50 Gy will improve the results in locally advancer anal cancer. Additional boost doses to the primary tumour usually range from 15 to 25 Gy, with higher doses applied for observed poor response. [12]

However, despite favourable disease-related outcomes and sphincter preservation, CRT is significantly associated with acute toxicity, which is in part due to the large radiation fields in order to include elective nodal regions, and to the lack of beam conformation. [59, 60] Evidently, as long as treatment dose is escalated, the irradiation of normal healthy tissue is also intensified. The

cornerstone randomized trials on CRT have reported a high rate of major dermatologic, hematologic and gastrointestinal acute toxicities, very likely due to the use of non-conformal radiation techniques such as AP/PA (antero-posterior/postero-anterior) parallel opposed fields or 4-field approaches. These techniques irradiate a large amount of normal tissues and, consequently, expose organs at risk (OARs) to undue radiation dose. In ACT II and RTOG 98-11, overall grade 3-4 acute toxicity occurred in more than 70% of the patients during CRT. This severe acute toxicity may be associated with excessive RT treatment interruptions, leading to a longer overall treatment time (OTT) and, consequently, treatment failure. [53-55, 57, 61]

The extension of the OTT in RT has been studied for several tumor sites and has included both planned (split-course regimens) and unplanned interruptions. Bese *et al.* reviewed and summarized the published data on the effect of treatment interruptions on outcome, however, no consensus was reached concerning either the duration and the position of the interruption. [62] Specifically in anal cancer, the impact of RT treatment interruption duration on LC was investigated in 90 patients treated by split-course CRT. [63] A significant decrease in LRC was found for patients with a longer OTT, and long interruption duration remained an independent prognostic factor, adversely affecting the LRC rate. On moderate-dose uninterrupted radiotherapy courses in association with chemotherapy, early gaps can be considered more acceptable because a sufficient period is available for compensation of the missed treatment days before completion of the RT schedule. [62]

Technological advances in RT have emerged since the randomized trials on anal cancer CRT. The two-dimensional (2D)-based RT planning progressed to computed tomography (CT)-guided three-dimensional (3D)-conformal RT (3D-CRT) treatments. These allow the radiation oncologist to identify target as well as normal structures on axial CT images, leading to improved treatment accuracy and delivery. More recent developments in RT, such as intensity modulated radiation therapy (IMRT), volumetric modulated radiotherapy (VMAT), image-guided radiotherapy (IGRT) using cone-beam CT (CBCT) allow smaller margins and highly conformal plans, resulting in decreased radiation doses to the OARs, less treatment gaps and consequently no increases in the OTT. In general, IMRT has been used to improve the tolerability of CRT to anal cancer, with most series reporting favourable results. [16, 50, 59, 60, 64-66] High radiation doses using IMRT, and with no extension in the OTT, is suggested to improve results.

Brachytherapy (BT) can also boost a small volume, sparing the adjacent normal tissues. BT is a highly conformal treatment which is able to deliver a high dose to the primary tumour, sparing surrounding normal tissues and the contralateral mucosa and sphincter. Delivery of a high-dose boost to the primary-tumour area is therefore feasible with this technique. [12, 67]

Delivery of radiotherapy in anal cancer is complex because of the varying size and shape of the target volume, and the proximity to dose-sensitive critical structures, such as small bowel, rectum, bladder, femoral heads, perineum and external genitalia. Several studies of IMRT in anal canal carcinoma have reported significant reduction in the doses delivered to the OARs. [12] The most common acute toxicities are dermatologic, gastrointestinal and hematologic, but pelvic bone marrow (PBM) is not yet taken into account during treatment planning. It has been described that anal cancer patients treated with IMRT have favourable outcomes and toxicity, suggesting IMRT plans can be optimized to reduce PBM irradiation, but it is not well known whether this technique will reduce hematologic toxicity (HT). [64]

2. Pelvic Bone Marrow

2.1 Bone Marrow Anatomy and Distribution in adults

The skeletal system has four components: bones, cartilage, tendons and ligaments. The skeleton is usually thought of as the structure of the body, but the skeletal system has many other functions as well including blood cell production. Many bones contain cavities filled with red bone marrow which gives rise to blood cells and platelets (Plts). In addition to the small spaces within spongy and compact bone, the diaphysis of long bone has a large internal space called the medullary cavity. The cavities of spongy bone and the medullary cavity are filled with marrow (Figure 3).

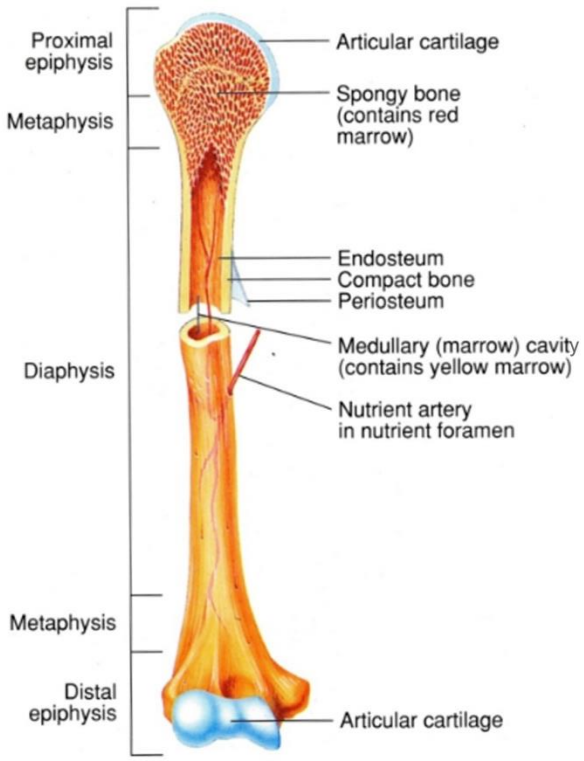

Figure 3 – Long bone structure and bone marrow location. Adapted from [68].

There are two types of bone marrow (BM): "red" marrow and "yellow" marrow. Red marrow is the site of red and white blood cell formation,

characterized by hemopoietic tissue surrounded by a framework of reticular fibers. Yellow marrow is mostly adipose tissue and does not produce blood cells. In the fetus, the spaces within bones are filled with red marrow. The conversion of red marrow to yellow marrow begins just before birth and continues into adulthood. Yellow marrow completely replaces the red marrow in the long bones of the limbs, umerus and femur. Elsewhere varying proportions of yellow and red marrow are found. In some locations, "red" marrow is completely replaced by "yellow" marrow; in others there is a mixture of red and yellow marrow. This is what happens in part of the ilium, which may contain 50% red marrow and 50% yellow marrow. This, together with being a large bone and being easily accessed, are the reasons why it is used as a source of donated red bone marrow. [1]

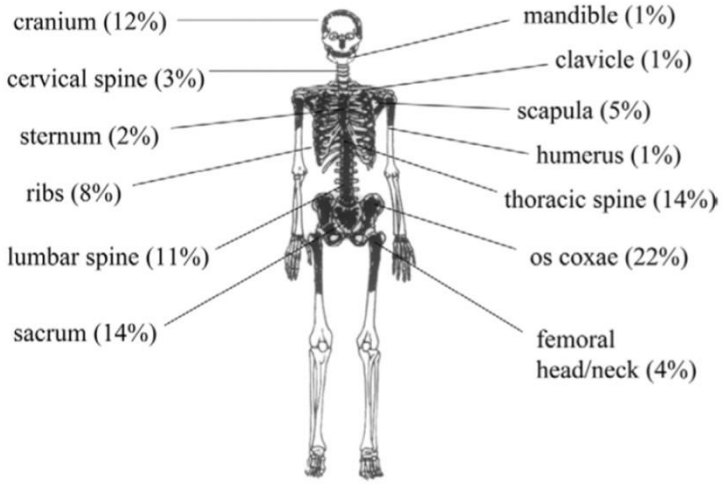

Figure 4 - Distribution of bone marrow in an adult. Adapted from [69] .

The major functional sites for BM in the adult population are the pelvis and vertebrae (lumbar, more precisely) that account for approximately 60% of the total amount. Pathologic studies have demonstrated that pelvic bone marrow (PBM) is composed of hematologically active "red" marrow and inactive "yellow" marrow. [70-72] Pelvic bones may contain up to 40% of the total functional BM (Figure 4). [5, 73-75] In pelvic RT hematologic events are very common because up to 50% of a patient's total hematopoietically active BM is within the conventional treatment fields. [72, 74]

The whole bone consists of, as previously described, higher density cortical/compact bone and lower density trabecular/spongy bone, the latter of

which contains the active marrow cavity. Thus, on CT scans, the marrow cavity corresponds to the lower Hounsfield Unit (HU) portions of the bone. Some published studies in which the PBM was delineated as the whole bone and correlated to the risk of HT have suggested that the dose to the marrow cavity may specifically better predict this risk. On the other hand, Cheng *et al.* demonstrated that whole bone delineation is superior to marrow cavity contouring in predicting HT according to Lyman-Kutcher-Burman (LKB) model. [76] The definition of PBM is crucial in this sense and may affect the strength of the relationship between BM and dosimetric parameters.

2.2 Irradiation of the PBM, Acute Toxicity and PBM Sparing in RT

Standard pelvic irradiation typically irradiates a substantial amount of bone marrow resulting in the depletion of hematopoietic stem cells which are needed to repopulate erythrocytes, leukocytes, and platelets. [77] As a result, pelvic irradiation has the potential to increase hematologic toxicity (HT), which can limit tolerance for chemotherapy and radiotherapy, namely leukopenia, neutropenia, anemia and thrombocytopenia, noted in blood counts. Myelosuppression from systemic chemotherapy is the predominant cause of HT. [78] In the US Intergroup trial (RTOG 87-04), 18% of patients experienced acute G ≥4 HT. [53, 70, 79]

Lumbosacral spine (LSS), a sub-site of PBM according to Mell *et al.* division, contains a substantial amount of BM that is significantly irradiated with RT techniques for both cervical and anal cancer due to its close proximity to the primary tumour and pelvic lymph nodes. [64] A study by Bazan *et al.* showed that the dosimetric impact of both LSS and PBM was important in predicting HT. [76] Concurrent CRT is associated with grade 3-4 HT in approximately 60% of patients. [59, 61] HT is related to infections, bleeding and fatigue and can lead to radiation treatment gaps and chemotherapy dose reduction. [16, 80, 81] Therefore, reduction of HT may lead to improved tolerance and delivery of CRT.

In addition, limited data is available regarding the correlation between dosimetric parameters to PBM and acute HT. Mell *et al.* demonstrated a correlation between several PBM dosimetric parameters and blood count nadirs in

patients with anal cancer. [64] In addition to the radiation dose, the volume of BM irradiated is an important factor. As the volume of actively proliferating BM irradiated increases, the total hematopoietic output decreases precipitously. Depending on the dose delivered and volume irradiated, it can also take a considerable period for the BM to recover. [73] Bazan *et al.* reported a significant association of PBM dose with HT, using normal tissue complication probability (NTCP) modelling. [82] Data from cervix cancer suggests that low dose radiation parameters, such as the volume of PBM receiving 10 Gy are correlated with grade 2 HT. [83] In a retrospective analysis, Rose *et al.* found that patients in whom ≥95% of the BM received 10 Gy had higher rates of grade 3 leukopenia than did those in whom <95% of the BM received this dose. [84] Additionally, Albuquerque *et al.* found that a volume of BM receiving 20 Gy was most strongly predictive of G≥2 HT. When >80% of the pelvic bone received 20 Gy, the risk of G≥2 HT was increased by a factor of 4.5. [83]

Though, BM tolerance to CRT remains poorly understood. BM is not included in the tables by Emami *et al.*, and the LKB model has not been applied to BM. [85, 86] The hematopoietic stem cells in the bone marrow that are continuously replacing circulating peripheral blood cells are among the most radiosensitive cells in the body. Data exist suggesting that radiation doses as low as 2–4 Gy delivered within 1–3 days can cause a significant reduction in bone marrow cellularity and proliferation, and doses of 30–40 Gy in conventional fractionation can lead to complete ablation of the BM. [51, 53, 57, 87] BM behaves as a parallel organ.

One strategy to limit HT is to use IMRT, reducing the volume of BM irradiated during CRT while maintaining adequate target volume coverage. IMRT has been shown to reduce radiation dose to other OARs such as the bowel, bladder and genitalia, compared with 3D-CRT plans, in the treatment of anal cancer. IMRT would minimize acute and late toxicity by virtue of sparing nontarget structures, without compromising locoregional control. [50]

Yang *et al.* reported potential dosimetric constraints for pelvic bony structures that can be used for pelvic RT planning, in particular IMRT planning. Through detailed dosimetric investigations, they have come to the conclusion that the volume of coxal (comprising bilateral ilium, ischium and pubic) and sacral (extending from the L5-S1 junction to coccyx) BM receiving at least 45 Gy (V45) emerged as significant predictors for lower white blood cell count (WBC),

absolute neutrophil count (ANC) and hemoglobin (Hg) cell count ratio at nadirs. Dose constraints employing these variables should, therefore, be considered using in IMRT planning, especially in patients aged ≥59 years old. [88]

However, the impact of IMRT in delivering a “low dose bath” to normal tissue needs to be considered in this tumour type, particularly in the context of concurrent CRT. This impact is most pronounced in highly chemoradiation sensitive tissue such as BM, whose increased irradiation has been shown to increase likelihood of early hematologic adverse events. [64, 69]

Aims of the study

1. Aims

Anal cancer is a rare malignant disease of the GI tract that accounts for an estimated 0.5% of all cancers in the United States. [13-15] The standard treatment for anal SCC is definitive CRT. This treatment is associated with a high rate of acute toxicity, due to the CRT scheme with 5-FU/MMC that, despite favourable disease-related outcomes and preservation of sphincter function may result in unintended treatment interruptions leading to a reduction in therapeutic efficacy. [88, 89]

Myelosuppression is a major side effect of anal cancer treatment and its main cause is the destruction of BM stem cells. [75] Thus, reduction of hematologic toxicity (HT) is an important goal. The large volume of irradiated highly sensitive bone marrow (BM) during conventional pelvic-inguinal RT is a significant contributor to HT, as up to 50% of a patient's total hematopoietically active BM is within the conventional treatment fields, in the pelvis. [74] RT techniques to anal cancer have been progressing from early 2D to 3D treatment planning, and more recently to IMRT. This treatment technique has the potential to reduce normal tissue irradiation and allows conformal radiation doses and tumor dose escalation in anal cancer patients. [59] However, the impact of IMRT in delivering a "low dose bath" to normal tissue needs to be considered, particularly in the context of CRT. [64, 69, 75]

Therefore, the main purpose of this study was to identify dosimetric predictors of acute hematologic toxicity in patients with squamous cell carcinoma of the anal canal treated with definitive chemoradiotherapy. In order to achieve this main aim, this project comprises the following set of goals:

- to assess dosimetric factors associated with pelvic irradiation-related HT;
- to investigate whether the irradiated volume of pelvic bone marrow (PBM) and specific subsites is a dosimetric predictor of acute HT;
- to create dosimetric constraints that can reduce the risk of HT to be used in routine planning practice.

Materials and Methods

1. Patient Selection

This study was approved by the Institutional Review Board [Comissão de Ética para a Saúde - CES IPO: 3/017] of Portuguese Oncology Institute - Porto, Portugal.

All patients with the diagnosis of anal squamous cell carcinoma (anal canal or anal margin), with locoregional disease only, treated with IMRT or VMAT techniques, and concurrent chemotherapy with 5-FU/MMC, with definitive intent, at the Radiotherapy Department of the aforementioned Institute were selected between July 2012 and August 2017.

Medical records were reviewed to identify each patient's demographic information, treatment data and treatment-related toxicity reports during CRT. Patient age, gender, tumour staging and HIV status were collected. Data was assessed for anal cancer patients who underwent radiotherapy treatments. Tumor stage was defined according to the 7th edition American Joint Committee on Cancer (AJCC) indications and patients with clinical stage T1-4 N0-3 M0, with whole pelvis and nodal irradiation with 45 Gy were enrolled in the study.

Patients undergoing palliative RT treatment or any treatment other than CRT; with pelvic irradiation dose other than 45 Gy; treated with conventional fields of 3D-CRT; chemotherapy other than 5-FU/MMC were excluded from the analysis.

2. Treatment Details

2.1 Radiotherapy

In order to define the target volumes, the patient must undergo a planning CT, in which the dose calculation will be made according to each voxel's HU. Beam configuration is then arranged, in order to deliver the prescription radiation dose to the target volume, trying to spare most healthy tissue and OARs as possible. The treatment planning system provides isodose lines distribution and dose-volume histograms (DVH) so as to evaluate the treatment plan. The specifications of these procedures are as follows:

- Planning CT: Patients were immobilized in the prone position with both knee rest and ankle support in order to prevent from hip rotation, indexed to the simulator table to ensure daily reproducible setup. For planning purposes, a non-contrast planning CT scan was performed and 3 mm-slice thickness axial images were obtained from the upper lumbar spine to the mid-femur. The origin coordinate was marked on the patient's skin under laser guidance in the pelvic region for daily position. The image datasets were transferred to the Eclipse planning system (Varian Medical System, Palo Alto, CA, USA).

- Volume delineation: For contouring purposes, the gross tumor volume (GTV) consisted of the gross primary tumor and the enlarged and involved lymph nodes. The clinical target volume (CTV) corresponded to the GTV and regional lymph nodes (mesorectal, pre-sacral, latero-pelvic and inguinal). Out of this structure the planning target volume (PTV) was created by an isotropical expansion of 10 mm, accounting for organ motion and set up errors. Boost volumes were created likewise. Normal tissues contoured at the time of RT planning included the bladder, small bowel and femoral heads.

- IMRT/VMAT planning: Two different treatment techniques were available, IMRT and VMAT. For IMRT, plans were generated with 7 to 9 modulated fields with inverse optimization algorithm, depending on patient's anatomy, and VMAT plans were generated using double-arc approach (Figure 5). Both IMRT and VMAT treatments were planned for Varian Trilogy or Novalis Tx linear accelerators (LINACs), with 6 MV photons. Inverse planning was used; dose constraints used to

guide inverse planning were individually adjusted. Whole pelvis irradiation and additional doses to boost volumes (and BT) were prescribed according to clinical indication, but due to variable boost doses and techniques only pelvic and/or inguinal irradiation was taken into account for this study. RT plans were also optimized using individualized normal tissue dose constraints, minimizing dose in particular to the small bowel, bladder and femoral heads, in order for the dose to all OARs to be kept as low as possible. Constraints were placed on femoral head dose but dose to other pelvic bone structures was unconstrained. Plans were evaluated according to isodose lines distribution in axial slices and according to PTV and OARs dose-volume parameters given by DVH. QUANTEC/Emami tables were taken into account for OARs dose evaluation. [85, 90]

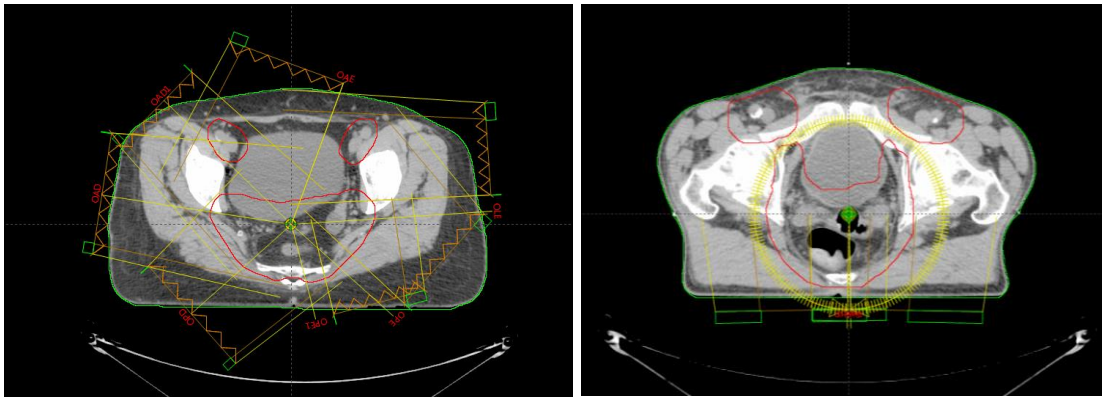

Figure 5 – Example of IMRT and VMAT treatment fields.

- Radiotherapy treatment delivery: Daily orthogonal kV on-board imaging (OBI) was performed before treatment to assure set-up accuracy after patient positioning. RT treatment was delivered 5 days/week with 1 fraction/day. RT treatment interruptions were avoided in order not to increase the overall treatment time (OTT). However, when this occurred, compensation for missed treatment days was made by treatment delivery on Saturday (one or more) or two fractions in one day with at least 8h between fractions, during the interruption week or the following.

2.2 Chemotherapy

Patients received concomitant chemotherapy, consisting of 5-FU (1000 mg/m^2/day) given as continuous infusion (days 1 and 29) combined with MMC (10 mg/m^2) given as bolus (days 1 and 29). A total of two concurrent cycles were planned for each patient. Chemotherapy re-schedule or discontinuation was performed based on patient's toxicity. Blood cell counts were routinely performed prior to each chemotherapy administration.

3. Bone Marrow Delineation

For each patient, the external contour of all bones within the pelvis was delineated on the planning CT scan, as a representation of the bone marrow (BM), at Eclipse treatment planning system. The external contour was chosen, rather than the low-density regions within the bones, to ensure reproducibility and to minimize dependence of the contours on CT windowing and levelling. Pelvic bone marrow (PBM) was divided into three subsites (Figure 6):

- lumbosacral bone marrow (LSBM), extending from the superior border of the L5 vertebral body to the coccyx;
- iliac bone marrow (IBM), extending from the iliac crest to the superior edge of femoral head;
- lower pelvis bone marrow (LPBM), consisting of the pubes, ischium, acetabula, and proximal femur, extending from the superior border of the femoral heads to the inferior border of the ischial tuberosities.

Sub division of BM was based on previously published work by Mell et al. The method of delineating BM using the external surface of bone is consistent with the RTOG 04-18 clinical trial as well as the aforementioned published work. [64, 69, 79]

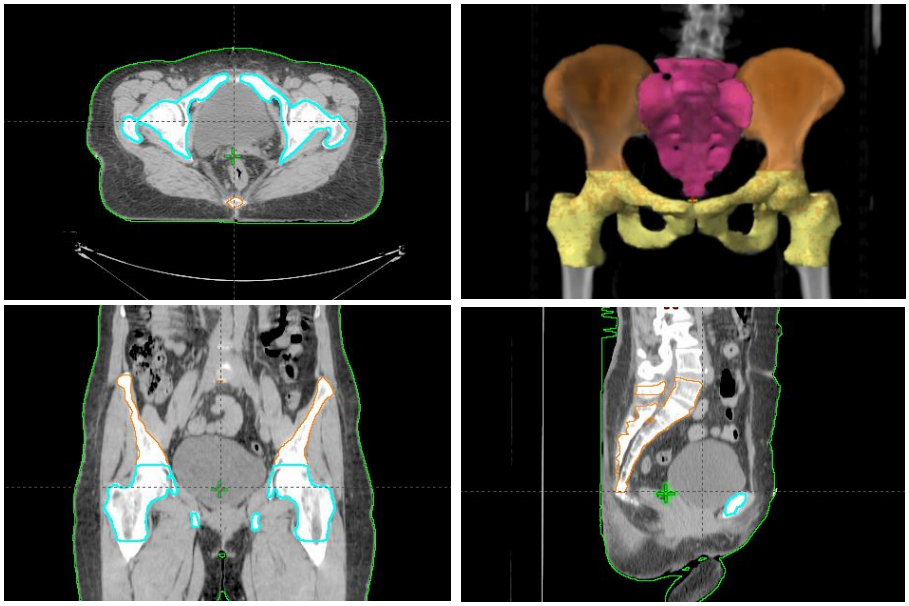

Figure 6 – Pelvic bone marrow and specific subsites delineation (axial, 3D, coronal and sagittal views).

Cumulative dose-volume histograms (DVH) were then generated, and the following parameters were retrospectively recorded for PBM and each subsite: volume, mean and maximum doses and the volume of each subsite receiving at least 5, 10, 20, 30 and 40 Gy (V5-40Gy). Dose metrics were collected from the treatment planning system Eclipse (Varian Medical Systems, Palo Alto, CA).

4. Hematologic Toxicity Assessment

All patients had weekly complete hematologic blood counts before and during CRT treatment. Acute hematologic toxicities (HT) were assessed and graded according to National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) v4.03 (See Table 3). [91]

Endpoints evaluated in the present analysis were hemoglobin (Hg), white blood cell count (WBC), absolute neutrophil count (ANC) and platelets count (Plts) baseline and nadir values after the first chemotherapy cycle and during the 45 Gy irradiation of the pelvis, and the highest grade toxicity grade for all blood cells during RT. In addition to absolute counts, blood count ratios were calculated by dividing nadir counts by baseline count. HT was defined as each acute hematologic adverse events grade 3 or 4 (HT3+).

Table 3 – Adverse events grading. Adapted from CTCAE v4.03. [91]

Adverse Event	*Grade 0*	*Grade1*	*Grade 2*	*Grade3*	*Grade 4*
Anemia	Hg NV: (F) 12–16 (M) 14–18 g/dL	Hg: 10–NV g/dL	Hg: 8–10 g/dL	Hg: <8 g/dL	–
Leukopenia	WBC NV: 4–11 x10^9/L	WBC: 2–NV x10^9/L	WBC: 2–3 x10^9/L	WBC: 1–2 x10^9/L	WBC: <1 x10^9/L
Neutropenia	ANC NV: 2.5–7.5 x10^9/L	ANC: 1.5–NV x10^9/L	ANC: 1–1.5 x10^9/L	ANC: 0.5–1 x10^9/L	ANC: <0.5 x10^9/L
Thrombocytopenia	Plts NV: 150–450 x10^9/L	Plts: 75–NV x10^9/L	Plts: 50–75 x10^9/L	Plts: 25–50 x10^9/L	Plts: <25 x10^9/L

Hg, Hemoglobin; NV, normal values; F, female; M, male; WBC, white cell count; ANC, absolute neutrophil count; Plts, platelets.

5. Statistical Analysis

Variables considered as continuous for the analysis were: age, PTV volumes, RT doses and PBM and each subsites volume, mean and maximum doses and the volume receiving at least 5, 10, 20, 30 and 40 Gy (V5-40Gy). Days of RT treatment interruption was also considered a continuous variable, as well as the days of overall treatment time increase. Variables considered as categorical were: gender, tumor location (anal canal vs anal margin), stage, HIV status, treatment approach (IMRT vs VMAT), chemotherapy dose reduction/discontinuation, hematologic toxicity grading, RT treatment interruption and overall treatment time increase.

Association between categorical variables HT grading and treatment interruption was tested using the chi-square independence test or the Fisher test, as appropriate. The distribution of continuous variables V5-40 Gy was compared between HT groups using the Mann-Whitney non-parametric test, and their correlation with Hg, WBC, ANC and Plts nadirs was investigated. All statistical analyses were performed using R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria). A p-value <0.05 was considered significant.

Results

1. Patient and Treatment Characteristics

A total of 30 anal SCC patients were selected for this retrospective study, treated with combination therapy with definitive intent between July 2012 and August 2017. This study analysis is focused on pelvic/inguinal irradiation with 45 Gy only. Patient and tumor characteristics are showed in Table 4.

Table 4 – Patient and tumor characteristics.

	No. of patients	*%*
Age, years		
Median (range)	58 (36-76)	
Gender		
Female	25	83.3
Male	5	16.7
HIV status		
Positive	1	3.3
Negative	29	96.7
Primary tumor site		
Anal canal	29	96.7
Anal margin	1	3.3
T stage		
T1	1	3.3
T2	12	40
T3	17	56.7
T4	0	0
N stage		
N0	9	30
N1	6	20
N2	8	26.7
N3	7	23.3
M stage		
M0	30	100
M1	0	0
Global stage		
I	1	3.3
II	9	30
IIIA	17	56.7
IIIB	3	10

* HIV, human immunodeficiency virus.

Median age was 58 years (range: 36–76) and patients were mainly female (83.3%), HIV-negative (96.7%), T2–T3 stage (96.7%) and with positive nodal stage (N1–N3, 70%).

The prescribed dose for whole pelvis irradiation was 45 Gy in 1.8 Gy fractions. Mean pelvic PTV volume was 2241.2 cm^3 for all 30 patients (range 1519.5–2840.8 cm^3). Patients were mainly treated with VMAT technique (86.7%). Treatment characteristics are summarized in Table 5.

Table 5 – Treatment characteristics.

	No. of patients		*%*
RT technique			
IMRT	4		13.3
VMAT	26		86.7
RT interruptions			
No gaps	12		40
Gaps < 3 days	4		13.3
Gaps ≥ 3 days	14		46.7
Mean (range), days		5.8 (1–11)	
OTT			
No increases	14		46.7
Increased OTT	16		53.3
Mean (range), days		5.1 (1–8)	

* OTT, overall treatment time.

A total of 18 patients (60%) underwent RT treatment interruptions with a mean of 5.8 days of RT interruption (range 1–11 days). 77.8% of these (14 patients) interrupted for 3 or more days. RT interruptions were caused by hematologic toxicities (neutropenia and/or thrombocytopenia) (60.9%), logistic causes (LINAC maintenance or holiday) (26%) and non-treatment related cause (upper gastrointestinal bleeding requiring hospitalization) (13%).

In 14 patients (46.7%) OTT had no increases but 16 patients (53.3%) underwent OTT increases, with mean increases of 5.1 days (range 1–8 days). RT treatment interruptions ≥3 days were found to be associated with an increase in OTT ($p<0.01$).

2. Toxicity Profile

The baseline and nadir Hg, WBC, ANC and Plts counts and count ratios are shown in Table 6, together with acute hematologic toxicity. In analysis of acute toxicity, grade 3 or higher hematologic toxicity (HT3+) was noted in 18 patients (60%). Maximum detected acute HT were as follows: anemia-G3: 3.3%; leukopenia-G3: 36.7%; leukopenia-G4: 6.6%; neutropenia-G3: 24.1%; neutropenia-G4: 20.7%; and thrombocytopenia-G3: 13.3%; thrombocytopenia-G4: 6.6%. Neutropenia was the most common acute HT3+ during RT treatment (44.8%), followed by leukopenia (43.3%) and thrombocytopenia (19.9%). Only 3.3% of the patients experienced grade ≥3 anemia during RT.

Table 6 – Acute toxicity.

Hematologic parameters	Baseline counts *Median (range)*	Nadir counts *Median (range)*	Nadir count ratios *Median (range)*
Hg	12.9 g/dL (9.5-15.3)	10.8 g/dL (6.2-12.7)	0.83 (0.41-0.64)
WBC	6.94 x10^9/L (2.03-15.70)	2.15 x10^9/L (0.63-3.62)	0.26 (0.11-0.64)
ANC	3.76 x10^9/L (1.08-11.85)	1.09 x10^9/L (0.10-2.74)	0.20 (0.03-0.83)
Plts	248 x10^9/L (74-355)	68 x10^9/L (8-192)	0.30 (0.03-0.73)

				HT3+	
Acute toxicity	Grade 0 *N (%)*	Grade 1 *N (%)*	Grade 2 *N (%)*	Grade 3 *N (%)*	Grade 4 *N (%)*
Anemia	1 (3.3)	17 (56.7)	11 (36.7)	1 (3.3)	-
Leukopenia	0 (0)	6 (20)	11 (36.7)	11 (36.7)	2 (6.6)
Neutropenia*	1 (3.5)	10 (34.5)	5 (17.2)	7 (24.1)	6 (20.7)
Thrombocytopenia	1 (3.3)	13 (43.3)	10 (33.3)	4 (13.4)	2 (6.7)

* One patient had missing values on absolute neutrophil count. Hg, hemoglobin; WBC, white blood cell count; ANC, absolute neutrophil count; Plts, platelets; HT3+, hematologic event G3-4.

Blood count nadirs occurred in mean at 16 days after CRT start. A total of 4 out of the 30 patients (13.3%) had chemotherapy discontinuation (did not receive the second cycle of chemotherapy) because of G3/4 toxicity.

No association was found between baseline blood counts (Hg, WBC, ANC and Plts) and RT treatment interruption (*p*-value: 0.7084, 1, 1 and 0.4920, respectively).

RT treatment interruption was associated with neutropenia G≥3 and thrombocytopenia G≥3 (p<0.01) and an association was found between OTT increase and HT3+ (p<0.01). Figure 7 shows RT interruptions and its respective cause, as well as the day nadir occurred.

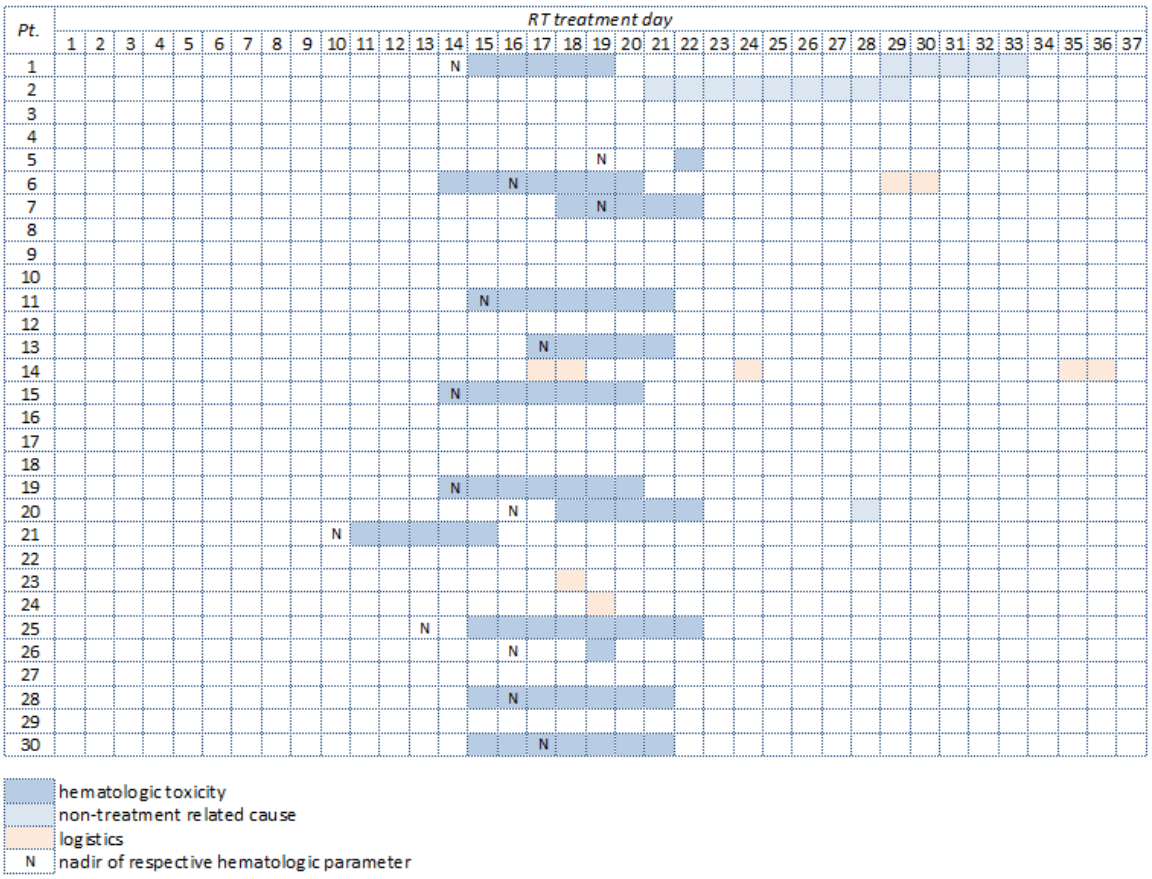

Figure 7 – RT treatment interruptions and respective hematologic nadir day.

3. BM dosimetric parameters

Volume and dosimetric parameters to pelvic bone structures are shown in Table 7.

Table 7 – Dosimetric parameters.

	Mean value	*SD*
PBM		
Volume (cm^3)	1275	190
Mean dose (Gy)	28	2
Maximum dose (Gy)	51	1
V5 (%)	86	5
V10 (%)	78	5
V20 (%)	68	9
V30 (%)	54	10
V40 (%)	33	7
LSBM		
Volume (cm^3)	330	44
Mean dose (Gy)	27	4
Maximum dose (Gy)	50	1
V5 (%)	79	8
V10 (%)	65	11
V20 (%)	58	13
V30 (%)	53	13
V40 (%)	45	8
IBM		
Volume (cm^3)	416	44
Mean dose (Gy)	23	4
Maximum dose (Gy)	50	1
V5 (%)	75	10
V10 (%)	65	10
V20 (%)	53	8
V30 (%)	39	6
V40 (%)	21	5
LPBM		
Volume (cm^3)	518	108
Mean dose (Gy)	33	1
Maximum dose (Gy)	50	2
V5 (%)	100	1
V10 (%)	99	2
V20 (%)	86	10
V30 (%)	59	8
V40 (%)	33	9

* SD, standard deviation; PBM, pelvic bone marrow; LSBM, lumbosacral bone marrow; IBM, iliac bone marrow; LPBM, lower pelvis bone marrow.

4. Predictors of HT

An analysis was performed to identify predictors of acute HT3+. The statistically significant dosimetric parameters of PBM and each subsite for acute hematologic toxicity are summarized in Table 8.

Table 8 – Dosimetric predictors of acute hematologic toxicity.

			p-value
PBM	Mean dose ≥28 Gy	ANC nadir and HT3+	*0.03904; <0.01*
	Max dose ≥51 Gy	HT3+	*0.02954*
	V30 ≥54%		*0.04434*
	V40 ≥33%		*0.03883*
LSBM	Mean dose ≥27 Gy	ANC nadir and HT3+	*0.02749; <0.01*
	V10 ≥65%	HT3+	*0.01655*
	V20 ≥58%		*0.01655*
	V30 ≥53%		*0.01655*
	V40 ≥45%	ANC nadir and HT3+	*0.02749; <0.01*
IBM	Mean dose ≥23Gy	HT3+	*0.02954*
	V5 ≥75%		*0.01219*
	V10 ≥65%		*0.01219*
	V20 ≥53%		*0.02566*
	V30 ≥39%		*0.01219*
LPBM	Mean dose ≥33 Gy	ANC nadir and HT3+	*0.0348; 0.01219*
	Max dose ≥53 Gy	HT3+	*0.02954*
	V30 ≥59%	ANC nadir and HT3+	*0.01902; <0.01*

* PBM, pelvic bone marrow; LSBM, lumbosacral bone marrow; IBM, iliac bone marrow; LPBM, lower pelvis bone marrow; Max, maximum.

Associations were found between:

- mean dose to PBM and ANC nadir (p=0.03904);
- HT3+ and mean dose (p=0.01219), maximum dose (p=0.02954) and V30 (p=0.04434) and V40 (p=0.03883) of the PBM.

In the lumbosacral BM (LSBM) subsite, associations were found between dosimetric parameters and hematologic toxicities as follows:

- ANC nadir and V40 (p=0.02749) and mean dose (p=0.02749);
- HT3+ and V10 (p=0.01655), V20 (p=0.01655), V30 (p=0.01655), V40 (p=0.00754) and mean dose (p=0.00754) to LSBM.

Relating to iliac BM (IBM), associations were found between:

- HT3+ and V5–V10 (p=0.01219), V20 (p=0.02566), V30 (p=0.01219) and mean dose (p=0.02954) to IBM.

Concerning the lower pelvis subsite of BM (LPBM), several dosimetric parameters were found to be associated with hematologic toxicities as follows:

- ANC nadir and V30 (p=0.01902) and mean dose (p=0.0348);
- HT3+ and V30 (p<0.01), mean dose (p=0.01219) and maximum dose (p=0.02954) to LPBM.

Discussion

1. Discussion

Concurrent chemoradiation is the current standard of care of patients with anal cancer, as pointed out by improving clinical outcomes, such as disease-free survival and sphincter preservation, over surgery [51] and radiotherapy alone [53-58]. However, HT remains an issue for this subgroup of patients, with reported rates of HT grade ≥3 up to 61% in the RTOG 98-11, [61] a study that involved conventional radiation techniques. Other work published by Salama *et al.* [60] reported a similar rate of this toxicity (HT3+: 58.5%) in a group of patients treated with IMRT, with no specific constraints to BM. Acute HT3+ rates appear similar in RTOG 98-11 (62%), RTOG 05-29 (58%), and the study by Mitra *et al.* (63%), which suggests that pelvic bone marrow is similarly suppressed by CRT, regardless of radiation approach. [92] Though, IMRT can provide a more favourable toxicity profile, as described by Kachnic *et al.* [65], who reported G3 and G4 acute HT rates of 49% and 12% with dose-painted IMRT. In our results, we found G3 and G4 acute HT rates of 53% and 23%, respectively. HT3+ was noted in 60% of patients, similarly to the rates published by Mitra *et al.* [92]

In our series, patients were treated with both IMRT and VMAT approaches, which might be a potential selection bias and may also influence toxicity profile given the different dosimetric outcomes. CRT with VMAT technique was recently described by Franco *et al.* [93], with reported acute HT and treatment gap rates much lower to those previously published. IMRT has been demonstrated by Bazan *et al.* and Choung *et al.* to decrease the acute toxicity profile in patients with anal cancer compared with 3D-CRT, with a consequent further advantage in terms of reduction in treatment gaps. [94, 95]

Kachnic *et al.* [65] reported treatment gaps in 40% of patients with a median interruption of 2 days. Among them, gaps >3 days corresponded to 35%. In our study, RT treatment interruptions occurred in 60% of patients, mostly due to HT (60.9%), with a mean interruption of 5.8 days and 78% of these interrupted for three or more days. All hematologic causes (neutropenia and/or thrombocytopenia) were grade 3 or 4.

Leukopenia and neutropenia however, were the most recurrent HT reported in the analysis, consistent with Salama *et al.* and Mitra *et al.* [60, 92] results. An

association was found between treatment interruptions and neutropenia and thrombocytopenia G≥3, however, baseline blood counts failed to predict RT interruptions. In most patients, treatment interruption did not match the day nadir occurred. This was due to one of two reasons: either the blood counts were collected few days prior to the medical appointment, and the treatment was only then interrupted; or the low blood count values kept dropping, reaching the nadir few days after treatment interruption. Among patients who had RT treatment interruptions, 88.9% resulted in an increased OTT.

Due to Department policy of keeping the OTT, more important than evaluating RT treatment interruptions is to evaluate the OTT increase. Compensation for missed treatment days is usually made by treatment delivery on Saturday (one or more) or two fractions in one day with at least 8 hours between fractions, during the interruption week or the following, in order to avoid the extension of OTT. OTT increase in unplanned RT interruptions has been studied for several tumor sites and summarized by Bese *et al*. [62] Early gaps are considered acceptable because a sufficient period is available for compensation of the missed treatment days before completion of the RT schedule. In our study, OTT increases occurred in 16 patients (53.3%), with mean increases of 5.1 days. We also found that OTT increase was significantly associated with HT3+ ($p<0.01$).

Bese *et al*. also reported that, regardless of the fractionation schedule and primary tumor site, an interruption of about 1 week was found to be associated with a reduction in LC rates of 10–12%, and a gap of 1 day could reduce the LC rate by about 1.4%. Moreover, OTT increase by 10 days was found to result in a 10–20% decrease in the 5-year relapse-free survival rate. Still, no unanimity has yet been established specifically for anal cancer. [62] According to tumor control probability (TCP) model by Muirhead *et al*., in late stage anal tumors, a dose escalation from 50 to 55 Gy improves 2-year LC rate from 50 to 80%. [96] High radiation doses using IMRT, with no extension in the OTT, seems to be suggested in order to improve outcomes. [67] However, dose escalation on patients susceptible to treatment interruptions due to HT needs to be balanced, as more toxicity will be added.

BM is an important dose-limiting cell renewal tissue as BM stem cells are extremely radiosensitive, resulting most of the times in myelosuppression. [75] It is already known that the major functional sites for BM in the adult population are the pelvis and vertebrae, accounting for approximately 60% of the total amount,

where pelvic bones may contain up to 40% of the total functional BM. [5] Thus the importance of pelvic irradiation in determining HT during combination therapy in anal cancer patients. Though, most of the published results relating HT with dose-volume parameters in PBM arise in the context of cervical cancer. The extent of radio-induced BM damage has been vastly demonstrated to be correlated with irradiated BM volume, particularly through associating blood cell count decrease and acute HT to dosimetric parameters of pelvic bony structures. [57, 69] Table 9 summarizes the published dosimetric predictors and associated acute hematologic toxicity effect.

Table 9 – Published dosimetric predictors of acute hematologic toxicity.

Author	*Dosimetric predictor*	*HT effect*
Mell *et al.* [69] *	PBM-V10 ≥90%; PBM-V20 ≥75%	lower WBC nadir
	LSBM-V10 (PBM-V10 ≥90%)	lower ANC nadir; G≥2 neutropenia
	PBM-V10, PBM-V20, LSBM-V20, LPBM-V10 and LPBM-V20	increased G≥2 leukopenia
Rose *et al.* [84] *	PBM-V10 ≥95%; PBM-V20 >76%	increased G≥3 leukopenia
Albuquerque *et al.* [83]*	PBM-V20 ≥80%	4.5-fold increased odd of G≥2 HT
Cheng *et al.* [76] •	LSBM-V5-30; PBM-V30	increased G≥3 HT
Mell *et al.* [64] •	PBM-V5-20; LSBM-V10-20	decreased WBC and ANC nadirs
Franco *et al.* [97] •	LSBM-V40 ≥41%	increased G≥3 HT
Bazan *et al.* [52] •	PBM-mean dose	increased HT
Rose *et al.* [72] *	BM-V30	lower WBC nadir
Zhu *et al.* [98] *	PBM-V20, PBM-V30, PBM-V40	reduction of WBC and ANC counts

*, cervical cancer; •, anal cancer.

Mell *et al.* [69] found an association between PBM-V10 ≥90% and PBM-V20 ≥75% (particularly LSBM-V10 and LSBM-V20) and lower WBC nadir, consistent with the findings published by Rose *et al.* [84] (PBM-V10 ≥95% and PBM-V20 >76% were associated with increased G≥3 leukopenia). LSBM-V10 was identified as the only predictor for ANC nadir, suggesting a constraint of PBM-V10 <90% in order to reduce HT. The same study showed PBM-V10 and V20, LSBM-V20 and LPBM-V10 and LPBM-V20 were good predictors to develop G≥2 leukopenia. A higher PBM-V10 was also found to be a predictor of G≥2 neutropenia. [69] Albuquerque *et al.* [83] found that patients undergoing cervical CRT with PBM-V20 ≥80% had a 4.5-fold increased odds of developing G≥2 HT. Specifically for anal cancer, Cheng *et al.* [76] observed an association between several low-dose

parameters of PBM and LSBM and a higher chance to develop G≥3 HT. The most consistent predictors were LSBM-V5-20 Gy. Mell *et al.* [64] found that an increased volume of PBM receiving doses of 5-20 Gy and the volume of LSBM receiving 10-20 Gy is significantly associated with decreased WBC and ANC nadirs; however, no significant associations between any of the parameters with G≥3 HT were made. Oppositely to these publications on low-dose parameters, Franco *et al.* [97] showed that V40 (LSBM) was a strong predictor of G≥3 HT, suggesting the use of a threshold at 41% for LSBM-V40 to limit HT. Bazan *et al.* [52] demonstrated that the mean radiation dose to PBM may be an important dosimetric parameter with regard to HT, given that PBM most likely behaves as a parallel organ.

In our series, no significant association was found between the irradiation of PBM and Hg, WBC and Plts nadirs. PBM-mean dose was, though, found to be associated with ANC nadir and HT3+. HT3+ was also correlated with maximum dose and V30 and V40 of the PBM. In the lumbosacral BM (LSBM) subsite, V40 and mean dose were found to be associated with ANC nadir and HT3+. HT3+ was also correlated with V10, V20, and V30. An association was found between HT3+ and IBM-V5-30 and IBM-mean dose. Concerning the lower pelvis subsite of BM (LPBM), V30 and mean dose were found to be correlated with both ANC nadir and HT3+, and LPBM-maximum dose was also found to be associated with HT3+.

These data suggest that specific BM sub-regions can be mainly responsible for hematopoiesis than the whole pelvic bone itself, testifying PBM has a parallel functional organization. Pathologic studies have shown that BM is composed of hematologically active “red” marrow and inactive “yellow” marrow. [70-72] Nearly 50 % of active BM is located within the pelvis and lumbar spine. [5, 72-75] Rose *et al.* [72] observed active BM was mainly located in the lumbar vertebrae, sacrum and pubic bones, while inactive BM was more frequently located in the ilium, ischium and proximal femur, in cervical cancer patients treated with concurrent CRT. This way, the definition of PBM is crucial and may affect the strength of the relationship between BM and dosimetric parameters. As demonstrated by Cheng *et al.* [76], whole bone delineation is superior to marrow cavity contouring in predicting HT, according to Lyman-Kutcher-Burman model.

Additionally, our results suggest the significance of doses ≤30 Gy received by BM subsites (LPBM and IBM) in the occurrence of HT. First reports by Mell *et al.* did not show correlation between doses higher than 30 Gy to BM and HT [64, 69], while Cheng *et al.* [76] found a borderline significance between PBM-V30 and

LSBM-V30 and G≥3 HT. Furthermore, Rose *et al.* [72] reported a significant correlation between active BM-V30 and WBC nadir in their positron emission tomography (PET)-based study. A longitudinal study by Zhu *et al.* [98] in patients undergoing CRT for cervical cancer demonstrated that increased PBM-V20, V30 and V40 were significantly associated with a higher weekly reduction of WBC and ANC counts.

The current point of interest lies in trying to limit the dose to BM. Our patients were treated with no specific constraints towards PBM sparing and it is possible that if a wider effort was employed on reducing BM irradiation, HT could be reduced further. Liang et al. [99] suggested that additional reduction in acute HT could be achieved with reduced IMRT field sizes by intentional sparing of functional PBM. Brixey *et al.* [100] showed that IMRT decreased the number of G≥2 leukopenia compared with conventional RT in 36 patients undergoing CRT, resulting in less delays in chemotherapy for HT. The same investigators used subsequently a BM-sparing IMRT technique and significantly reduced the volume of BM receiving more than 18 Gy compared with both unconstrained IMRT and conventional 4-field RT techniques. [101] According to our results, it is important to delineate PBM and each subsite for planning purposes, and to use dose constraints during the optimization. For PBM: mean dose <28 Gy, maximum dose <51 Gy, V30 <54% and V40 <33%; for LSBM: mean dose <27 Gy, V10 <65%, V20 <58%, V30 <53%, V40 <45%; for IBM: mean dose <23Gy, V5 <75%, V10 <65%, V20 <53%, V30 <39%; and for LPBM: mean dose <33 Gy, maximum dose <53 Gy and V30 <59%. This intends to reduce HT3+.

BM-sparing IMRT planning approaches should take into account low doses to PBM and specific sub-regions (LSBM and IBM), but medium-high dose constraints can also play a role (IBM and LPBM in our study). The consideration of these constraints within IMRT planning strategies, generating a more abrupt and selective dose fall-off between target volumes and LSBM may moderate acute HT profile, as suggested by Franco and Robinson and their co-workers. [93, 102] Other solution lies in individualizing target structures delineation based on PET or MRI scans, additionally to CT scans. As reported by Rusten *et al.*, though PET seems to have lower inter-observer variability, both imaging techniques produced similar GTVs for RT planning. [103] Target specific setup is another possibility, as it would likely result in reduced treatment volumes and consequently reduced toxicity (Durrant *et al.* [104]).

Conclusion and future perspectives

1. Conclusion and future perspectives

In our study, PBM-mean dose was found to be associated with ANC nadir and HT3+. HT3+ was also correlated with maximum dose and V30 and V40 of the PBM. In the lumbosacral BM (LSBM) subsite, V40 and mean dose were found to be associated with ANC nadir and HT3+. HT3+ was also correlated with V10, V20, and V30. An association was found between HT3+ and IBM-V5-30 and IBM-mean dose. Concerning the lower pelvis subsite of BM (LPBM), V30 and mean dose were found to be correlated with both ANC nadir and HT3+, and LPBM-maximum dose was also found to be associated with HT3+.

Therefore, in order to reduce acute hematologic toxicity, it is suggested to limit the dose to BM using specific constraints towards PBM sparing, especially mean dose to pelvic bones. We found several significant dosimetric predictors, such as PBM-mean dose ≥28 Gy, LSBM-mean dose ≥27 Gy, LSBM-V40 ≥45% and LPBM-V30 ≥59%.

In the future, it is suggested that these dosimetric constraints to PBM and each subsite should be included to routine practice, using templates for planning optimization, for example. We intend to prospectively evaluate the differences in acute hematologic toxicities, and its impact in treatment interruptions and OTT increase. Survival rates and the effect of RT treatment interruptions should also be studied.