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Original Article

External validation of a composite bio-humoral index in anal cancer patients undergoing concurrent chemoradiation

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ABSTRACT

Background and purpose: A prognostic scoring system based on laboratory inflammation parameters, [Hemo-Eosinophils-Inflammation (HEI) index], including baseline hemoglobin level, the systemic inflammatory index and eosinophil count was recently proposed in patients with squamous cell carcinoma of the anus (ASCC). HEI was shown to discriminate disease-free (DFS) and overall (OS) survival in ASCC patients treated with concurrent chemoradiation (CRT). We tested the accuracy of the model on a multicentric cohort for external validation.

Materials and methods: Patients treated with CRT were enrolled. The Kaplan–Meier curves for DFS and OS based on HEI risk group were calculated and the log-rank test was used. Cox proportional hazards models were used to assess the prognostic factors for DFS and OS. The exponential of the regression coefficients provided an estimate of the hazard ratio (HR). For model discrimination, we determined Harrell's C-index, Gönen & Heller K Index and the explained variation on the log relative hazard scale.

Results: A total of 877 patients was available. Proportional hazards were adjusted for age, gender, tumor-stage, and chemotherapy. Two-year DFS was 77 %(95 %CI:72.0–82.4) and 88.3 %(95 %CI:84.8–92.0 %) in

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the HEI high- and low- risk groups. Two-year OS was 87.8 %(95 %CI:83.7–92.0) and 94.2 %(95 %CI:91.5–97). Multivariate Cox proportional hazards model showed a HR = 2.02(95 %CI:1.25–3.26; p = 0.004) for the HEI high-risk group with respect to OS and a HR = 1.53(95 %CI:1.04–2.24; p = 0.029) for DFS. Harrel C-indexes were 0.68 and 0.66 in the validation dataset, for OS and DFS. Gonen-Heller K indexes were 0.67 and 0.71, respectively.

Conclusion: The HEI index proved to be a prognosticator in ASCC patients treated with CRT. Model discrimination in the external validation cohort was acceptable.

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Squamous cell carcinoma of the anus (ASCC) is considered a rare cancer, with an annual incidence of 0.5–2.0 new cases in 100.000 individuals [1]. Concurrent chemo-radiation (CRT) is the standard curative therapeutic option, as investigated within prospective randomized phase III trials [2,3]. Due to the relatively infrequent occurrence of ASCC, the identification of robust predictive and prognostic factors has always been challenging [4]. This fact represents a gap in the era of personalized medicine, hampering the attempts to deliver targeted approaches to anal cancer patients, such as escalated or de-escalated treatment strategies based on a reliable risk stratification [5]. Most of the prognostic factors explored are related to the tumor, such as primary tumor size, nodal involvement, and Human Papilloma Virus (HPV) status, while patient-related features comprise gender, ethnicity, and the baseline level of hemoglobin [6–9]. In recent years, a growing research interest was focused on the interplay between cancer, the immune system and inflammation, as different bio-humoral prognostic scores were identified. Among them, the absolute leukocyte and neutrophil count, the Neutrophil-Lymphocyte ratio (NLR), the Platelet-Lymphocyte ratio (PLR) and the Systemic Index of Inflammation (SII), have been tested in ASCC patients [10–14]. A recent multicentric observational study investigated the validity of a prognostic model based on the Hemo-Eosinophils Inflammation (HEI) Index in ASCC patients treated with concurrent CRT [15]. The model is based on the combination of baseline hemoglobin level, eosinophil count and SII and was demonstrated to predict for disease-free (DFS) and overall (OS) survival in this setting of patients. These preliminary results require validation within an external cohort, before being proposed in daily clinical practice, to comply with the TRIPOD statements [16]. The present study aimed at validating the prognostic value of the HEI index in this clinical setting.

Material and methods

Patient population

The study sample for the present analysis included patients previously enrolled within the RAINSTORM study, conducted by the study group for gastrointestinal malignancies of the Italian Association of Radiotherapy and Clinical Oncology (AIRO) to evaluate the pattern of care and clinical outcomes of ASCC patients treated with Intensity-modulated-radiotherapy (IMRT) [17]. Patients with available baseline blood count were selected. Clinical data were collected into electronic data files by each participating centre co-investigator and double-checked at the data management centre. Written informed consent for treatment was obtained from all patients. The study was conducted according to the Declaration of Helsinki and the protocol was approved by the Scientific Council and the Board of Directors of AIRO (Project identification code: 16/2021). Patients were treated between 2007 and 2020 at 25 Radiation Oncology departments, affiliated with AIRO. Briefly, all patients had a histological diagnosis of ASCC, after endoscopic examination and biopsy assessment and were staged with pelvic magnetic resonance, chest computed tomography and/or whole body ^{18}FDG-PET, following the 7th Edition of the American Joint Committee on Cancer staging manual. Patients with clinical stages T1-T4, N0-N3, M0 were included. All patients underwent definitive CRT. Chemotherapy consisted of 5- fluorouracil (5-FU) (1000 mg/m^2/day) given as continuous infusion for 96 h (days 1–5 and 29–33) combined with mitomycin C (MMC) (10 mg/m^2) given as a bolus (days 1 and 29). Mitomycin C was capped at 20 mg maximum [18]. A minority of patients received 5-FU 200 mg/m^2 given as continuous infusion for 24 hours combined with Cisplatin 80 mg/m^2 (days 1 and 21) or Capecitabine 825 mg/m^2 twice daily for 5 days/week combined with Cisplatin 60–70 mg/m^2 every 3 weeks [19].

Radiation was delivered with IMRT, using either static or volumetric approaches, up to a total median prescription dose to the primary tumor of 55 Gy (range: 45–75 Gy), depending on tumor size and clinical stage at diagnosis [17]. Elective volumes were given a median dose of 45 Gy (range: 36–48 Gy). Patients were treated with either a simultaneous integrated or sequential boost dose [17,20].

Data collection

Response to treatment was assessed, using the RECIST criteria, at 3 months after treatment and thereafter at 6 months after CRT, for final evaluation. Clinical and laboratory data were retrieved through electronic medical record review. The following baseline variables were collected between 1 and 14 days before the treatment: white blood cell (cell/ml), lymphocyte (cell/ml), neutrophil (cell/ml), eosinophils (cell/ml), platelet count (cell/ml), hemoglobin (gr/dl). SII was defined as platelet $\times$ neutrophil/lymphocyte**.**

HEI-index

As previously described, the HEI Index was obtained by combining the inflammation indicators and laboratory parameters obtained at baseline, namely hemoglobin value, SII and absolute eosinophil count. A weight = 1 was given to each of the following variables: Hb < 12 g/dl, SII > 560 and eosinophil count $\geq$ 100/μL. Accordingly, patients were stratified into two different risk groups as follows: the low-risk group (no or maximum 1 negative prognostic factor present) and the high-risk group (either 2 or 3 negative prognostic factors observed) [15].

Statistical analysis

The original dataset, denominated derivation dataset, is the one used in the study by Rimini et al [15]. The validation dataset was assembled for the present study, using the newly collected data. For both the validation and derivation datasets, we computed the descriptive statistics expressed as absolute frequency and percentage (%) for categorical and as median [q1 = first quartile;q3 = third quartile] for continuous variables. The association between categorical variables was investigated using Pearson's Chi-squared test. The normality assumption was assessed using the Shapiro-Wilk's test. Since the null hypothesis was rejected, the Mann-Whitney U test was performed. The Kaplan–Meier curves for DFS and OS in

both HEI risk groups were calculated, and the log-rank test was used to test the difference between Kaplan Meier estimates. DFS was defined as the time interval between the start of CRT to the date of the disease relapse, death, or last follow-up examination. OS was defined as the time interval between the start of CRT to the date of death for any cause. Univariate and Multivariate Cox proportional hazards models were applied to evaluate the impact of prognostic factors on DFS and OS. All the analyses were based on complete cases and no missing imputation procedure was applied. Only the available data with respect to the considered parameters was computed for each patient. The exponential of the regression coefficients from the Cox model provided an estimate of the hazard ratio (HR) and the 95 % confidence interval (95 % CI). The original model included the HEI Index and was adjusted for age, gender, clinical stage and chemotherapy, which are considered clinical variables with a strong impact on clinical outcomes. This means that, in the multivariate analysis, the HEI parameter HR was corrected using the clinical parameters listed above. Radiation dose was quite heterogeneous in the original dataset and hence it was not included in the original model. To validate the prognostic model published by Rimini et al, we applied several methods, as described by Royston et al [21]. At first, we calculated the prognostic index (PI) defined as: $\sum_i \beta_i x_i$. We first determined the model calibration slope (i.e., regression coefficient) on the PI in a Cox regression model within the validation cohort and performed a likelihood ratio test of this slope being equal to 1. When the slope equals 1, the model can be considered valid. As a measure of model discrimination in the validation and derivation cohort, we determined Harrell's c-index, Gönen & Heller K Index and the explained variation on the log relative hazard scale based on the D statistic (R^2_D). C-index or Gönen & Heller K Index of 1 indicates perfect discrimination. De Long's test was used to compare the Area Under the Curve (AUC) of the Receiver Operating Characteristics (ROC) curves, considering the HEI Index as the outcome predictor at 60 month follow up. For calibration we compared the two estimates of the baseline survival function into the validation dataset with an approximation of the survival probability obtained from the derivation dataset. Predicted survival was computed for each patient with the use of our prognostic model according to the equation that follows from the proportional hazards assumption: $S(t) = S_0(t)^{e^{(PI-PI_0)}}$ where S(t) is the probability that a patient with PI will still be alive t years later, and $S_0(t)$ is the survival function for a hypothetical patient with PI0 (corresponding to the average covariate values). All statistical analyses were performed using R statistical software (version 3.1.2.; R Foundation for Statistical Computing, Vienna, Austria). All p-values were two-tailed and a p-value < 0.05 was considered indicative of a statistically significant association.

Results

A total of 877 patients was available for the present analysis. Table 1 reports the descriptive statistics for the variables included in both the derivation and validation cohorts. The median age in the validation cohort was 62.6 years (IQR = 16.34). Most of the patients were female (70.6 %) and affected with stage III ASCC (54.8 %). Most of them were treated with a chemotherapy regimen based on fluoropyrimidines and MMC (92.1 %). Stratification according to the HEI index split the cohort into almost numerically equivalent subgroups. Table 1 also reports on other variables not included in the initial model, such as individual bio-humoral parameters and radiation dose. No difference was found in the listed parameters between the derivation and validation datasets, apart from age category, in which a higher proportion of patients aged over 70 years was observed in the validation dataset (70.5 % vs 52.2 %; p < 0.001).

In the univariate Cox hazard regression model applied on the validation set, age ≥ 70 years was a significant predictor for worse OS (HR:1.54; 95 %CI: 1.14–2.08; p = 0.004) and DFS (HR:1.61; 95 % CI: 1.02–2.53; p = 0.040), together with clinical stage III at diagnosis for both OS (HR:2.10; 95 %CI: 1.52–2.91; p < 0.001) and DFS (HR:2.21; 95 %CI: 1.31–3.72; p = 0.003) (Table 2).

Fig. 1 reports Kaplan-Meier curves for DFS and OS stratified by HEI risk score over 5 years. The HEI index was found to be a parameter discriminating risk categories with a HR: 1.73 (95 %CI:1.22–2.44; p = 0.002), for OS, and HR: 2.37 (95 %CI:1.48–3.78; p < 0.001), for DFS. See Table 2 and Fig. 1 for details. Table 3 shows the multivariate analyses with respect to OS and DFS, for the validation and the derivation datasets. The HRs are > 1 for all variables, except for chemotherapy, in both datasets. Concerning OS, in the validation dataset, we found a significant HR for age (≥70 vs < 70 years, HR:1.67, 95 %CI: 1.05–2.64), gender (Male vs Female, HR:1.60, 95 %CI: 1.01–2.59), clinical stage (III vs I-II, HR:2.05, 95 % CI: 1.20–3.48), and HEI (High-Risk vs Low-Risk HR:2.02, 95 %CI: 1.25–3.26).

Concerning DFS in the validation dataset, the variables used for the construction of the model which provided a statistically significant contribution were age (≥70 vs < 70 years, HR:1.60, 95 %CI: 1.08–2.38), clinical stage (III vs I-II, HR:2.20, 95 %CI: 1.43–3.40), and HEI (High-Risk vs Low-Risk HR:1.53, 95 %CI: 1.04–2.24).

Discrimination has been assessed with several measures whose values and corresponding standard errors are presented hereby. (Table 4). Harrell c-index for OS was 0.68 (0.027) in the validation dataset and 0.76 (0.054) in the derivation. The Gönen & Heller K was 0.67 (0.057) in the validation dataset, and 0.70 (0.028) in the derivation. The values of c, K, and the Explained Variation - $R2_D$ are similar between the validation and derivation datasets for OS. Concerning DFS, results for Explained Variation - $R2_D$ are different in size between the two datasets (Table 4). In each dataset, the model shows a good discrimination performance but exhibits a slight reduction in the validation sample.

At 60 months, the AUC of the ROC curves, using the HEI Index as the independent variable and OS as the outcome measure, are 0.597 (95 % CI: 0.538–0.656) for validation and 0.630 (0.512–0.749) for derivation datasets (DeLong's test p-value = 0.602). For DFS, the univariate model for HEI results in AUC = 0.577 (95 % CI: 0.527–0.627) for validation and AUC = 0.654 (95 % CI: 0.529–0.779) for derivation datasets (DeLong's test p-value = 0.262).

In the Cox regression model, the slope for the PI in the validation dataset, with respect to OS, is 0.789 (0.150) and is not significantly different from 1 (p = 0.122). The discrimination is not preserved for DFS (p < 0.001). Fig. 2 reports the calibration analysis for OS and DFS. A good calibration for OS is shown especially from 24 months to 60 months.

Discussion

The HEI Index was confirmed to be an independent prognostic factor with respect to both OS and DFS in anal cancer patients treated with CRT. As elaborated by Glynne Jones et al, prognostic factors are intended to be specific measurable characteristics that can be easily obtained and measured during clinical observation within a certain population to be potentially correlated to measures of clinical outcomes [8]. In anal cancer patients, prognostic factors have been investigated within prospective studies or retrospective cohorts (4, 8, 22). Some of the seminal randomized phase III trials, which set the standard for the treatment of anal cancer, such as the European Organization for Research and Treatment of Cancer trial 22861 (EORTC 22861), the Radiotherapy and Oncology Group trial 98–11 (RTOG 98–11) and the Anal Cancer Trial-I study (ACT-I), explored the role of prognostic factors in this setting

Table 1
Descriptive statistics of the patients into the validation and derivation dataset. Data are expressed as absolute frequency (n) and percentage (%) and as median and [q1 = fist quartile, q3 = third quartile].

Characteristics		Validation (N = 877)		Derivation (N = 308)		p-value
		n	%	n	%	
Age	**< 70 yr**	242	29.5	145	47.9	< 0.001
	≥ 70 yr	579	70.5	158	52.2	
Gender	**Female**	622	70.9	231	75.0	0.195
	Male	255	29.1	77	25.0	
Stage	**I-II**	346	40.3	142	46.3	0.079
	III	513	59.7	165	53.8	
Chemotherapy	**MMC-based**	690	88.5	249	82.5	0.119
	CCDP-based	90	11.5	45	14.9	
HEI Index	**Low-risk**	325	51.2	168	54.6	0.368
	High-risk	310	48.8	140	45.5	
Pre-treatment Hb	**< 12 g/dL**	575	80.2	251	81.5	0.692
	≥ 12 g/dL	142	19.8	57	18.5	
Pre-treatment SII	**≤ 560**	333	47.5	126	40.9	0.062
	> 560	368	52.5	182	59.1	
Pre-treatment Eosinophil	**< 100/μL**	170	25.0	89	28.9	0.226
	≥ 100/μL	510	75.0	219	71.1	
RT dose (Gy)	**median [q1, q3]**	54.0 [54.0, 59.0]		54.0 [54.0, 55.0]		0.622

Legend: Hb: Haemoglobin; SII: Systemic Inflammatory Index; RT: Radiotherapy; HEI: Hemo-Eosinophils Inflammation.

Table 2
Univariate Cox hazard model for OS and DFS outcome into the validation dataset.

Characteristics	OS		DFS	
	HR 95 % CI	*p-value*	HR 95 % CI	*p-value*
Age (ref. < 70 yr)				
≥70 yr	1.54(1.14, 2.08)	*0.004*	1.61(1.02, 2.53)	*0.040*
Gender (ref. Female)				
Male	1.35(1.00, 1.83)	*0.050*	1.46(0.91, 2.35)	*0.113*
Chemotherapy (ref. MMC-based)				
CCDP-based	0.65(0.35, 1.20)	*0.168*	0.44(0.16, 1.20)	*0.110*
Stage (ref. I-II)				
III	2.10(1.52, 2.91)	*<0.001*	2.21(1.31, 3.72)	*0.003*
HEI (ref. Low-risk)				
High-risk	1.73(1.22, 2.44)	*0.002*	2.37(1.48, 3.78)	*< 0.001*

[1]HR = Hazard Ratio, CI = Confidence Interval.
Legend: CDDP: cisplatin; MMC: Mitomicyn C; OS: overall survival; DFS: disease-free survival; HR: Hazard Ratio; CI: Confidence Interval; HEI: Hemo-Eosinophils Inflammation.

within secondary analyses [8,22,23]. They are related to both tumor and patient. Specifically, the EORTC 22861 has shown that male gender, nodal involvement, and skin ulceration are independent predictors of loco-regional recurrence and OS [23]. In the RTOG 98–11 trial, authors reported a significant correlation between male gender, nodal involvement, and the rate of loco-regional recurrence and established a threshold for primary tumor size at 5 cm as an independent predictor for both DFS and OS [22]. Mature results of the ACT-I trial provided evidence for palpable lymph nodes and male gender as predictive and prognostic factors for loco-regional recurrence and OS and showed that lower baseline hemoglobin levels could predict the risk of cancer-related death and death from any cause [8]. Biological information needs to further complement the clinical factors to pave the way for effective personalized treatment strategies in this clinical setting. Recent refinements in the molecular characterization of ASCC, lead to the development of biological models stratifying prognosis according to HPV status and the presence of tumour-infiltrating lymphocytes (TIL) [24]. The prognostic role of TIL in p16 positive tumours suggests a role for the immune response in ASCC [25]. The strong expression of immune marker expression is associated with HPV16 infection and can predict for improved local control and DFS [26]. Baseline immune inflammation indicators are easy to access parameters, being obtainable from a peripheral blood sample, and can be used as prognosticators in cancer. This is particularly suited for ASCC, where the balance between inflammation and immune response was shown to trigger response to treatment and to impact on clinical outcomes [26]. This is the reason why we investigated the HEI Index to allocate anal cancer patients in prognostic categories after definitive concurrent CRT [15]. The HEI Index combines a) SII, b) baseline eosinophil count and c) hemoglobin level and can stratify patients with respect to DFS and OS [15]. The SII represents the balance between the pro-inflammatory activity induced by the tumor and the antitumor immune response elicited by the host [14]. SII is derived by combining the platelet count and the NLR and represents a reliable surrogate for the systemic inflammatory response, since it comprises the 3 main subpopulations of blood cells. SII has been demonstrated to be correlated to treatment response and progression-free survival (PFS) in ASCC [14]. A SII increase can be observed in 3 conditions: neutrophilia, lymphopenia and thrombocytosis, suggesting a high pro-inflammatory status and an exhausted immune response, which are microenvironmental conditions hampering the response to treatment. [14]. Neutrophilia can prompt secretion of vascular endothelial growth factors, and angiogenetic cytokine and therefore accelerate tumour development and seeding at distant sites [10]. Conversely, lymphopenia is associated with a more severe clinical behaviour and immune escape of tumour cells from TILs [11]. In anal cancer patients, baseline leukocytosis (defined as leukocyte > 10.000/ul) and neutrophilia (defined as neutrophil

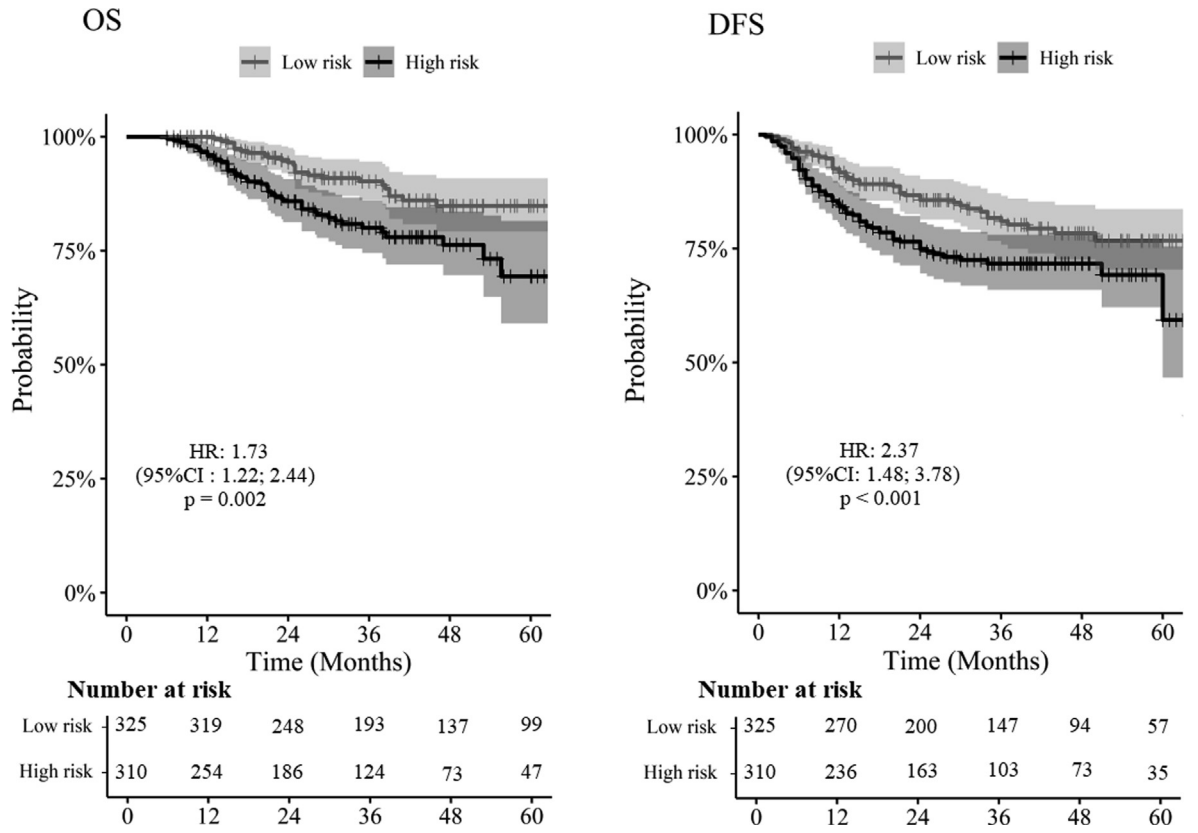

Fig. 1. Kaplan–Meier curves for overall (OS) and disease-free survival (DFS) and survival in high- and low-risk groups according to the HEI Index in the validation dataset.

Table 3
Hazard Ratios and relative 95% Confidence Interval for OS and DFS resulted from multivariate Cox regression analysis.

Characteristics	OS		DFS	
	Validation	Derivation	Validation	Derivation
Age (≥70 yr vs < 70 yr)	1.67(1.05, 2.64)*	1.92(0.88, 4.16)*	1.60(1.08, 2.38)*	2.25(1.19, 4.26)*
Gender (Male vs Female)	1.60(1.01, 2.59)*	1.79(0.89, 3.58)*	1.42(0.96, 2.09)	1.19(1.43, 4.72)
Chemotherapy (CCDP-based vsMMC-based)	0.48(0.17, 1.32)	0.25(0.08, 0.79)*	0.53(0.26, 1.10)	0.34(0.15, 0.76)*
Stage (III vs I-II)	2.05(1.20, 3.48)*	1.97(0.87, 4.42)	2.20(1.43, 3.40)*	1.39(0.76, 2.54)
HEI Index (High-Risk vs Low-Risk)	2.02(1.25, 3.26)*	2.97(1.36, 6.50)*	1.53(1.04, 2.24)*	2.59(1.42, 4.72)*

Legend: CDDP: cisplatin; MMC: Mitomicyn C; OS: overall survival; DFS: disease-free survival; HEI: Hemo-Eosinophils Inflammation.
* $p < 0.05$ derived from Cox regression analysis.

Table 4
Discrimination measures and standard error (SE) for OS and DFS for the validation and derivation datasets.

	OS		DFS	
	Validation	Derivation	Validation	Derivation
Harrell c-index (SE)	0.68 (0.027)	0.76 (0.054)	0.66 (0.026)	0.80 (0.049)
Gönen & Heller K (SE)	0.67 (0.057)	0.70 (0.028)	0.71 (0.048)	0.74 (0.021)
Explained Variation - R^2_D (SE)	0.06 (0.403)	0.17 (0.193)	0.06 (0.453)	0.21 (0.129)

Legend: OS: overall survival; DFS: disease-free survival; SE: standard error.

count > 7.500/ul) were found to be significantly associated with DFS, PFS and OS, independently of tumor and nodal stage at diagnosis [10,11]. Platelets can induce circulating tumor cell epithelial-mesenchymal transition and promote extravasation to metastatic sites [14]. Circulating platelets actively signal to tumor cells, via TGFβ and NF-κB, to promote their malignant potential outside the primary microenvironment, inducing prometastatic phenotype [14]. The second parameter included in the HEI Index is baseline eosinophil count [15]. Eosinophils are a crucial component in the interplay between inflammation, cancer, and immunity [15]. High baseline levels of eosinophils have been demonstrated to be associated with a higher likelihood of recurrence in ASCC [15]. The third parameter included in the HEI Index is baseline hemoglobin level, which could predict complete response to CRT in a retrospective cohort of ASCC patients [9]. The likelihood to achieve a complete response increases by 5.6 % for every single unit (g/dl) increase in baseline hemoglobin level. In the same series, baseline hemoglobin was found to be an independent predictor of OS. In the ACT-I randomized phase III trial, baseline hemoglobin level was shown as an independent prognostic factor for anal cancer-

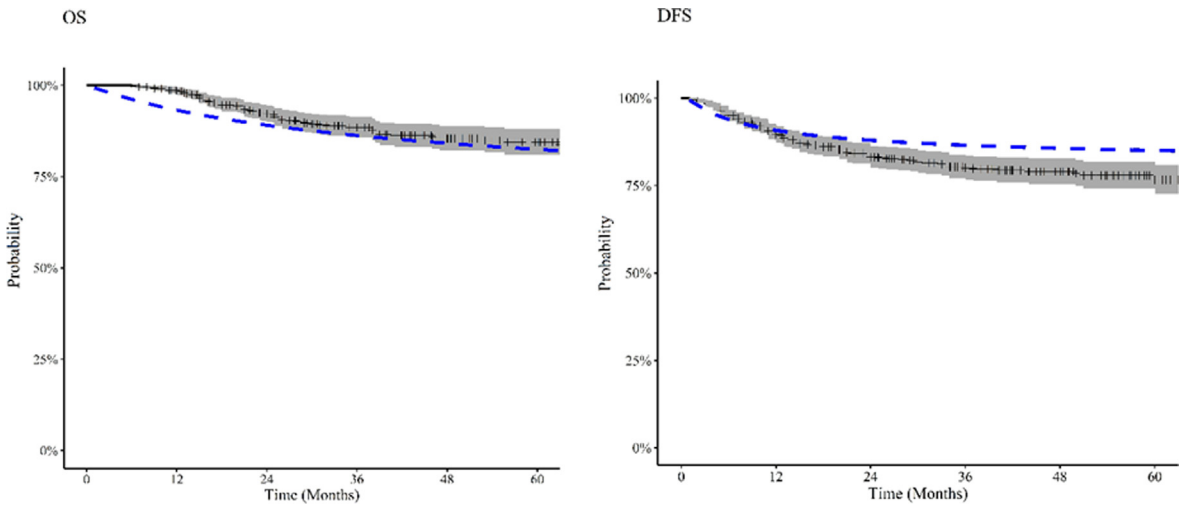

Fig. 2. Calibration plot for the estimates of the baseline survival function in the validation (grey curve) and derivation dataset (blue smoothed curve) for OS and DFS outcome.

related death. After adjusting for sex and lymphnode status, Glynne-Jones et al demonstrated that, on average, a single-unit (g/dl) increase in hemoglobin was associated with a 19 % reduction in the risk of anal cancer death [8].

In our previous study, the HEI Index was found to be an independent prognostic factor for both OS (HR: 2.97; 95 %CI:1.36–6.50; p < 0.001) and DFS (HR: 2.59;95 %CI:1.42–4.72; p < 0.001), after adjusting for well-established clinical factors (age, gender, clinical stage, chemotherapy) [15]. Median DFS for the Low-Risk group was not reached, while it was observed to be 79.5 months (95 %CI:45.20–79.54) in the High-Risk group [15]. There was a statistically significant difference between groups in terms of DFS with more than triple the risk of recurrence and death (HR:3.22; 95 %CI:2.04–5.10; p < 0.001) for patients in the High-Risk group based on the HEI Index and more than triple the likelihood to achieve complete remission after RT-CT for those in the Low-Risk group (OR:3.2;95 %CI:1.79–5.73; p < 0.001) [15]. Median OS was not reached in either group.

To externally validate the HEI Index in patients affected with ASCC, we employed a validation dataset comprising patients enrolled within a retrospective observational study and treated in Italy with IMRT-based concurrent CRT between 2007 and 2020 [17]. Derivation and validation dataset were not found to be statistically different with respect to most of the patient-, tumor- and treatment-related characteristics having a potential correlation with clinical outcomes. A higher proportion of patients in the derivation dataset was above 70 years of age compared to the derivation dataset. However, age was one of the factors which our model was adjusted for, allowing us to overcome the potential impact of this imbalance on the estimated outcomes.

In the validation dataset, the HEI Index was confirmed to be an independent prognostic factor for both OS (HR:2.02;95 %CI:1.25–3.26; p < 0.05) and DFS (HR:1.53;95 %CI:1.04–2.24; p < 0.05), being able to discriminate ASCC patients in risk categories with a doubling of the risk of death and a 50 % increase in the risk of failure and death for patients allocated to the High-Risk group according to the HEI Index. However, the risk estimates found in the validation dataset are lower compared to those observed in the derivation set for both OS (HR: 2.02 vs 2.97) and DFS (HR: 1.53 vs 2.59). Nevertheless, with respect to OS, discrimination was found to be acceptable with a similar size for all concordance measures that were employed. Calibration for OS was also good, when considering the observation period comprised between 2 and 5 years after treatment. Conversely, with respect to DFS, the size of the parameters was found to be different, particularly for the explained variance. This led to a discrimination that is not preserved for this specific outcome and less precision in the calibration ability. Potential explanations for the lower discrimination performance of the model in the validation dataset with respect to DFS include the differences in the number of centers and the number of patients per center between the derivation and validation datasets and the different timeframe of treatments between datasets, but mostly the presence of residual confounding. Specifically, in the multivariate model, the effect of the HEI Index on DFS was adjusted only for the clinical variables that were selected in the original study (Age, Gender, Clinical stage and Chemotherapy regimen) for internal consistency. Other impactful variables, such as comorbidities, radiotherapy dose, overall treatment time, treatment interruptions, and chemotherapy dose reduction, were not controlled for since they were not included in the original model [27,28]. This may have had an influence on the risk estimates and have hampered the discrimination performance of the model in the validation dataset with respect to DFS.

Nevertheless, the HEI Index was confirmed to be a biomarker with prognostic relevance in ASCC treated with CRT within the external validation cohort. We think this is an important finding which adds up to the prognostication toolkit in ASCC. Prognostic factors for outcomes following anal cancer treatment are limited [29]. Establishing HEI as a prognostic biomarker may further enrich the portfolio of clinical variables to be potentially selected in future prognostic modelling studies [29]. It may also help in better allocating ASCC patients into risk group for tailored stratification which may inform personalized treatment and follow up strategies. Nevertheless, a further evaluation of the potential role of the HEI Index in risk stratification for ASCC patients is deemed necessary within a prospective trial before implementation in clinical practice.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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