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Anal cancer

Leukocytosis and neutrophilia predicts outcome in anal cancer

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ABSTRACT

Objective: Leukocytosis and neutrophilia could be the tip of the iceberg in the inflammatory tumor microenvironment. We aimed to validate their prognostic significance in a cohort of patients treated with definitive chemoradiation for anal squamous cell carcinoma (SCC).

Materials & methods: Clinical records from all consecutive patients treated in a single institution between 2006 and 2016 with curative-intent radiotherapy were retrospectively analyzed. Leukocytosis and neutrophilia, defined as leukocyte or neutrophil count over 10,000 and 7500/mm^3, respectively, were studied in terms of overall survival (OS), progression (PFS), locoregional (LFS) and distant (DFS)-free survival.

Results: We identified 103 non-metastatic HIV-negative patients, with concurrent chemotherapy use in 78%. Twelve and 8% displayed baseline leukocytosis and neutrophilia, respectively. Estimated 3-year OS and PFS were 88% and 67%, respectively. In univariate analysis, both leukocytosis and neutrophilia were strongly associated with inferior OS, PFS, LFS and DFS ($p < 0.01$). In multivariate analysis, leukocytosis and neutrophilia remained strongly associated with patient outcome ($p < 0.01$), independently from tumor T and N-stage. Anemia was an independent predictor of worse OS and PFS, while chemoradiation overall treatment time below 50 days improved PFS.

Conclusion: Leukocytosis and neutrophilia are strong prognostic factors for OS, PFS, LFS and DFS in anal cancer treated with chemoradiation. These biomarkers could help identify patients with higher risk of tumor relapse that require treatment intensification.

Introduction

Standard treatment for locally advanced (stage II and III) anal SCC is concurrent chemoradiotherapy (two cycles of 5-FU – mitomycin in a 28 day cycle) with a dose of 45 Gy in 1.8 Gy/fractions or 44 Gy in 2.0 Gy/fractions to the prophylactic pelvic volume, with a sequential additional 14–20 Gy dose delivered to the tumor volume [1]. Despite appropriate therapeutic management, 5 years OS is 66% [2]. Documented prognostic factors are male gender, nodal involvement and tumor size [3]. Anemia may also correlate with PFS, OS and distant relapse [4]. Yet, more powerful biomarkers are needed to improve patient stratification and outcome.

Inflammation, key component of tumor initiation and progression, has been involved in various steps of oncogenesis [5]. Tumor microenvironment is partially composed of inflammatory cells that orchestrate the neoplastic process, promoting tumor proliferation, survival and migration [5]. In the field of radiotherapy, inflammation is also a key component [6].

Tumor-related leukocytosis has been reported in up to 20% of patients with non-small cell lung cancer and approximately 10% in patients with cervical cancer; it may be associated with immune suppression, promotion of tumor angiogenesis and metastatic process [7,8]. In breast cancer, clinical inflammatory T4d-stage tumors are associated with poor prognosis and high tumor invasiveness

Abbreviations: 5FU, 5-fluorouracil; AJCC, American Joint Committee on Cancer; CRP, C – reactive protein; DFS, distant failure free survival; CTV, clinical target volume; EBRT, external beam radiotherapy; G-CSF, granulocyte colony-stimulating factor; GTV, growth tumor volume; Gy, gray; HIV, Human Immunodeficiency Virus; HPV, human papillomavirus; IMRT, intensity-modulated radiotherapy; LFS, locoregional failure free survival; MRI, magnetic-resonance imaging; MV, mega voltage; NLR, neutrophils to lymphocytes ratio; OARs, organs at risk; OS, overall survival; PET-CT, positron-emission tomography CT; PFS, progression free survival; SAA, serum amyloid A; SCC, squamous cell carcinoma; TAMs, tumor-associated macrophages; TANs, tumor-associated neutrophils; TILs, tumor-infiltrating lymphocytes.

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[9]. Biological inflammatory biomarkers (CRP, SAA) may also be predictive of patients' outcome in breast cancer [10].

Recent data suggested that leukocytosis and neutrophilia could be predictive for cervical cancer patients [11,12]. Anal and cervix SCCs share many biologic similarities, primarily because they both are associated with chronic HPV infection [13].

In the current study, the prognostic significance of systemic leukocytosis and neutrophilia on survival, local and distant recurrence was analyzed in a single center cohort of patients homogeneously treated with curative-intent conservative chemoradiation.

Materials and methods

Patients and tumors

We examined clinical records of consecutive patients treated in our institution between 2006 and 2016 for a histologically confirmed anal SCC, treated in a conservative and curative intent with radiation therapy. Explorations at diagnosis included rectal endoscopic ultrasound, computed tomography (CT), exploring thoracic, abdominal and pelvic regions, and pelvic magnetic-resonance imaging (MRI). Positron-emission tomography CT (PET-CT) was prescribed in patients with >T1 tumor and/or nodal involvement [1]. Disease staging was defined according to the 2002 American Joint Committee on Cancer (AJCC) anal cancer staging manual, sixth edition.

We recorded and analyzed distinctly patients with Human Immunodeficiency Virus (HIV) positive status at diagnosis if they were treated with initial curative intent concurrent chemoradiation, and patients with initial metastatic disease treated with standard chemoradiation with a locoregional control intent, followed by chemotherapy. We excluded patients treated in a palliative intent with hypofractionated chemoradiation, patients who received neoadjuvant chemotherapy, and patients presenting chronic inflammation (such as inflammatory bowel diseases), treated for an immune disease, acute or chronic infection (except HIV).

Treatment characteristics

Patients received pelvic external beam radiotherapy (EBRT) (45 Gy in 25 fractions of 1.8 Gy or 44 Gy in 22 fractions of 2 Gy) in a prophylactic volume including bilateral iliac and inguinal nodes areas. Most patients treated before 2009 underwent a 3D conformal technique from a 6 MV photon linear accelerator with an isocentric technique; those treated after 2009 underwent intensity-modulated radiotherapy (IMRT) techniques, with either step-and-shoot or helical tomotherapy [14]. A sequential 15–20 Gy boost was delivered to the clinical target volume (CVT), typically defined from gross tumor volume (GTV) plus a 1–2 cm margin. Organs at risk (OARs) were bladder, rectum, small bowel, and pelvic bones. All OARs were delineated to generate dose-volume histograms and maximum-tolerated doses and volumes. Treatment was performed with a linear accelerator of at least 6 MV.

Patients with >T1 tumor and/or node involvement received concurrent chemotherapy with mitomycin C (10 mg/m^2 on days 1 and 29) and 5-fluorouracil (5FU) (1000 mg/m^2 on days 1–4 and 29–32) or equivalent capecitabine dose [1].

Patients with T1N0 diseases were treated with exclusive radiotherapy following specific recommendations [1,15]. Patients with a small tumor without node involvement were treated with a sequential brachytherapy boost with interstitial implantation delivered through one single application using an iridium (Ir-192) source with dosimetry and implantation done according to the Paris system. The treatment planning objective was to deliver at least 60–65 Gy to the reference isodose rate (85% of the minimal dose rate between planes) taking into account the dose delivered by EBRT after converting doses into biological effective doses normalized to a radiobiologically weighted dose equivalent of 2 Gy/fraction ($\alpha/\beta = 10$ Gy). HIV-positive patients were treated with similar modalities, as demonstrated safe and efficient [16].

Complete blood count analysis

Patients underwent systematic complete blood cell counts weekly during chemoradiation. Pretreatment blood samples taken before any chemotherapy were employed in the current analysis. Leukocytosis and neutrophilia, defining biological inflammation, were set as blood count over 10,000/mm^3 and 7500/mm^3 respectively, while anemia was defined as hemoglobin count below 13.0 g/dL. We tested these three parameters for statistical correlation with OS, PFS, LFS and DFS.

Follow-up and statistical analysis

Follow-up was scheduled at 6 weeks, every three months during two years, then every 6 months. Systematic rectal endoscopic ultrasound examination was performed 3 months after CRT completion, then every six months. MRI and PET-CT were performed at the discretion of the physician. Surgery was performed in case of isolated local failure, after a disease restaging through PET-CT. Factors associated with tumor relapse were examined. Survival times were calculated from the time of chemoradiation completion and survival rates were estimated using the Kaplan–Meier method. Univariate analyses were carried out using log rank tests. Multivariate analyses were performed for variables with p value < 0.1 in univariate analysis, according to the Cox method. Statistical analyses were performed using SPSS statistics 23.0® (Statistical Package for Social Science) for Macintosh (an IBM company software, Chicago, Illinois, USA) and R (version 3.3.2).

Results

A total of 103 consecutive HIV-negative and non-metastatic patients with anal SCC were included for analysis. Median age was 61 years (range: 33–86 years). Fifty-seven patients had T3-T4 disease (55%), and 57 patients (55%) had pelvic involved nodes. All patients had a 0 or 1 performance status according to the World Health Organization (WHO) prognosis status classification.

On initial blood count, before the first week of EBRT, 57 patients (59%) had anemia. Leukocytosis and neutrophilia were found in 12 patients (12%) and 8 patients (8%) respectively. There was a trend toward an association of leukocytosis with a larger tumor at diagnosis (T1 or T2 vs. T3 or T4) ($p = 0.061$). Initial biology was missing in six patients: they all were treated with radiotherapy alone for T1 (2 patients, 40%) or "small T2"<2 cm (3 patients, 60%) disease without node involvement at diagnosis; none of them relapsed or died after a mean 42.9-month follow-up.

All patients received a pelvic irradiation, extended to the iliac and inguinal bilateral areas, delivered with 3D-conformational technique in 48 patients (47%) and IMRT in 55 patients (53%). There was no significant impact of technique on OS ($p = 0.899$) or PFS ($p = 0.523$). Eighty (78%) patients received concurrent chemotherapy, based on 5FU – mitomycin in 61 patients (76%), 5FU – cisplatin in 15 (19%). Four patients underwent other concurrent chemotherapy regimens: cisplatin + 5FU + cetuximab ($n = 2$), carboplatin ($n = 1$), or capecitabine ($n = 1$).

Median total dose after sequential boost to the anal tumor was 60 Gy (40–74 Gy), delivered with interstitial brachytherapy in 10 (10%) patients (6 patients with T1-stage, 60%). Median dose of sequential boost to macroscopically involved lymph node metastases was 15 Gy (9–20 Gy, 1.8–2 Gy per fraction). Median overall treatment time was 47 days (range: 24–84 days).