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Profiling complete regression after pre-operative therapy in gastric cancer patients using clinical and pathological data☆

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ABSTRACT

Introduction: The consistent use of pre-operative treatment before surgery for gastric cancer (GC) has resulted in increased rates of complete response. However, factors associated with response have been scantly investigated.

Methods: Patients with GCs treated between 2017 and 2022 undergoing pre-operative treatment followed by resection were included. Clinicopathological data were analyzed for the association with tumor regression grades (TRG); secondary outcomes included the short-term overall (OS), disease-free (DFS) and disease specific survival (DSS).

Results: Among 108 patients, 35.1% had an intestinal histotype GC, and 70.4% were treated with FLOT. Complete tumor regression (TRG1) was documented in 6.5% of patients. Univariable analyses documented that a higher pre-operative albumin ($p = 0.04$) and the expression of HER2 ($p = 0.01$) were associated to TRG1. In the multinominal regression model, the log-odds of being classified as TRG1 increased with the expression of HER2 by 170.247 times and with higher pre-operative albumin by 34.525 times, while with a higher Charlson Index and a diffuse hystotipe reduced it by 25.467 times and 3759.126 times, respectively. Among 49 patients (mean follow-up: 17.1 months), TRG1-2 was associated to better OS, DFS and DSS curves compared to TRG 3-5 (respectively $p < 0.01$, p 0.007 and $p < 0.01$), altogether with the reported negative impact of comorbidities in OS and DSS multivariable analyses (respectively p 0.04 and p 0.006). The random survival forest further confirmed the impact of HER2 and comorbidity on DSS.

Conclusion: A better clinical profile, HER2 expression and intestinal histotype significantly correlated with GC regression. A complete-major response was an independent factor for survival.

1. Introduction

Gastric adenocarcinoma is the 3rd cause of cancer death worldwide. In 2020, the latest figures by the Global Cancer Observatory reported in Europe some 13,600 gastric cancer (GC) cases, representing 12.5% of the global incidence [1]. In Europe GC is often diagnosed as an advanced disease, due to the lack of standard screening policies, thus most patients are treated with a perioperative chemotherapy approach. This management represents the gold standard for medically fit patients clinically staged as cT2 or higher, and/or cN positive, cM0, given the competitive survival gain reported in comparison with upfront surgery followed by adjuvant treatment [2].

The shift in favor of peri-operative protocols over upfront resection, commenced in Europe fifteen years ago, with the benefits in survival demonstrated by the English MAGIC trial, and the results were further improved ten years later with the German FLOT4 study [3,4]. The introduction and the subsequent evolution of perioperative therapy protocols led to remarkable rates of pathologic complete response, reported as up to 16% [4] and few strategies were proposed to consolidate the effects of chemotherapy and increase the rate of responders, including the administration of adjunctive pre-operative cycles [5,6].

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To-date, a limited number of studies investigated the clinicopathologic features associated with complete response in retrospective series of GCs, identifying the expression of HER2 [7], as well as a non-signet/intestinal histology [[4,8]] as associated with a greater tumor regression.

Nevertheless, the rate of patients undergoing peri-operative treatments and experiencing serious (grade 3-4) adverse events was reported also as significantly high, ranging from 25% to 40% [4], thus the identification of variables associated with complete response could aid in implementing treatment strategies aimed at both at maximizing the benefits of pre-operative therapy in responders and reducing unnecessary treatments in others.

Based on this background, the research hypothesis of this study was that we could define a profile of patients responding to pre-operative therapy based on a combination of clinical and pathological variables. The primary aim was thus the identification of a framework of features correlated with complete tumor response in patients with GC who underwent surgery after pre-operative treatment. The secondary aim was the evaluation of survivals in responders comparing others.

2. Materials and methods

This is a retrospective cohort study aiming to describe patients with GC who responded to pre-operative treatments. The protocol was notified to the Institutional IRB and designed adhering to the STROBE criteria for observational studies [9]. All consecutive patients who underwent gastric resection at our Unit between January 2017 and May 2022 were considered eligible for inclusion. The Unit is part of the Surgical Department of Fondazione Policlinico Universitario A. Gemelli in Rome, a University Research Hospital, ranking as the first Institution for GC volumes of resections/year in Italy [10,11], and appointed as comprehensive cancer center in 2019. Included patients were those with a diagnosis of GC with any clinical stage, undergoing pre-operative treatment and subsequent surgical resection. Exclusion criteria were Siewert type I GCs, patients with genetic syndromes including CDH1 mutations, R2 resections, patients with >30% missing data and those treated with non-resective palliative procedures (i.e. gastro-jejunal by-pass).

All clinical, operative, pathological and molecular data were recorded in a prospectively maintained data-base, retrieved and analyzed for the purpose of this study.

Clinical and operative data included sex, age (years), BMI, Charlson Index, location of the tumor [12], clinical staging, Bormann classification, type of pre-operative treatment (FLOT *vs* other regimens; number of cycles) and type of surgical procedure (open surgery, mini-invasive -MIS- laparoscopy, robotics, or MIS-assisted). Post-operative hospital stay (days) and 30-days complications (Clavien-Dindo Classification) were also recorded. Laboratory data were obtained upon surgical admission and included pre-operative albumin (g/dl), hemoglobin (g/dl), neutrophil and lymphocytes count.

Pathology data included the AJCC pathologic stage [13], number of retrieved and positive lymph-nodes, tumor diameter (mm), hystotype according to the Lauren's and WHO classification, and tumor regression (see below).

Molecular data included the expression of HER2 documented using immunohistochemistry (IHC). IHC evaluations were performed on pre-treatment endoscopic biopsies (when available) or on samples obtained from the surgical specimens in those GC with minor or without tumor regression, since by definition a complete pathologic response has no tumoral tissue available for the molecular characterizations. At our Institution, other IHC tests, as PDL-1, are performed in the setting of Institutional protocols and clinical studies (see below), whereas mismatch repair/microsatellite instability high (MSI-H) status has been consistently reported since early 2020, thus this variable was excluded from the investigation.

Patients' management. Following endoscopy and GC diagnosis, the pre-operative assessment is conducted in all patients using contrast-enhanced chest/abdomen CT scan and with staging laparoscopy in all advanced T/N stages or in cases with unclear peritoneal involvement based on imaging. In all patients included in this series, the management was established with a gastrointestinal oncologist; a formal MDT dedicated to GC including surgeons, oncologists, radiation therapists, pathologists and radiologists has been introduced at the Institution in 2019 and since then it has been held once a week to discuss all referrals. In brief, all non-metastatic patients with GC, fit for treatments and staged as cT2 or higher and/or those staged cN + are scheduled for a peri-operative treatment, with preferred FLOT regimen (if unfit for the taxan-based therapy, patients were candidate to ECF or FOLFOX schemes). Tumors located at the cardia are treated with pre-operative chemoradiation if Siewert I/II.

In adjunct to the current standards of care, during MDT discussions GC patients are screened and selected for: a) a randomized controlled trial (RCT) comparing FLOT *vs* FLOT/Ramucirumab in locally advanced non-metastatic esophagogastric adenocarcinoma (enrolling at our Institution since 2019) [14]; b) a protocol including pre-operative anti CTLA-4 plus anti-PD-L1 agents for locally advanced MSI-H GC (enrolling since 2021) [15]; and c) a RCT comparing prophylactic cytoreductive surgery plus HIPEC *vs* standard resection for locally advanced cT3-4/N+ non metastatic GC (enrolling since 2021) [16]. Also, and before commencing pre-operative treatments, patients are screened for malnutrition, using anthropometry and body composition measures [17] and treated with oral/enteral nutritional supports if required. Finally, GC patients Stage IV routinely undergo HER2 IHC evaluation before chemotherapy, that is prescribed consequently [18,19].

At the end of the pre-operative treatment, patients are re-staged using contrast enhanced CT scan, re-evaluated in a multidisciplinary discussion and scheduled for surgery if appropriate (stable/responsive disease), in selected cases also aiming to conversion surgery. Endoscopy is usually performed at the end of neoadjuvant treatment in GC located at the cardia to define the esophagogastric junction macroscopic involvement, and plan the surgical resection accordingly. Surgical resection usually includes a D2 nodal dissection, and laparoscopic gastrectomy is performed routinary in patients with early-stage GCs [[12,20]].

Outcome of interest. The primary outcome of interest was a complete tumor response, defined as a pathologic diagnosis of ypT0/Mandard tumor regression grade (TRG) 1 in the surgical resection specimen [21]. Secondary outcomes included overall survival (OS), defined as any cause of death, disease free survival (DFS), defined as the first recurrence after surgical resection, and disease specific survival (DSS), defined as death due to GC, all calculated from the date of resection.

Statistics. Categorical data were reported using frequencies and percentages and continuous variables using mean values and standard deviations (SD) or medians and ranges. Missing data were handled with statistical imputation using a non-parametric Random Forest method. In the entire cohort, clinical and pathological variables were tested for normality and univariable analyses were performed using the Kruskal-Wallis test for a possible correlation with TRG grades.

Also, to analyze the primary outcome, and to define a set of variables containing most of the information, a principal component analysis (PCA) of quantitative variables was performed to select those clinicopathological and molecular variables that could differentiate GCs with TRG1, TRG3 or TRG5 (complete regression - partial regression - absence of regression). As a first step, the degree

of correlation among different variables was tested and then 2D and 3D PCA dimensions were evaluated. Pre-operative variables presenting a greater contribution in the first two PCA dimensions were selected and analyzed using an ordinal multinomial logistic regression.

The selected features were combined to obtain a Decision Trees (DT) algorithm to differentiate responders *vs* non responders using a Random Forest (RF) analysis. For this analysis, the data set was randomly partitioned (80% training set - 20% test set) and implemented with a 10 k-fold cross validation method to include a simple DT per each fold. The RF classification model was designed by the aggregation of many DTs, setting the accuracy at 75% and the complexity at 0.03, and evaluated for sensibility, specificity and reliability using the Cohen's kappa coefficient. Finally, the model was checked for the control over the prediction using the confusion matrix.

Survival analyses with the end-points of OS, DFS and DSS were conducted with the Kaplan–Meier method and log-rank test and a Cox multivariable logistic regression. Only patients treated before 2021 were selected for the latter analysis and covariates were chosen on the basis of the multinominal model. Hazard ratios were illustrated in a spline model on continuous exposures. Survivals were also evaluated using a supervised machine-learning approach to generate a random survival forest analysis (RSF). RSF foresees a partition of the dataset (training set and validation set), which was implemented by a 10 k-fold resampling to generate up to 500 tree survival models. Models were evaluated for the out of bag (OOB) prediction error (1-C) - to express the rate of misclassification- and the best splits were assessed using the Gini impurity and analyzed for precision, recall and F1 score.

All tests were two-tailed, and a p-value of <0.05 was considered statistically significant. All the analyses were performed using R software and packages detailed in Supplementary Materials.

3. Results

Out of the 338 GCs patients who underwent surgery during the investigation period, 108 were selected based on study criteria, Fig. 1. Of these, the majority of the patients were males (66.7%), with moderate/severe comorbidity indexes (mean Charlson Index 5.5 ± 2.9) and normal BMI (mean BMI 24.9 ± 4.0). GCs were classified as Lauren's intestinal type in one third of the cases and cancers were mostly located in the upper stomach (56.5%); in these cases, the esophago-gastric junction involvement was 85.3%. In the vast majority of the population, GCs presented as locally advanced and had clinical nodal metastases in more than 85.0% of the cases. One quarter of the series had distant metastases at diagnosis (mostly with minimal peritoneal disease: mean peritoneal carcinosis index -PCI- 3.4, range 1–6). Seventy percent of the patients were treated with a FLOT-based regimen, among the remaining 32 patients, 14 patients were treated with FOLFOX schemes, 6 with ECF schemes, and the others included in trials as detailed above. The mean number of pre-operative cycles was 5.2 in the whole study cohort. However, it should be noted, that the mean number of pre-operative cycles was of 4.3 in the series treated with FLOT and 7.1 in GCs treated with other regimens; in this latter group, 4 patients were treated with pre-operative chemoradiation (Supplementary Table 1). Finally, 6 patients reported a dose reduction or chemotherapy suspension due to toxicities. Mean pre-operative laboratory values upon surgical admission displayed mild anemia (mean Hb 11.8 ± 1.7 g/dl), whereas mean neutrophil and lymphocyte counts were mostly within normal ranges. Among the 9 patients who presented a HER2 3+ expression, 4 GCs (44.4%) were metastatic, and among this subgroup, 3 were treated with anti-HER agents, whereas 1 patient was enrolled in the protocol with neo-adjuvant immunotherapy [15].

The greater percentage (59.3%) of patients were treated with total gastrectomy, and 23 patients were treated with a mini-invasive (MIS) procedure, using laparoscopy or robotics as a full procedure or MIS-assisted. About 14% of the series had a post-operative complication requiring a radiological or surgical treatment, whereas 2 patients were readmitted after discharge: one patient due to bleeding from a splenic artery pseudo-aneurism and one for dyspnea. Median post-operative hospital stay was of 8.0

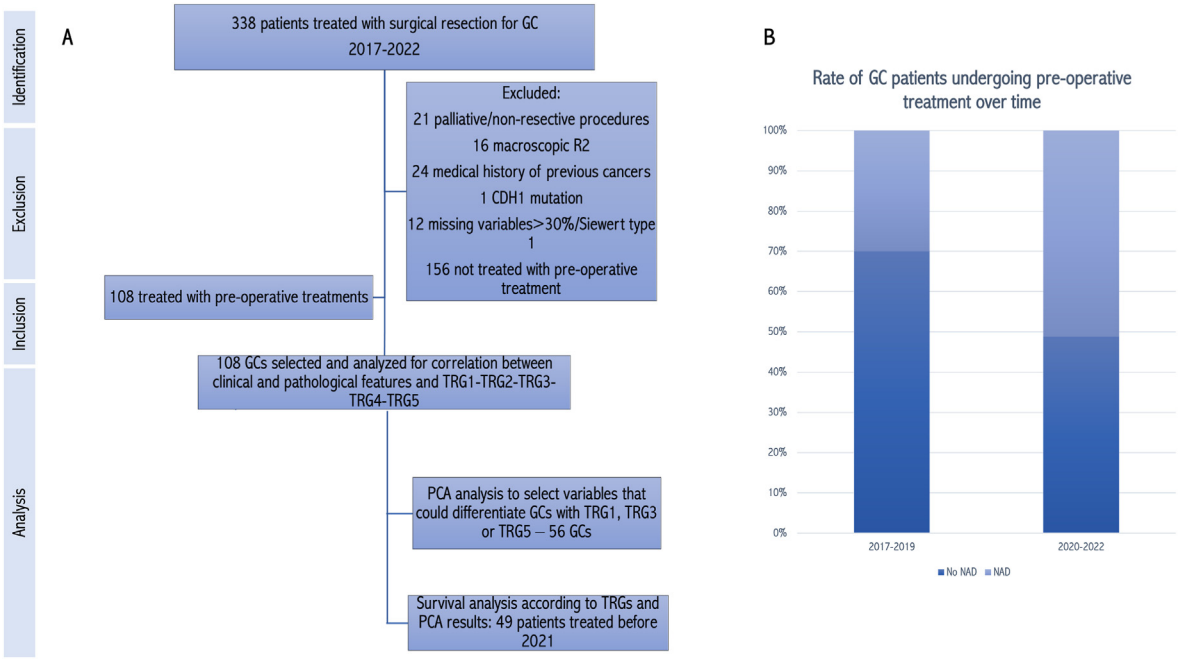

Fig. 1. A. Study flow-chart of included and excluded patients showing also steps of statistical analyses; GC: gastric cancer; TRG: tumor regression grade; PCA: principal component analysis **B.** Patients treated with pre-operative treatment before and after the introduction of a dedicated multidisciplinary team meeting (MDT) in 2019.

Table 1
Univariable analysis with non-parametric tests comparing baseline patients' clinical, pathological and molecular features among different tumor regression categories.

	TRG1 7 patients		TRG2 9 patients		TRG3 31 patients		TRG4 43 patients		TRG5 18 patients		P-Value
Age											0.29
Mean; SD	65.1	14.2	59.7	11.2	60.6	9.5	64.4	10.6	63.5	9.2	
Median; IQR1-IQR3	71.0	59.0–73.5	56.0	50.0–72.0	63.0	53.0–66.5	67.0	58.5–72.0	63.0	59.2–71.8	
Sex											0.23
Male	3	43.0%	7	78.0%	19	61.0%	33	77.0%	10	56.0%	
Female	4	57.0%	2	22.0%	12	39.0%	10	23.0%	8	44.0%	
Charlson Index											0.31
Mean; SD	3.6	1.7	5.2	2.7	5.4	3.2	5.8	2.8	5.8	2.8	
Median; IQR1-IQR3	4.0	2.5–4.5	5.0	3.0–7.0	4.0	3.0–6.5	5.0	4.0–7.0	5.0	4.0–7.5	
BMI											0.43
Mean; SD	24.6	3.2	26.5	4.1	25.7	3.3	24.8	4.2	23.6	4.3	
Median; IQR1-IQR3	23.8	22.8–25.0	26.0	25.0–29.4	26.3	23.2–27.7	24.0	21.9–28.6	24.0	20.7–26.9	
Tumor Location n - (%)											0.46
Lower GC	1	14.0%	5	56.0%	8	26.0%	12	28.0%	6	33.0%	
Middle GC	0	0.0%	0	0.0%	6	19.0%	6	14.0%	3	17.0%	
Upper GC	6	86.0%	4	44.0%	17	55.0%	25	58.0%	9	50.0%	
Esophago-Gastric Junction GC n - (%)											0.79
No	4	57.0%	5	56.0%	18	58.0%	19	44.0%	10	56.0%	
Yes	3	43.0%	4	44.0%	13	42.0%	24	56.0%	8	44.00%	
Bormann Classification n - (%)											0.21
Fungating/ulcerated	3	43.0%	4	44.0%	20	64.5%	32	74.4%	14	78.0%	
Polypoid	3	43.0%	4	44.0%	10	32.3%	7	16.3%	4	22.0%	
Others	1	14.0%	1	12.0%	1	3.2%	4	9.3%	0	0.0%	
cT Stage n - (%)											0.51
cT2	1	14.0%	1	12.0%	3	9.7%	3	7.0%	0	0.0%	
cT3	1	14.0%	4	44.0%	8	25.8%	13	30.2%	4	22.2%	
cT4	5	72.0%	4	44.0%	20	64.5%	27	62.8%	14	77.8%	
cN stage n - (%)											0.77
cN0	0	0.0%	0	0.0%	3	9.7%	4	9.3%	1	5.6%	
cN positive	7	100.0%	9	100.0%	28	90.3%	39	90.7%	17	94.4%	
cM Stage n - (%)											0.22
cM0	5	71.4%	6	66.7%	21	67.7%	37	86.0%	11	61.1%	
cM positive	2	28.6%	3	33.3%	10	32.3%	6	14.0%	7	38.9%	
Neo-adjuvant treatment type n - (%)											0.51
FLOT	3	43.0%	6	66.7%	23	74.2%	32	74.4%	12	66.7%	
Others	4	57.0%	3	33.3%	8	25.8%	11	25.6%	6	33.3%	
Number of neo-adjuvant treatment cycles											0.16
Mean; SD	5.7	2.9	6.6	6.0	5.4	2.7	4.6	2.1	5.4	2.6	
Median; IQR1-IQR3	4.0	4.0–5.9	4.0	4.0–4.0	4.0	4.0–5.5	4.0	4.0–4.0	4.0	4.0–5.7	
Pre-operative Hb (g/dl)											0.54
Mean; SD	11.9	1.4	12.0	1.3	12.2	1.7	11.7	1.6	11.3	2.0	
Median; IQR1-IQR3	11.8	11.5–12.8	12.2	11.3–12.7	12.1	11.3–13.2	11.9	11.0–12.8	10.9	9.9–12.8	
Pre-operative Neutrophil count (x10^9/L)											0.47
Mean; SD	3.9	2.4	3.5	1.3	3.6	1.5	4.1	1.8	4.4	2.1	
Median; IQR1-IQR3	3.4	2.3–4.3	2.9	2.6–4.5	3.5	2.5–3.9	3.4	3.0–4.7	4.3	2.6–5.7	
Pre-operative Lymphocyte count (x10^9/L)											0.09
Mean; SD	1.9	0.8	1.8	0.6	1.9	0.7	1.8	0.6	1.5	0.4	
Median; IQR1-IQR3	1.7	1.4–2.5	1.6	1.5–2.1	1.7	1.4–2.0	1.7	1.4–1.9	1.4	1.2–1.6	
Pre-operative Albumin (g/dl)											**0.04**
Mean; SD	39.3	3.7	40.3	2.7	38.5	3.9	36.9	3.9	36.5	4.5	
Median; IQR1-IQR3	38.0	36.5–41.0	41.0	38.1–42.0	38.0	37.0–41.5	37.0	34.5–39.0	36.0	34.2–39.8	
Surgical Resection n - (%)											0.34
Sub-Total Gastrectomy	1	14.3%	5	55.6%	9	29.0%	11	25.5%	5	27.8%	
Total Gastrectomy	5	71.4%	4	44.4%	18	58.1%	26	60.5%	11	61.1%	
Esophago-Gastrectomy	1	14.3%	0	0.0%	4	12.9%	6	14.0%	2	1.1%	
Intra-operative HIPEC n - (%)											0.18
No	7	100.0%	7	78.0%	26	84.0%	41	95.0%	14	78.0%	
Yes	0	0.0%	2	22.0%	5	16.0%	2	5.0%	4	22.0%	
Tumor's diameter (mm)											**0.004**
Mean; SD	23.2	17.2	25.1	16.4	37.6	22.7	44.8	22.6	58.2	31.2	
Median; IQR1-IQR3	30.0	10.0–35.1	20.0	15.0–35.0	40.0	21.0–48.9	40.0	28.5–60.0	57.5	30.0–79.5	
Lauren Classification n - (%)											0.19
Intestinal	5	71.4%	5	55.6%	13	41.9%	25	58.1%	9	50.0%	
Mixed	2	28.6%	3	33.3%	3	9.7%	7	16.3%	6	33.3%	
Diffuse	0	0.0%	1	11.1%	15	48.4%	11	25.6%	3	16.7%	
WHO Poorly Cohesive											0.38
No	6	85.7%	8	88.9%	19	61.3%	33	76.7%	13	72.2%	
Yes	1	14.3%	1	11.1%	12	38.7%	10	23.3%	5	27.8%	
Signet Ring Cells Features											0.15
No	7	100.0%	6	66.7%	16	51.6%	30	69.8%	12	66.7%	
Yes	0	0.0%	3	33.3%	15	48.4%	13	30.2%	6	33.3%	

Table 1 (*continued*)

	TRG1 7 patients		TRG2 9 patients		TRG3 31 patients		TRG4 43 patients		TRG5 18 patients		P-Value
Mucinous Features											0.65
No	7	100.0%	7	77.8%	26	83.9%	33	76.7%	15	83.3%	
Yes	0	0.0%	2	22.2%	5	16.1%	10	23.3%	3	16.7%	
HER2 Expression											**0.01**
Mean; SD	1.3	0.9	1.2	1.1	0.8	0.7	0.5	0.4	0.3	0.2	
Median; IQR1-IQR3	1.3	0.7–1.5	1.0	0.3–1.5	0.0	0.0–1.0	0.0	0.0–0.6	0.0	0.0–0.4	
ypT n - (%)											**<0.01**
ypT0	7	100.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	
ypT1	0	0.0%	3	33.3%	2	22.3%	0	0.0%	0	0.0%	
ypT2	0	0.0%	4	44.4%	5	16.2%	8	18.6%	1	5.6%	
ypT3	0	0.0%	2	22.3%	19	61.3%	20	46.5%	2	11.1%	
ypT4	0	0.0%	0	0.0%	5	16.2%	15	34.9%	15	83.3%	
ypN n - (%)											**<0.01**
ypN0	6	85.7%	6	66.7%	8	25.9%	10	23.2%	3	16.7%	
ypN1	1	14.3%	1	11.1%	13	41.9%	8	18.6%	2	11.1%	
ypN2	0	0.0%	1	11.1%	5	16.1%	11	25.6%	3	16.6%	
ypN3	0	0.0%	1	11.1%	5	16.1%	14	32.5%	10	55.6%	
ypM n - (%)											0.07
ypM0	6	86.0%	7	78.0%	23	74.0%	38	88.0%	10	56.0%	
ypM1	1	14.0%	2	22.0%	8	26.0%	5	12.0%	8	44.0%	
Positive Metastatic Nodes											**<0.01**
Mean; SD	0.0	0.0	2.4	5.9	4.0	7.2	6.6	7.5	9.7	12.0	
Median; IQR1-IQR3	0.0	0.0–0.0	0.0	0.0–1.0	2.0	0.5–4.0	5.0	1.0–10.5	8.5	2.2–10.8	
Lymph-Node Harvest											0.49
Mean; SD	31.4	7.9	42.2	15.2	34.1	14.8	34.1	16.7	36.0	16.0	
Median; IQR1-IQR3	32.0	27.5–36.5	38.0	30.0–54.0	31.0	23.5–44.0	30.0	25.0–37.5	39.5	22.5–44.8	

TRG: tumor regression grade - GC: gastric cancer.

days. Overall, in the cohort, the effect of pre-operative treatments resulted in a 6.5% of TRG1.

Profiling GC regression. For this analysis, an imputation was performed to overcome a modest percentage of missing data (mean rate of missing data for each variable 5.7% ± 0.1%; median 2.8%; range 0.0%–25.9%, Supplementary Table 2 and Supplementary Fig. 1).

Univariable analyses were then performed to correlate patients' characteristics and GC features with the different TRGs (Table 1). These analyses documented a less tumor regression (higher TRG) with lower pre-operative albumin levels (median value: 38.0 in TRG1 *vs* 36.0 in TRG5, $p = 0.04$) and lower expression of HER2 ($p = 0.01$). Greater tumor diameter, higher ypT, ypN stages and number of positive metastatic nodes also correlated with higher TRG ($p < 0.01$).

In order to obtain a framework of features describing patients with better response to pre-operative therapy, we then focused on GCs with complete (TRG1), partial (TRG3) and absence of regression (TRG5), investigating in total 56 patients. As a primary step, a good correlation among the quantitative variables was documented

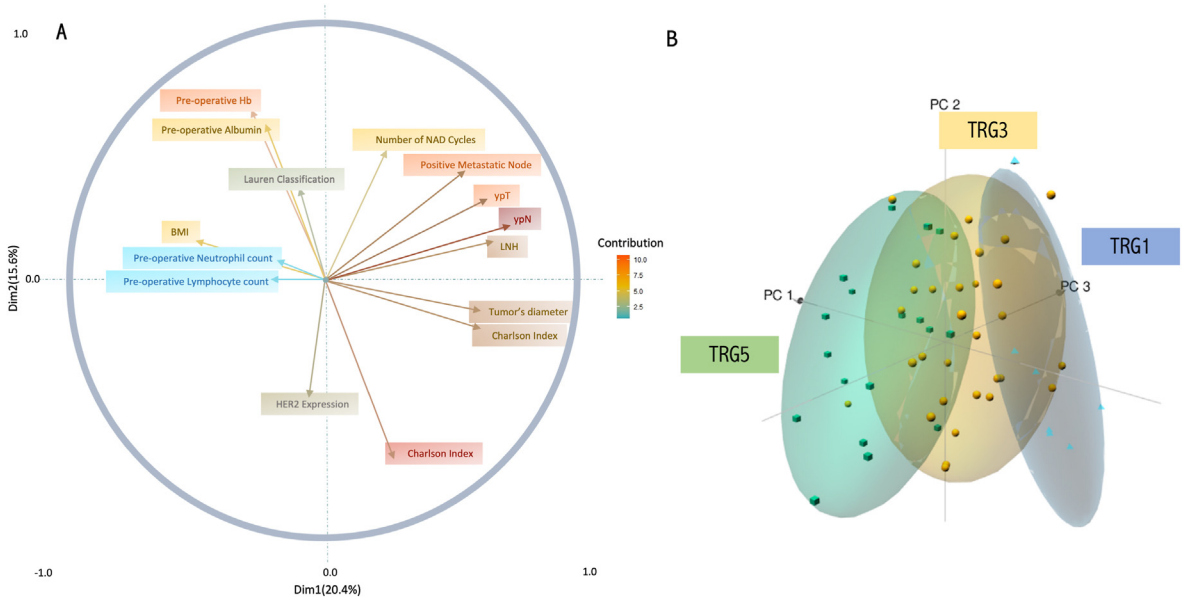

Fig. 2. A. Principal component analysis of variables that could differentiate tumor regression grade (TRG) sub-groups TRG1 *vs* TRG3 and TRG5; **B.** Spatial representation and 3D partition of the 3 TRG groups in the principal component analysis. NAD: neo-adjuvant treatment; Hb: hemoglobin.

Table 2
Multinomial logistic regression[§].

Dependent variable	TRG1	TRG3
Age		
Log-ODDS	0.362	0.058
SE	−0.658	−0.068
HER2		
Log-ODDS	**170.247****	**1.387***
	−9.188	−0.695
BMI		
Log-ODDS	**−29.939****	0.142
	−1.679	−0.13
Number of neo-adjuvant treatment cycles		
Log-ODDS	−2.155	−0.056
	−1.859	−0.168
Pre-operative Hemoglobin (Hb)		
Log-ODDS	**−16.636****	0.045
	−1.968	−0.246
Charlson Index		
Log-ODDS	**−25.467****	0.07
	−2.974	−0.157
Pre-operative Albumin		
Log-ODDS	**34.525****	0.217
	−2.079	−0.124
Lauren Classification		
Log-ODDS	**−3759.126****	2.204
	0	−1.165
Constant	**−471.566****	−16.358
	−4.253	−8.897
Akaike Inf. Crit.	88.19	88.19

[§]reference TRG5; *p < 0.05; **p < 0.01.

(Supplementary Fig. 2), and following, a PCA was performed (Supplementary Fig. 3).

Fig. 2 shows the positive (strong impact) and negative (poor impact) contributions of the variables in differentiating the 3 groups of TRGs in the PCA. With this respect, pre-operative laboratory values provided mixed contribution (negative contribution: pre-operative neutrophil and lymphocyte counts; positive contribution: pre-operative albumin and Hb levels), whereas age, Charlson Index and the other pathologic feature had a strong positive impact in discriminating these 3 groups. A 3D visual partition of the 3 TRG groups based these variables is also shown in Fig. 2.

Finally, those pre-operative variables presenting a greater contribution in the first two PCA dimensions (Supplementary Table 2) were selected and computed in a multinomial logistic regression model. As shown in Table 2, significant results were documented for HER2 expression, BMI, pre-operative Hb, Charlson Index, pre-operative albumin, and Lauren classification.

In brief, the log-odds of being classified as TRG1 *vs* TRG5 was increased by 170.247 times, as the expression of HER2 increased from 0-negative to HER2 3+. The same trend applied to pre-operative albumin level (80% of TRG5 patients had very low albumin levels), whereas opposite results were documented for the Charlson Index (greater in the TRG5 population) and Lauren classification. In particular, the log-odds of being classified as TRG1 *vs* TRG5 decreased by 3759.126 times when the hystotipe was diffuse instead of intestinal. The multinomial regression also reported a significant association of the BMI and pre-operative hemoglobin levels with a negative log-odds for TRG1 *vs* TRG5, but it has to be noted that no clear trend for these variables was documented among TRG categories (i.e. mean BMI 24.6 in TRG1 *vs* 25.7 in TRG3 and 23.6 in TRG5).

These variables were therefore combined in a RF model, to differentiate TRG1 *vs* TRG5 patients. Even though the expression of HER2 was documented to categorize patients, the DT presented just one split, with sub-optimal statistical values (sensibility 50.0%, specificity 100.0% and Cohen's kappa coefficient 0.5), possibly due to the small sample of patients (Supplementary Fig. 4).

Survivals. Forty-nine patients were analyzed with a mean follow up of 17.1 months. Kaplan–Meier curves were obtained comparing TRG1-TRG2 (complete/major responders) *vs* TRG3-TRG4 (partial responders) *vs* TRG5 (non-responders). As shown in Fig. 3, OS, DFS and DSS were improved in responders compared to the other groups (log-rank test respectively: $p = 0.04$, $p < 0.0001$ and $p = 0.043$). At the multivariable Cox analyses, a complete-major response was independently associated with survival (TRG1-2 *vs* TRG5 for OS, DFS and DSS, respectively HR 1.27E+08, 95%CI 2.3E+07-7.1E+08, $p < 0.01$; HR 51.7, 95%CI 2.9–924.4, p 0.007; HR 1.1E+08, 95%CI 1.9E+07 -6.8E+08, $p < 0.01$).

Also, an increased Charlson Index independently correlated with worse OS and DSS, although with limited HR (respectively OS: HR 1.25, 95%CI 1.00–1.56, p 0.04 and DSS: HR 1.38, 95%CI 1.09–1.74, p 0.006, Table 3). This correlation is further documented in Fig. 4, plotting comorbidity indexes with the relative death rates in OS and DSS and showing that when the Charlson index increases (x-axis) increases also the relative death rate (y-axis).

Finally, survivals were evaluated using RSF: the OOB ranged between 0.27 and 0.37, and the best discrimination model was obtained for DSS (Supplementary Fig. 5 and 5). Using root analysis, the model discriminated DSS based on the favorable impact of HER2 expression. In cases of HER2 negativity, DSS was discriminated by a Charlson Index greater or equal than 7.5 (negative impact) or lower than 7.5 (positive impact) (Fig. 5 and Supplementary Materials).

4. Discussion

With this study, we profiled regression grades based on clinical and pathological features. The univariable analyses pointed to clinical (increased albumin level), pathological (lower tumor's diameter, pT, pN stages) and molecular characteristics (increased HER2 expression), and this profile was further enriched by the PCA categorization and the multinominal model. From a clinical point of view, the profile was consistent with that of a patient with a good performance status (higher albumin level, lower Charlson Index) with specific tumor's features (intestinal hystotype and HER2 positivity), who presented with better response after pre-operative therapy. Other associations, as BMI and pre-operative hemoglobin levels, are more difficult to comment given the fluctuation presented in the TRG3 category. A similar finding was also reported in a small series of Stage 3 GCs, where authors found that pre-treatment obesity and BMI did not affect the TRG categories [22].

The correlation between immune/nutritional profile with regression has been previously investigated in literature, although always in relatively small series. In a recent cohort of 30 GC patients treated with sintilimab and XELOX, the systemic immune-inflammatory index and prognostic nutritional index were reported lower in the TRG0-2 AJCC stages [23]. Other examples include the evaluation of pre-neoadjuvant laboratory values: in particular lower neutrophil-lymphocytes ratio correlated with improved TRG categories [24]. Consistently with our findings, a greater prevalence of intestinal histotype was reported in the TRG 1-2 subgroup in large series of locally advanced GCs treated with pre-operative chemotherapy [25,26]. Also, the presence of signet ring cells has been correlated with lower regression grades [26,27].

Unfortunately, it was not possible to investigate mismatch-repair complex status in our cohort, however, literature in this field provided controversial results when correlating this variable with regression grades. Indeed, in 2 large series of French and Chinese patients, the histological response after neoadjuvant in GC patients was not statistically different in proficient *vs* deficient

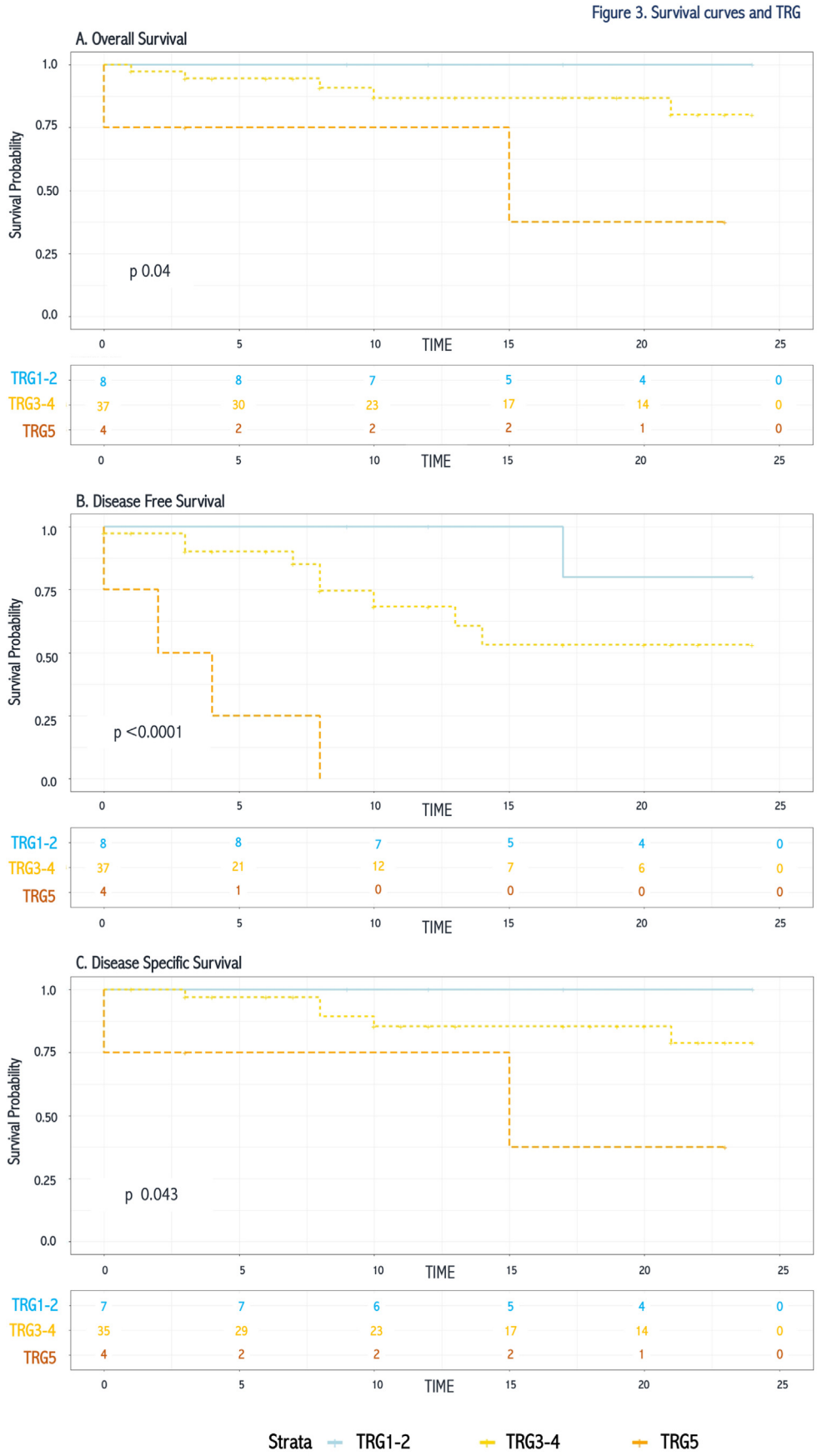

Fig. 3. Survival curves and tumor regression grade (TRG) sub-groups: **A.** Overall survival; **B.** Disease Free Survival; **C.** Disease specific survival.

Table 3
Cox logistic multivariable regression survival analysis.

	Coef.	HR	Lower CI 0.95	Upper CI 0.95	Pr(>|z|)
Overall Survival					
TRG1-2 *vs* TRG 3-4	18.51	1.09E+08	1.94E+07	6.15E+08	**<0.01**
TRG1-2 *vs* TRG5	18.66	1.27E+08	2.26E+07	7.14E+08	**<0.01**
Lauren Classification (Intestinal *vs* Diffuse)	−1.21	0.30	0.03	2.55	0.26
BMI	−0.16	0.85	0.68	1.06	0.14
Charlson Index	0.23	1.25	1.00	1.56	**0.04**
Pre-operative Hemoglobin (Hb)	0.20	1.22	0.76	1.96	0.41
Pre-operative Albumin	−0.13	0.88	0.70	1.11	0.28
HER2	−89.81	0.00	0.00	–	0.99
Disease Free Survival					
TRG1-2 *vs* TRG 3-4	0.95	2.59	0.25	26.64	0.42
TRG1-2 *vs* TRG5	3.94	51.73	2.89	924.39	**0.007**
Lauren Classification (Intestinal *vs* Diffuse)	0.06	1.07	0.18	6.09	0.93
BMI	−0.01	0.98	0.82	1.17	0.87
Charlson Index	0.18	1.19	0.96	1.48	0.09
Pre-operative Hemoglobin (Hb)	−0.09	0.91	0.60	1.38	0.66
Pre-operative Albumin	−0.11	0.89	0.72	1.10	0.29
HER2	−0.38	0.68	0.27	1.67	0.40
Disease Specific Survival					
TRG1-2 *vs* TRG 3-4	18.76	1.41E+08	2.35E+07	8.44E+08	**<0.01**
TRG1-2 *vs* TRG5	18.55	1.14E+08	1.89E+07	6.80E+08	**<0.01**
Lauren Classification (Intestinal *vs* Diffuse)	−1.44	0.24	0.03	2.08	0.19
BMI	−0.09	0.91	0.71	1.17	0.46
Charlson Index	0.32	1.38	1.09	1.74	**0.006**
Pre-operative Hemoglobin (Hb)	0.25	1.28	0.77	2.13	0.34
Pre-operative Albumin	−0.15	0.86	0.68	1.08	0.19
HER2	−89.67	1.14E-39	0.00	–	0.99

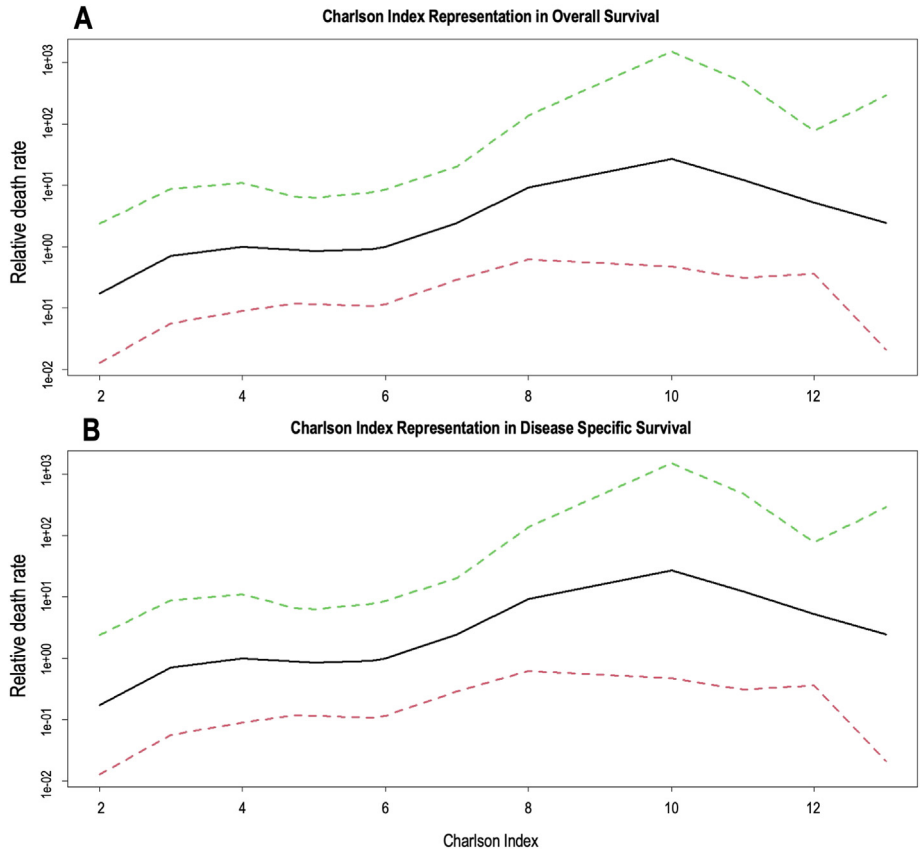

Fig. 4. Spline model and Cox proportion hazard. **A.** Overall survival: x-axis: Charlson index values and y-axis: relative death rate (dashed lines shows 95%CI); **B.** Disease Specific survival: x-axis: Charlson index values and y-axis: relative death rate (dashed lines shows 95%CI).

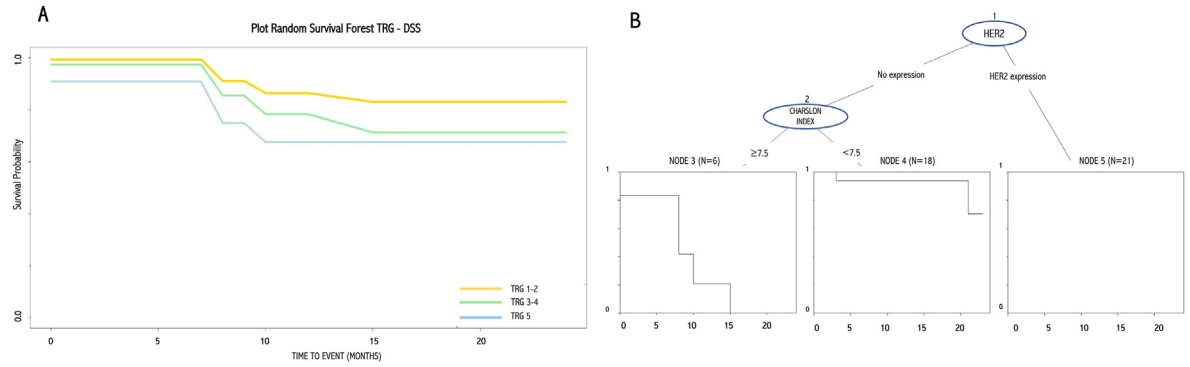

Fig. 5. Random Survival Forest analysis: **A.** Disease Specific Survival (DSS); **B.** Root analysis of the Disease Specific Survival.

patients [28,29], whereas others reported a statistically significant association between MSI-H and poor histological response [30].

In line with our results, HER2 expression was documented as correlated with improved regression grades also by past authors [31], and in our series this was one of the most influent features on response to treatment and on survival.

Consistently with past findings, TRG category had a strong impact on survival in our series, although there has been some controversy on the long-term significance of a pathologic regression [[25,27,32–38]].

Limits of our research were the relatively small cohort analyzed and the lack of other molecular features which could help in better describing the profile of patients presenting tumor regression. Moreover, there was heterogeneity due to the mixed schemes of pre-operative treatments performed before surgery, however, we focused more on the effects of these treatments from a clinical point of view, rather than comparing different schemes.

Among its strengths is the complex statistical work-out used to elaborate data, in particular in relation to PCA and RFS. PCA is a method to reduce the dimension of large set of variables to a small set containing most of the information. The aim of the PCA method is to summarize, extrapolate and analyze the total (common and unique) variance among variables. During this process PC scores are generated, these scores are derived from each case (row) on each factor (column) (as seen in Supplementary Table 3). On the other hand, a RFS [39] focuses on regression and classification problems. RFS was introduced to extend Random Forest (RF) to the setting of right-censored survival data. The implementation of RSF follows the same principals as RF: in brief, survival trees are generated using bootstrapped data, random feature selection is used when splitting tree nodes, trees are grown deeply, and last, survival forest ensemble us calculated by averaging terminal node statistics."

On the other hand, the added value of this research is that we aimed to provide a clinical fingerprint of patients presenting response to pre-operative treatment. Our results are consistent with the idea that patients would benefit of the corrections of all modifiable risk factors before pre-operative and surgical treatments. It's possible that this correction could balance other non-modifiable factors, as molecular and pathologic features, and it can result in greater rates of tumors' regression and improved survivals.

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Ethical compliance

All procedures performed in studies involving human participants were in accordance with the ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The research protocol has been notified to the Institutional IRB.

Data access statement

Research data supporting this publication are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Alberto Biondi: Data curation, Writing – original draft, preparation. **Laura Lorenzon:** Conceptualization, Methodology, Formal analysis. **Gloria Santoro:** Methodology, Formal analysis. **Annamaria Agnes:** Literature review, Writing – original draft, preparation. **Antonio Laurino:** Data collection, litterature review. **Roberto Persiani:** Writing – review & editing. **Domenico D'Ugo:** Supervision, Data interpretation, Validation.

Declaration of competing interest

None of the authors has any relevant potential financial conflicts of interest related to this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejso.2023.06.021.

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