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Biomarkers in anal cancer

External validation of leukocytosis and neutrophilia as a prognostic marker in anal carcinoma treated with definitive chemoradiation

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ABSTRACT

Purpose: To validate the prognostic value of leukocyte disorders in anal squamous cell carcinoma (SCC) patients receiving definitive concurrent chemoradiation.

Materials and methods: Bi-institutional clinical records from consecutive patients treated between 2001 and 2015 with definitive chemoradiation for anal SCC were retrospectively reviewed. Prognostic value of pretreatment leukocyte disorders was examined, with focus on patterns of relapse and survival. Leukocytosis and neutrophilia were defined as leukocyte or neutrophil count exceeding 10 G/L and 7 G/L, respectively.

Results: We identified 133 patients, treated in two institutions. Eight% and 7% displayed baseline leukocytosis and neutrophilia, respectively. Estimated 3-year overall survival (OS) and progression-free survival (PFS) were 88% and 77%, respectively. In univariate analysis, both leukocytosis and neutrophilia were associated with worse OS, PFS ($p < 0.01$), locoregional control (LRC) and Distant Metastasis Control (DMC) ($p < 0.05$), also after stratification by each institution. In multivariate analysis, leukocytosis and neutrophilia remained as independent risk factors associated with poorer OS, PFS, LRC and DMC ($p < 0.05$).

Conclusion: This study validates leukocytosis and neutrophilia as independent prognostic factors in anal SCC patients treated with definitive chemoradiation. Although prospective confirmation is warranted, it is suggested that the leukocyte and neutrophil count parameters are clinically relevant biomarkers to be considered for further clinical investigations.

With increasing annual incidence 1.8/100,000 men and women per year, anal squamous cell carcinoma (SCC) was responsible in 2016 for ~8080 new cases (0.5% of all cancer cases), and ~1080 deaths (0.2% of all cancer death) in USA (SEER database). Since the 1990s, standard treatment moved from surgery to conservative chemoradiotherapy [1]. Concomitant chemotherapy is based on Mitomycin and 5-fluorouracil (5FU) in locally advanced tumors, with brachytherapy as an alternative for sequential radiation boost [2].

Established prognostic factors are male gender, tumor stage, nodal involvement, and anemia [3,4]. Still, relapses often occur in patients who have few prognostic factors, which calls for more powerful biomarkers. Anal SCC is strongly associated with human papillomavirus (HPV) infection in 80–85% of patients [5,6]. Conditions increasing HPV infection appear to affect the epidemiology of this tumor, e.g., human immunodeficiency virus (HIV) chronic infection and immunosuppression [2].

Tumor-related leukocytosis and particularly neutrophilia is a paraneoplastic syndrome reported in various malignant advanced tumor types, and is associated with poor survival [7]. While activated neutrophils have been shown to kill tumor cells, there is

Abbreviations: 5FU, 5-fluorouracil; AJCC, American Joint Committee on Cancer; DMC, Distant Metastasis Control; CTV, clinical target volume; EBRT, external beam radiotherapy; GTV, growth tumor volume; Gy, gray; HIV, human immunodeficiency virus; HPV, human papillomavirus; IMRT, intensity-modulated radiotherapy; LRC, locoregional control; MRI, magnetic resonance imaging; MV, megavoltage; NLR, neutrophil to lymphocyte ratio; OARs, organs at risk; OS, overall survival; PET-CT, positron-emission tomography CT; PFS, progression free survival; SCC, squamous cell carcinoma; TAN, Tumor Associated Neutrophils; TGF-β, Transforming Growth Factor beta.

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growing evidence for neutrophil activation driving tumor progression and metastasis through stimulation of thrombosis, angiogenesis, stromal remodeling, and impairment of T cell-dependent antitumor immunity [8]. Neutrophil heterogeneity in cancer promotes cancer control and progression, while antineoplastic treatments (surgery, chemotherapy, radiotherapy and growth factors) may also influence neutrophil response [8]. This fragile balance warrants further investigations.

We recently reported that leukocytosis and neutrophilia before chemoradiation could predict outcome in patients with anal and cervical SCC. These preliminary results require validation in an external cohort to be implemented in clinical daily practice [9,10].

This study aimed to validate the prognostic value of leukocyte and neutrophil count in patients with anal SCC undergoing exclusive chemoradiation in a bi-institutional retrospective series.

Materials and methods

Patients and tumors

We reviewed clinical records of consecutive patients undergoing definitive chemoradiation for histologically proven anal SCC in two French centers: (i) Paoli-Calmettes Institute (PCI, Marseille, France) between May 2000 and November 2014; (ii) Tenon Hospital (Paris, France) between October 2011 and June 2015. Data extraction was performed independently in each institution.

Clinical work-up 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. Disease staging was defined according to the 2010 American Joint Committee on Cancer (AJCC) anal cancer staging manual, seventh edition, defining T-stage by tumor size.

We excluded patients treated with palliative intent by hypofractionated chemoradiation or who received prior chemotherapy, patients with initial metastatic disease or neoadjuvant chemotherapy, and patients with a history of previous neoplasia. We also excluded patients presenting chronic inflammation, acute or chronic infection (including human immunodeficiency virus), or under medication for an immune disease or any treatment that may confound our analysis (corticosteroids, G-CSF, antibiotics for a recent infection).

Treatment characteristics

Patients treated in Tenon Hospital underwent pelvic external beam radiotherapy (EBRT) (49.5 Gy in 30 fractions of 1.65 Gy) in a prophylactic volume including bilateral iliac and inguinal nodes areas. Regardless of disease stage, 60 Gy/30 fractions (2 Gy daily) were prescribed to the anal gross tumor PTV, while gross nodal PTVs were prescribed 54 Gy/30 fractions (1.8 Gy daily) with intensity-modulated radiotherapy (IMRT) planning with simultaneous integrated boost (SIB) technique.

Patients treated in PCI received EBRT, 45 Gy in 25 fractions of 1.8 Gy in a prophylactic volume, and a sequential 15–20 Gy boost was delivered to the PTV encompassing anal tumor and macroscopically involved nodes. Patients treated before 2010 underwent a 3D conformal technique from a 6 MV photon linear accelerator with an isocentric technique. Those treated after April 2010 had an IMRT planning technique. At physician discretion, patients underwent a sequential brachytherapy boost with interstitial implantation delivered through one single application using an iridium (Ir-192) source with dosimetry and implantation according to the Paris system, aiming to deliver at least 60–65 Gy to the reference isodose rate, taking into account the dose delivered by EBRT.

Gross tumor volume (GTV) consisted of all macroscopic disease, both primary tumor (GTV-T) and lymph nodes (GTV-N) taking into account MRI and PET information after performing coregistration. GTVs were then isotropically expanded, adding 15–20 mm (CTV-T) and 10 mm (CTV-N) to generate the corresponding clinical target volumes (CTVs) modified in order to exclude surrounding osseous and muscular tissues. Prophylactic CTV encompassed the mesorectum and appropriate draining lymphatic regions (inguinal, external iliac, internal iliac, obturator, presacral and perirectal). A subsequent 6–10 mm isotropic margin was added to generate the planning target volume (PTV), accounting for organ motion and setup errors.

Before 2010, concurrent chemotherapy was based on cisplatin (80 mg/m^2 every 3 weeks), and 5-fluorouracil (5FU) (800 mg/m^2 on days 1–4) or equivalent capecitabine dose. After 2010, concurrent chemotherapy was based on 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 [2]. Patients with T1N0 diseases were treated with exclusive radiotherapy following specific recommendations [2,11].

Complete blood count analysis

Pretreatment blood samples taken before the start of chemoradiation were employed in the current analysis. Leukocytosis and neutrophilia were defined as blood count over 10 G/L and 7 G/L, respectively. Anemia was defined as hemoglobin count below 13.0 g/dL; thrombocytosis as platelet count over 400 G/L; lymphopenia as lymphocyte count below 1.0 G/L; and monocytosis as monocyte count over 1.0 G/L. neutrophil to lymphocyte ratio (NLR) cutoff was defined as 4, like in most reported studies [12].

Follow-up and statistical analysis

Follow-up was scheduled 6 weeks after CRT completion, every three months during two years, then every 6 months until 5 years. Rectal endoscopic ultrasound examination, 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 with PET-CT. Differences in patient characteristics regarding baseline leukocytosis were compared with Fisher's test, Wilcoxon–Mann's and student's-t test, and by variance analysis. Factors associated with tumor relapse were examined. Survival times were defined as the time between the diagnosis and the first event (time of death for OS, time of recurrence or death for PFS, time of loco-regional recurrence (LRC: locoregional control) and time of distant metastasis (DMC: distant metastases control) estimated by the Kaplan–Meier method. Univariate analyses were carried out using log rank tests. Multivariate analyses were performed for variables with p value <0.2 in univariate analysis, according to the Cox method. Statistical analyses were performed using R (version 3.3.2).

Results

Patients and tumors

From 2000 to 2015, 133 consecutive patients with available blood count were included for analysis, 68 from Tenon Hospital (Paris), and 65 from PCI (Marseille). Median age was 62 years (range: 21–93 years). Most patients had T3-T4 disease (59%) and/or pelvic involved nodes (52%); 30 patients (23%) had $\geq$1 performance status according to the World Health Organization (WHO) classification.