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Original Article

# Evaluation of prognostic factors after primary chemoradiotherapy of anal cancer: A multicenter study of the German Cancer Consortium-Radiation Oncology Group (DKTK-ROG)

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ABSTRACT

*Background and purpose:* Prognosis after chemoradiotherapy (CRT) for anal squamous cell carcinoma (ASCC) shows marked differences among patients according to TNM subgroups, however individualized risk assessment tools to better stratify patients for treatment (de-) escalation or intensified follow-up are lacking in ASCC.

*Materials and methods:* Patients' data from eight sites of the German Cancer Consortium - Radiation Oncology Group (DKTK-ROG), comprising a total of 605 patients with ASCC, treated with standard definitive CRT with 5-FU/Mitomycin C or Capecitabine/Mitomycin C between 2004–2018, were used to evaluate prognostic factors based on Cox regression models for disease-free survival (DFS). Evaluated variables included age, gender, Karnofsky performance score (KPS), HIV-status, T-category, lymph node status and laboratory parameters. Multivariate cox models were separately constructed for the whole cohort and the subset of patients with early-stage (cT1-2 N0M0) tumors.

*Results:* After a median follow-up of 46 months, 3-year DFS for patients with early-stage ASCC was 84.9%, and 67.1% for patients with locally-advanced disease (HR 2.4, $p < 0.001$). T-category (HR vs. T1: T2 2.02; T3 2.11; T4 3.03), N-category (HR versus N0: 1.8 for N1-3), age (HR 1.02 per year), and KPS (HR 0.8 per step) were significant predictors for DFS in multivariate analysis in the entire cohort. The model performed with a C-index of 0.68. In cT1-2N0 patients, T-category (HR 2.14), HIV status (HR 2.57), age (1.026 per year), KPS (HR 0.7 per step) and elevated platelets (HR 1.3 per 100/nl) were associated with worse DFS (C-index of 0.7).

*Conclusion:* Classical clinicopathologic parameters like T-category, N-category, age and KPS remain to be significant prognostic factors for DFS in patients treated with contemporary CRT for ASCC. HIV and platelets were significantly associated with worse DFS in patients with early stage ASCC.

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Anal squamous cell carcinoma (ASCC) is a rare tumor entity with a steady rise in incidence [1]. Primary chemoradiotherapy (CRT) with 5-fluorouracil and mitomycin C (5-FU/MMC) remains the standard treatment for localized disease since the introduction of this treatment approach by Nigro et al. in the 1980s [2]. Radical surgery with abdominoperineal resection and a permanent colostomy is now reserved for patients with locally persistent or recurrent disease after CRT. The addition of sequential chemotherapy, either prior to or following primary CRT, or dose escalation of radiotherapy was tested in several randomized trials, but failed to improve oncological results [3–5].

When reviewing the literature, it is striking that outcome data reported according to TN subcategories is scarce. Treatment outcomes are favorable for small cT1-2N0M0 tumors, but local and/or distant recurrence occurs in up to 40% of patients with locally-advanced disease (cT3-4 and/or involved lymph nodes) [6,7]. Thus, both de-escalation and escalation strategies are currently investigated for early and advanced stage tumors, respectively [8]. Established clinical prognostic factors to better stratify patients for treatment alternatives include tumor size, lymph node involvement and gender [7,9,10]. Human papilloma virus (HPV)/p16 status and infiltration with CD8 + tumor infiltrating lymphocytes have recently been proposed as prognostic molecular markers [11–14]. Tumor infiltrating lymphocytes have been shown to be of predictive value particular for HPV positive patients [12]. Moreover, an association of peripheral blood leukocytosis and elevated CRP to albumin ratio with poor prognosis has been reported [15–20]. Also, low baseline hemoglobin levels were associated with reduced overall survival (OS) in retrospective studies and with a higher risk of anal cancer related death in the ACT1 trial [10,21–23]. Nevertheless, the combined influence of these parameters on outcome have not been assessed. Other composite blood based markers that have been reported to be associated with impaired prognosis are the systemic inflammatory index consisting of platelets, neutrophils and lymphocytes and the Hemo-Eosinophil-Inflammatory index consisting of hemoglobin and eosinophil levels [24,25].

In the present analysis of pooled real-world data from eight cancer centers of the German Cancer Consortium-Radiation Oncology Group (DKTK-ROG), we describe outcomes according to pretreatment clinicopathologic factors, including T-category, lymph node involvement, age, gender, Karnofsky performance score (KPS), human immunodeficiency virus (HIV)-status and peripheral blood values.

In rare tumor entities like ASCC, multicenter datasets are needed to make progress in the field and DKTK-ROG has an established track record in successful cooperation, especially in head and neck squamous cell carcinoma [26,27].

## Materials and methods

### *Patients and treatment*

Patients were treated with 5-FU/MMC or Capecitabine/MMC-based CRT between 2004 and 2018. Patients were staged according to TNM edition 7. Inclusion criteria were histologically proven ASCC, cT1-4 $cN_{any}M0$, CRT with curative intent, and a minimal cumulative dose to the tumor of 45 Gy. Radiotherapy dose was decided per departmental policy. A total of 639 patients were identified. After exclusion of patients with missing follow-up, 605 patients remained for analysis. This trial was approved by the ethical committees of all DKTK-ROG partner sites (Medical Faculty of Frankfurt University, Ethics committee, Centralized protocol number 458/17).

### *Statistical analysis*

Patients data were collected in the RadPlanBio infrastructure of the DKTK [28]. Survival times were calculated from start of CRT to the date of respective events or last follow-up. The variables evaluated included T-category, lymph node involvement, age, gender, KPS, HIV-status, and baseline blood values including white blood cells (WBC), platelets and hemoglobin. KPS and all blood values were analysed as continuous variables. The hazard ratio (HR) for a change of one step in KPS (e.g. 100 to 90) are calculated by exponentiating the HR with the factor 10. To report the impact of platelets by an increase of 100/nl the HR was exponentiated by 100. Cumulative incidences of local failure and distant metastasis were calculated.

Local failure was assessed either as a lack of clinical complete response (cCR) at restaging or any locoregional recurrence during follow-up after initial cCR. Missing covariates (<10%) were imputed using Additive Regression, Bootstrapping and predictive mean matching using the R package 'Hmisc'.

Disease-free survival (DFS) was calculated using the date of diagnosis of locoregional failure, distant metastases, or death of any cause as event. Overall survival (OS) was calculated with death of any cause as the event. Differences in survival were calculated using the log rank test or Cox regression analysis. The assumption of proportional hazard was verified by assessing the scaled Schoenfeld residuals.

Significant predictors for outcomes were selected by training a Cox model. A final model using only the predictors that were significant in the preliminary model was created and evaluated whether all factors still contributed to the outcome. Model performances were calculated using bootstrapping, which means that the dataset was tested 1000 times with randomly resampled data for each time. All statistical analysis was performed with R (Version 4) [29]. A $p$-value $\leq 0.05$ was considered significant.

**Table 1**
Patient's characteristics.

| | | Median (range) or *n* (%) |
|---|---|---|
| Age, years | | 59 (21–95) |
| Gender | Male | 211 (35) |
| | female | 394 (65) |
| HIV-Status | positive | 61 (10) |
| | negative | 526 (87) |
| | unknown | 4 (3) |
| T-Stage | T1 | 113 (19) |
| | T2 | 281 (46) |
| | T3 | 152 (25) |
| | T4 | 59 (10) |
| N-Stage | N0 | 358 (59) |
| | N1-3 | 247 (41) |
| Grading | G1 | 35 (6) |
| | G2 | 346 (61) |
| | G3 | 180 (32) |
| | unknown | 7 (1) |
| KPS | 100 | 329 (54) |
| | 90 | 195 (32) |
| | 80 | 51 (9) |
| | 70 | 20 (3) |
| | 60 | 10 (2) |
| *Radiotherapy* | | |
| RT modality | 3D | 230 (38) |
| | IMRT | 376 (62) |
| Total dose (Gy) | | 58.5 (45–68.4) |

Abbreviations: HIV, human immunodeficiency virus; KPS, karnofsky performance score; Gy, Gray; RT, radiotherapy;

## Results

Patient characteristics are summarized in Table 1. In total, 283 (46.8%) patients had early stage disease (cT1-2N0M0) and 322 (53.2%) patients presented with a locoregionally advanced tumor (cT3-4 and/or cN1-3). 61 (10%) of patients were HIV-positive (HIV+), of whom 55 were male ($p < 0.001$) and significantly younger (median 51 years of age vs. 60 years in the HIV-negative cohort, $p < 0.001$). All patients were treated with 5-FU/MMC or Capecitabine/MMC concomitant chemotherapy; 376 (62%) of patients received intensity modulated radiotherapy (IMRT) and 230 (38%) received 3D conventional radiotherapy (3DCRT). The median total dose to the primary tumor was 58.5 Gy (range, 45–68.4 Gy). The median dose to the elective lymph nodes was 50 Gy (range, 30.6–54 Gy).

Median follow-up was 46 months (interquartile range, 22–74 months). In the entire cohort, the 3-year cumulative incidence of local failure and distant metastasis was 12.0% and 9.7%, respectively, whereas 3-year DFS and OS rates were 75.7% and 87.2%, respectively. Patients with locally advanced ASCC had a 3-year cumulative incidence of local failure or distant metastasis of 18.1% and 15.4%, respectively, whereas for early stage ASCC these rates were 5.4% and 3.5% ($p < 0.0001$ for both, Fig. 1A, B). The 3-year DFS was 67.2% for locally advanced ASCC, and 85.1% for early stage ASCC ($p < 0.0001$ for both, Fig. 1C), and the 3-year OS was 82.8% and 92%, respectively ($p < 0.001$, Fig. 1D). Univariate analysis of baseline blood parameter is in line with published data, as elevated platelets or WBC and low hemoglobin levels are associated with inferior DFS (see Table 2).

To evaluate potential prognostic factors for DFS we conducted a multivariate cox regression analysis including clinicopathological and laboratory parameters that were associated with adverse prognosis in the literature (see Table 2). The only parameters that remained significant in multivariate analysis were T-category, lymph node status (cN0 vs cN1-3), age and KPS. Models for local failure and distant metastasis were also calculated. For local failure, T-category (cT1: ref; cT2: HR 5.34; cT3: HR 7.13; cT4: HR 10.68), lymph node status (cN0: ref; N1-3: HR 1.92), and KPS (HR 0.8 per step) remained the sole significant predictors in multivariate analysis; lymph node status (HR 2.13), platelets (HR 1.1 per 100/nl) and advanced T-category (cT1: ref; cT2: HR 3.17; cT3: HR 5.96; cT4: HR 4.92) remained significant adverse prognosis factors for distant metastasis (Supplementary Table 1). Similar data were observed for OS in the multivariate analysis (Supplementary Table 1).

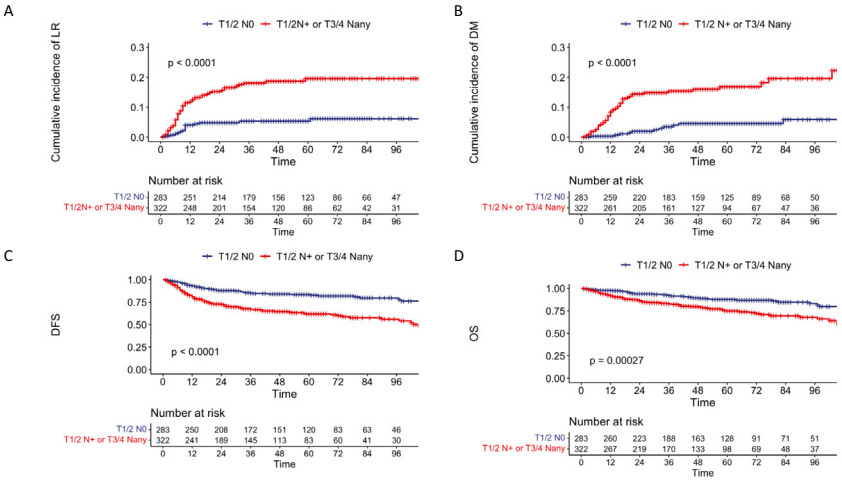


**Fig. 1.** Kaplan Meier Plots grouped according to early ASCC (cT1-2cN0) and advanced ASCC (cT1-2N1-3 or cT3-4c$N_{any}$) for cumulative incidence of local recurrence (A), distant metastases (B), disease-free survival (C) and overall survival (D). ASCC: anal squamous cell carcinoma.

**Table 2**
Uni- and multivariate cox regression for disease free survival (DFS) in the whole cohort.

| | Univariate Analysis | | Multivariate Analysis | |
|---|---|---|---|---|
| | HR (95% CI) | *p* | HR (95% CI) | *p* |
| T Stage | | | | |
| T1 | ref | | | |
| T2 | 2.4 (1.35–4.24) | **0.003** | 2.02 (1.13–3.62) | **0.02** |
| T3 | 3.3 (1.86–6) | **< 0.001** | 2.11 (1.13–4) | **0.02** |
| T4 | 5.9 (3.1–11.4) | **< 0.001** | 3.03 (1.47–6.27) | **0.003** |
| N + vs. N0 | 2.3 (1.71–3.17) | **< 0.001** | 1.8 (1.28–2.53) | **< 0.001** |
| HIV + vs. HIV- | 1.24 (0.78–1.99) | 0.36 | 1.39 (0.8–2.4) | 0.24 |
| Female vs. Male | 0.8 (0.59–1.11) | 0.19 | 0.94 (0.65–1.35) | 0.72 |
| Age (years) | 1.01 (1.001–1.027) | **0.03** | 1.02 (1.002–1.029) | **0.03** |
| KPS[a] | 0.96 (0.95–0.98) | **< 0.001** | 0.98 (0.96–0.99) | **0.001** |
| Platelets[a] (/nl) | 1.001 (1–1.001) | **0.003** | 1.0003 (1–1.001) | 0.48 |
| Hemoglobin[a] (mg/dl) | 0.9 (0.84–0.96) | **0.003** | 0.96 (0.89–1.03) | 0.25 |
| White blood cells[a] (/nl) | 1.1 (1.04–1.15) | **< 0.001** | 1.03 (0.98–1.09) | 0.2 |

HIV, human immunodeficiency virus; KPS, Karnofsky performance score;
[a] parameters were included as continuous variables

The final cox model for DFS included T-category, lymph node status, age and KPS. After bootstrapping, the model performed with a C-index of 0.68. As advanced T-category, lymph node involvement, advanced age and poor KPS remained the sole predictors of worse DFS in the entire cohort in multivariate analysis, we decided to evaluate prognostic factors only for the early stage group (cT1-2 cN0: 283 of 605 patients, 47%) because these patients generally have a good prognosis and identification of patients with dismal prognosis in this group would be of special interest. Due to the low number of events in this cohort we did not create separate models for local recurrence and distant metastasis but rather focused on DFS. We first created a cox model including T-category, gender, age, HIV-status, baseline WBC, platelets, hemoglobin levels and KPS (Supplementary Table 2). The final model included T-category (HR 2.14), age (HR 1.26 per 10 years), HIV (HR 2.57), platelets (HR 1.3 per 100/nl) and KPS (HR 0.7 per Step, Table 3). The final model was bootstrapped and performed with a C-index of 0.7. Because the patients in this study were treated during a rather large timeframe (2004–2018) which may be associated with stage migration [30] or change of treatment regimen and techniques, we did an additional sensitivity analysis for both cox models and included the year of the initiation of treatment as a parameter. There was no significant change regarding the outcome parameters (data not shown).

## Discussion

While detailed oncological outcome after CRT according to the different TNM categories has been reported from two of the major randomized trials (ACT II, RTOG 98-11), and one large retrospective series [7,31,32], we here report real world data from patients that were treated with contemporary CRT using mainly IMRT techniques.

We evaluated prognostic factors based on real-world data after a median follow-up of 46 months pooled from eight DKTK-ROG centers to predict DFS for the whole cohort as well as separately for early stage ASCC.

Albeit the prognosis of patients with early stage ASCC (T1-2N0) is excellent, approximately 35% of patients with ASCC experience high-grade late side effects after CRT [33], which is the main rationale for radiotherapy dose de-escalation currently explored within the ACT3 and ACT4 trials of the PLATO umbrella study [34]. The cox regression model of the subgroup of patients with early stage ASCC shows that 3-year DFS is worse in early-stage patients with HIV+ status, elevated baseline platelet count, poor KPS, or T2 (vs T1) tumors. Interestingly, baseline elevated platelets remained a significant adverse predicting factor for DFS in this early stage group of patients, whereas in the entire cohort this parameter was likely outperformed by T- and N-category. Also, HIV positivity was associated with worse outcome. The prognostic role of HIV is still a topic of debate and mixed findings have been reported, while current NCCN guidelines recommend that HIV+ patients receiving antiretroviral therapy should be treated with standard CRT. A recent meta-analysis demonstrated an impaired DFS in HIV+ compared to HIV-negative patients [35].

**Table 3**
Final multivariate model for DFS in cT1-2 cN0 patients.

| | HR (95% CI) | *p* |
|---|---|---|
| T Stage | | |
| T1 | ref | |
| T2 | 2.14 (1.11–4.13) | **0.024** |
| HIV+ vs. HIV- | 2.57 (1.07–6.15) | **0.034** |
| Age (Hazard per year) | 1.026 (1.002–1.051) | **0.034** |
| KPS | 0.97 (0.94–0.99) | **0.04** |
| Platelets (/nl) | 1.003 (1.0001–1.006) | **0.024** |

Our analysis is in line with subgroup analysis of the RTOG 9811 trial that also reported a significant reduction in 5-year DFS for patients with involved lymph nodes (cT2-3cN0 70%; cT2cN1-3 57%; cT3cN1-3 38%, cT4cN1-3 31%) or advanced T-category (cT2cN0 72%; cT3cN0 61%; cT4cN0 50%) [7]. We found no significant impact of baseline hemoglobin, WBC or platelets on DFS in the whole cohort, however, elevated platelets were associated with increased rate of distant metastasis. Preclinical evidence suggests that platelets play an important role in promoting metastatic spread [36], and an adverse impact of thrombocytosis has been reported in ASCC and other tumors including colorectal and cervical cancer [17,37–39]. Although elevated baseline WBC showed an adverse prognostic effect in the univariate analysis, which is in line to several retrospective studies in ASCC [15–17], it did not remain significant in the multivariate analysis. The ACT1 trial [10] and the national cancer database (NCDB) [40] have demonstrated better clinical outcome in females compared to males after primary CRT, albeit mixed findings have been reported [4,9]. We failed to identify male gender as a negative prognostic factor. As of today, no clear explanation for this finding has been presented and other possible biasing factors could play a role in these conflicting reports.

In locally-advanced disease (cT3-4 and/or cN1 per 8th edition of TNM), several treatment modifications are possible to improve outcomes and/or to avoid late side effects in patients with excellent prognosis. Strategies for RT dose escalation are currently under investigation in the ACT5 trial of the PLATO (ISRCTN88455282). ACT5 includes patients with locally advanced high-risk disease and investigates the role of CRT with dose escalation up to 61.6 Gy in 28 fractions. The most important negative prognostic factor in these patients is lymph node involvement. In ASCC, there is a strong rationale for combining immune checkpoint blockade with CRT [5,41]. In two phase Ib/II trials, treatment with PD1/PD-L1 inhibitors showed encouraging responses in recurrent/metastatic ASCC [42,43]. Several trials in localized ASCC that are currently recruiting are the NCI ECOG ACRIN randomized phase III trial that that evaluates adjuvant treatment with Nivolumab after combined CRT (clinicaltrials.gov: NCT03233711), the RADIANCE randomized phase II trial (clinicaltrials.gov: NCT04230759) of the German Anal Cancer Study Group [44] and the UK phase 1b/II trial CORINTH (NCT04046133).

There are several limitations to our study. First, despite the large cohort, the retrospective collection of data could lead to bias. Second, the HPV/p16 status of the vast majority of patients is unknown as it does not constitute part of routine pathology testing in clinical practice in Germany or worldwide, and was not factored in the analysis. We also were not able to retrieve the tissue samples of the majority of patients in order to assess HPV status ourselves. Third, we were not able to retrieve the size of the primary tumor, which could have be helpful in model development. Fourth, HIV-related information like peripheral blood CD3, CD4 cell counts or HIV viral load was lacking. Also, further external validation of our model according to the TRIPOD criteria by use of a different set of ASCC patients is needed [45]. Nevertheless, the hereby proposed cox regression models are based on real-world data from patients treated with contemporary CRT.

In conclusion, this is, a large retrospective study of a cohort of patients with ASCC treated with contemporary CRT that evaluates prognostic factors for DFS. The data from this large patient cohort of ASCC confirm the poor DFS in patients with locally-advanced disease and underline the importance of testing new treatment options such as immunotherapy in combination with CRT to improve clinical outcome in patients with this orphan disease.

Future projects from DKTK-ROG will center around discovery of molecular biomarkers from primary tumor tissue which in turn will be validated in a prospective manner.

## Disclosure of potential conflicts of interest

In the past 5 years, Dr. Krause received funding for her research projects by IBA (2016), Merck KGaA (2014–2018 for preclinical study; 2018–2020 for clinical study), Medipan GmbH (2014–2018). She is involved in an ongoing publicly funded (German Federal Ministry of Education and Research) project with the companies Medipan, Attomol GmbH, GA Generic Assays GmbH, Gesellschaft für medizinische und wissenschaftliche genetische Analysen, Lipotype GmbH, and PolyAn GmbH. For the present study, Dr. Krause confirms that none of the above mentioned funding sources were involved in the study design or materials used, nor in the collection, analysis and interpretation of data nor in the writing of the paper.

In the past 5 years, Dr. Michael Baumann received funding for his research projects and for educational grants to the University of Dresden by Bayer AG (2016–2018), Merck KGaA (2014-open) and Medipan GmbH (2014–2018). He is on the supervisory board of HI-STEM gGmbH (Heidelberg) for the German Cancer Research Center (DKFZ, Heidelberg) and also member of the supervisory body of the Charité University Hospital, Berlin. As former chair of OncoRay (Dresden) and present CEO and Scientific Chair of the German Cancer Research Center (DKFZ, Heidelberg), he has been or is responsible for collaborations with a multitude of companies and institutions, worldwide. In this capacity, he has discussed potential projects and signed contracts for research funding and/or collaborations with industry and academia for his institute(s) and staff, including but not limited to pharmaceutical companies such as Bayer, Boehringer Ingelheim, Bosch, Roche and other companies such as Siemens, IBA, Varian, Elekta, Bruker, etc. In this role, he was/is also responsible for the commercial technology transfer activities of his institute(s), including the creation of start-ups and licensing. This includes the DKFZ-PSMA617 related patent portfolio [WO2015055318 (A1), ANTIGEN (PSMA)] and similar IP portfolios. Dr. Baumann confirms that, to the best of his knowledge, none of the above funding sources were involved in the preparation of this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radonc.2021.12.050.

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