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Mariana Brochado Pinto e Moreira Fernandes

O impacto do número de gânglios linfáticos retirados na cirurgia no prognóstico de doentes com cancro gástrico / Impact of the number of lymph nodes harvested during surgery in gastric cancer patients prognosis

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NOME

Mariana Brochado Pinto e Moreira Fernandes

NÚMERO DE ESTUDANTE	E-MAIL
201104866	marianabpmfernandes@gmail.com

DESIGNAÇÃO DA ÁREA DO PROJECTO

Cirurgia Geral

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O impacto do número de gânglios linfáticos retirados na cirurgia no prognóstico de doentes com cancro gástrico / Impact of the number of lymph nodes harvested during surgery in gastric cancer patients prognosis

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Aos meus pais, à Ana e ao Ricardo
por acreditarem em mim mesmo
quando eu não acredito.

Impact of the number of lymph nodes harvested during surgery in gastric cancer patients prognosis

M. Fernandes [1]; V. Devezas, MD MSc [1,2]; B. Caldeira, MD MSc [2], J. Barbosa, MD PhD [1,2]; J. Costa-Maia, MD FACS FEBS [2]; H. Santos-Sousa, MD MSc PhD(c) [1,2]

[1] Faculty of Medicine, University of Porto, Porto, Portugal

[2] Department of Surgery, Centro Hospitalar São João, Porto, Portugal

Corresponding author:
Dr. Hugo Santos-Sousa
Department of Surgery,
Centro Hospitalar São João / Faculty of Medicine, University of Porto
Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal
+351.966933583
h.santos.sousa@gmail.com

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Abstract

Background: The number of lymph nodes (LN) retrieved could be an important prognostic factor in gastric cancer. Currently, the established cut-off for adequate staging is 15 LN, however this seems not always appropriate to improve survival. The aim of our study is to evaluate the effect of the number of LN harvested on gastric cancer prognosis.

Patients and methods: A retrospective analysis of a prospective database with 476 gastric cancer cases submitted to curative intent surgery, between January 2010 and December 2015, in an Upper GI Surgery Unit. We analyzed 288 patients that met the inclusion criteria for this study. Overall survival (OS), disease specific survival (DSS) and disease free survival (DFS) curves according to the number of LN retrieved were calculated and adjusted to pathological stage, type of tumor (EGC vs advanced) and pN.

Results: Most patients (86,1%) had more than 15 lymph nodes retrieved. Harvesting more than 15 LN had positive effects on the OS of patients with pStage I tumors, early gastric cancer and pN negative tumors. Retrieving more than 15 LN contributed to improvements in DSS and DFS of patients with pStage II. When more than 30 LN were retrieved the OS of gastric cancer patients was significantly better not only in pStage I tumors, early gastric cancer and pN-, but also in pStage III. Harvesting more than 30 LN was also associated with better DSS and DFS in pStage II gastric cancer patients. The number of LN retrieved was not associated with more morbidity, but slightly increased the surgery duration. The independent predictors for the number of LN retrieved were type of resection, type of lymphadenectomy, tumor location and number of LN invaded.

Conclusions: The number of LN harvested appears to have prognostic impact on gastric cancer survival and lymphadenectomy extension should be individually designed for each patient according to tumor stage.

Keywords

Gastric cancer, lymph nodes retrieved, prognosis, recurrence, TNM stage

Introduction

Gastric cancer is the fourth most frequent cancer in the world, with approximately one million people newly diagnosed per year, and the second leading cause of cancer-related death both in men and women [1]. It is particularly common in Asian countries where almost 75% of the cases occur**.** Despite a substantially decline of gastric cancer incidence and mortality worldwide, Portugal is still one of the European countries with highest mortality rates, perhaps as a result of the high prevalence of Helicobacter pylori infection [2].

Surgical resection combined with appropriate lymphadenectomy is the main treatment for patients with gastric cancer [3, 4]. Many features influence the prognosis after surgery. Besides depth of invasion, the number of lymph nodes (LN) metastasis is the most important prognostic factor for gastric cancer and has been considered a staging parameter in the Union for International Cancer Control/ American Joint Committee on Cancer (UICC/AJCC) [5, 6]. The examination of at least 15 lymph nodes has been traditionally recommended by AJCC to optimize staging, however the ideal number of LN that need to be examined in order to achieve a reliable staging for the strongest prognostic value is not certain [4-7].

The proper extent of lymphadenectomy during gastric cancer surgery has been the subject of a long discussion. In Asia, D2 lymphadenectomy has been considered the gold standard with improvements in the long-term survival rates [7]. Recently the Dutch trial also demonstrate that D2 dissection (without routine pancreatectomy and splenectomy) is associated with lower locoregional recurrence and disease-related death when compared to D1 lymphadenectomy [8]. It seems logical that a more extensive node dissection will harvest more LN to be examined pathologically, consequently increasing the staging accuracy [7]. However, the contribution of a higher number of LN harvested in the improvement of local regional disease control and survival has not been consistently established [7].

Recently the notion that negative lymph nodes (NLN) counts as a possible prognostic factor in gastric cancer is emerging. Although patients with NLN

gastric cancer have a superior overall survival rate when compared to patients with positive LN, some will have recurrence and 15% will die of the disease [9, 10]. However, little is known about the optimal number of examined LN in patients with NLN gastric cancer.

The aim of this study is to evaluate if the number of LN harvested during surgery of gastric cancer patients is related with better long-term survival rates in patients with positive LN and NLN, if the threshold of 15 LN required by the AJCC remains appropriate and if patients with different TNM stage would benefit from a different number of retrieved LN.

Patients and methods

Patient sample

This study retrospectively analyzed a prospective database of gastric cancer patients (n=476), who underwent surgical treatment in Upper GI Surgery Unit of Centro Hospitalar de São João – Faculty of Medicine, University of Porto (Porto, Portugal), between January 2010 and December 2015. The Institutional Review Board approved this study (CES 60-16).

Patients were selected from the database according to the following criteria: gastric adenocarcinoma and curative intent surgery. The exclusion criteria were: non-resectional surgery, palliative, pathological stage IV carcinomas, prophylactic and completion gastrectomies, atypical resections, other histologic types than adenocarcinoma and R2 resections. Patients lost for follow-up were not included in this analysis. After screening patients with the above criteria, a total of 288 cases were obtained (**Figure 1**).

Data collection

With the purpose of characterizing the population the following parameters were collected from each patient: age at time of surgery, gender, presence of comorbidities, American Society of Anesthesiologists (ASA) physical status classification and body mass index (BMI) score. In order to describe the clinicopathological profile: tumor location and size (cm), macroscopic appearance, histologic type (Laurén classification), growth pattern (Ming classification), TNM staging (7th edition, 2010), LN ratio and venous, perineural and lymphatic invasions were evaluated. The surgery approach, type of resection and lymphadenectomy and the presence of neoadjuvant therapy were used to characterize the therapeutic approach.

For short-term complications analysis, the following parameters were evaluated: post-operative morbidity (less than 30 days after surgery), including anastomotic leakage rate and Clavien-Dindo classification (specifically the need for intervention, Clavien ≥ IIIa), post-operative mortality, re-intervention, transfusion and readmission rates. Long-term complications (more than 30

days after surgery) presence and need for re-intervention were assessed. Some perioperative outcomes were categorized: surgery duration (minutes) and post-operative length-of-stay (LOS) in days.

The oncological-related outcomes were: type of resection (R), distance (cm) to proximal and distal margin on tumor specimen, number of lymph nodes invaded and recurrence rate and type. Resection was defined as curative (R0) when the tumor was completely removed with all the margins negative; incomplete resection was defined as residual gross disease (R2) or positive surgical margins [(R1), tumor less than 1 mm from any margin].

Clinical, radiologic, or endoscopic signs of disease were used in order to diagnose recurrence.

In survival analysis, overall survival (OS), disease-specific survival (DSS) and disease-free survival (DFS) were assessed. OS was defined as the period between the day of surgery and death of patient. Patients who had survived until the end of the observation period were censored at their last follow-up visit. Death from a cause other than gastric cancer was a censoring event in DSS. DFS was considered the period between the end of primary therapy and the first evidence of disease recurrence. Patient follow-up was completed in March 2017 for 100% of the population. Median follow-up was 28 (0-83) months.

All the data was reviewed by two of the authors (HSS, MF) for identification of data entry errors.

Perioperative management and surgical procedure

All the patients were submitted to upper endoscopy with biopsy and computerized tomography (CT) as diagnostic procedures for gastric cancer. The tumors were staged according to clinical, radiological (CT) or endoscopic (endoscopic ultrasonography) features. Staging laparoscopy was applied when considered necessary (mostly in local advanced tumors, when resectability was uncertain).

The preoperative clinical stage was used to select the surgical approach and the type of lymphadenectomy. In diffuse and proximally located tumors the

surgery performed was total gastrectomy with Roux-en-Y reconstruction. In distally located tumors the surgery selected was subtotal distal gastrectomy, using Billroth II and sporadically Roux-en-Y reconstructions, due to age and comorbidities. The extent of lymphadenectomy was classified based on the third version of Japanese Gastric Treatment Guidelines, 2010 (**Figure 2**).

In the postoperative period, all patients went to the post-surgical intensive care unit for post-operatively early extubation, pain control, vigorous respiratory therapy, early mobilization and ambulation. According to clinical evolution, food intake was permitted. During the first year after surgery the patients were followed up at 3-month intervals, during the second year at 6-month intervals and annually afterwards.

Statistical analysis

Statistical analysis was performed using SPSS® 21.0 for Mac (IBM Co., Armonk, NY, USA).

Normal distribution of continuous variables was assessed by visual analysis of histograms, normal Q-Q plots and both Kolmogorov-Smirnov and Shapiro-Wilk tests of normality. Student's t-test, non-parametric test Mann-Whitney U and Welch's t-test were used to compare means, and chi-square or Fisher's exact test were used to compare proportions, as appropriate. Odds ratio (OR) and 95% confidence intervals (CI) were calculated by logistic regression for the complications analysis. Linear regression was used for the analysis of the impact of the LN retrieved in surgery duration and for the predictors of the number of LN harvested.

Significance was assumed for *p* values inferior to 0.05. All *p* values given are results of 2-sided tests.

Cumulative survival curves for OS, DSS and DFS were calculated by Kaplan-Meier (KM) method and adjusted to pathological stage, type of gastric cancer (early gastric cancer versus advanced gastric cancer) and N stage. Log rank test was used to assess differences between groups. Hazard ratio (HR) and 95% confidence intervals (CI) were calculated by Cox regression and adjusted for possible confounders for the survival analysis. In order to define a better cut-

off for the number of LN retrieved, a new category was created based on the median (**Figure 3**) of the LN harvested in patients with a minimum number for an adequate staging (≥15 LN).

Results

Population baseline characteristics

Population baseline characteristics are described in **Table 1**. The average age at surgery was 66,94 ± 12,234 years and there was a predominance of male patients (58%). The mean body mass index was 25,852 ± 3,8102. Comorbidities were present in 82,3% of the patients and 84,7% were classified as ASA score II or III.

Most gastric cancers (67,7%) were located in the antrum and presented with a mean size of 4,444 ± 2,9584 cm. Based on Laurén classification the most frequent histologic type was intestinal (45%), and according to Ming classification the most common growth pattern was infiltrative (73,1%). Around 60% of the cases were advanced gastric cancers. The lymph node ratio was superior to 0,2 in 24%. Most patients had stage I cancer (45,1%), followed by stage III (31,6%) and stage II (23,3%). The most common surgery approach (54,5%) was open gastrectomy and the most frequent type of resection was Billroth II distal gastrectomy (54,9%), followed by total gastrectomy (38,2%) and Roux-en-Y distal gastrectomy (6,9%). D1+ and D2 lymphadenectomy were performed in 75% of the patients. Neoadjuvant therapy was applied in 9% of the cases.

Regarding the survival, mean OS was 52,043 ± 2,122 months. The 1-year, 2-year and 5-year OS were 81%, 71% and 51%, respectively. The mean DFS was 62,702 ± 2,079 months and the 1-year, 2-year and 5-year DFS were 86%, 77% and 70%, respectively. There were significant differences in OS according to pStage ($p<0,001$), EGC ($p<0,001$) and pN ($p<0,001$) (**Figure 4**). In DFS there were also significant differences according to pStage ($p<0,001$), EGC ($p<0,001$) and pN ($p<0,001$).

Comparative analysis <15 LN versus ≥15 LN retrieved

Most patients (86,1%) had ≥15 LN retrieved and only a minority (13,9%) had <15 LN retrieved. The patients were grouped by the number of examined LN

(<15 and ≥ 15) and clinical and pathologic characteristics were compared (**Table 1**).

In the group with <15 LN retrieved the patients had more comorbidities (95 versus 80,2%, *p*=0,024) and, although not statistically significant (*p*=0,077), it was observed that patients with highest ASA scores (III and IV) had an inferior number of LN retrieved. The group with ≥ 15 LN retrieved was significantly (*p*= 0,004) associated with more proximal and extensive tumor location (body – 31 versus 5%, fundus – 1,6 versus 0% and extensive – 3,6 versus 2,5%), while the <15 LN retrieved group was related to inferior tumor size (3,338 ± 3,2825 versus 4,611 ± 2,8768, *p*=0,001). The group with ≥ 15 LN retrieved correlates with more advanced gastric cancers (64,1 versus 42,5%, *p*=0,014), whereas the <15 LN retrieved group was associated with lower N stages (N0 – 72,5 versus 49,6% and N1 – 17,5 versus 15,7%, *p*=0,031), pathological stage I (70 versus 41,1%, *p*=0,003) and less cases of lymphatic permeation and perineural invasion (34,2 versus 59,5%, *p*=0,005 and 21,1 versus 39,9%, *p*=0,03 respectively). In what concerns therapeutic approach, in the group with ≥ 15 LN retrieved more total gastrectomies (42,3 versus 12,5%, *p*<0,001) and more D2 lymphadenectomies (38,3 versus 22,5%, *p*=0,016) were performed. In fact, in 91,3% of D2 lymphadenectomies more than or equal to 15 LN were harvested.

Patients with ≥15 LN harvested did not present significant differences in short and long-term complications when compared to patients with <15 LN retrieved (**Table 2**).

Related to perioperative outcomes (**Table 2**), though not statistically significant, the surgery duration was superior in the group with ≥ 15 LN retrieved (229,52 ± 51,991 versus 208,8 ± 72,006, *p*= 0,087).

Both groups presented similar oncological outcomes (**Table 2**)., except for the number of LN invaded which was significantly higher in the group with ≥ 15 LN retrieved (4,04 ± 6,996 versus 0,75 ± 1,765, *p*=0,001).

Survival analysis

Overall survival (OS)

Analysis of the effect of the number of LN retrieved

There was not an association (crude HR 0,986, 95% CI 0,971 – 1,001, *p*=0,072) between the number of LN retrieved and the OS. But when OS was adjusted to pathological stage, type of cancer (EGC versus AGC) and N stage, there was statistically significant association with the number of LN harvested (HR 0,97. 95% CI 0,954 – 0,986, *p*<0,001; HR 0,976, 95% CI 0,961 – 0,992, *p*=0,003; and HR 0,981, 95% CI 0,966 – 0,996, *p*=0,015, respectively) **(Table 3)**.

Comparative analysis < 15 LN *versus* ≥15 LN retrieved

The OS of patients with ≥15 LN retrieved was better than those with < 15 LN, but this difference was not statistically significant (*p*=0,124). When OS curves were adjusted to pathological stage (**Table 3**), the differences were statistically significant (HR 0,436, 95% CI 0,262 – 0,727, *p*=0,001). According to the KM curves, these differences were observed in pathological stage I (*p*<0,001), but not in stages II and III (**Figure 5**). When adjusted to the type of cancer (**Table 3**), the OS curves were statistically significant (HR 0,532, 95% CI 0,324 – 0,875, *p*=0,013). According to the KM curves (**Figure 6**), these differences were observed in early gastric cancer (*p*<0,001). The OS curves were statistically significant (HR 0,535, 95% CI 0,324 – 0,883, *p*=0,014) when adjusted to the N stage (**Table 3**). According to the KM curves (**Figure 6**), these differences were observed in pN- tumors (*p*=0,002).

Comparative analysis < 15 LN *versus* 15 - 29 LN *versus* ≥30 LN retrieved

The OS of patients with 15 to 29 LN retrieved and with ≥30 LN retrieved was not statistically better than that of patients with <15 LN retrieved (*p*=0,65 and *p*=0,21). Although, when adjusted to pathological stage (**Table 3**) the differences were statistically significant in both groups (HR 0,56, 95% CI 0,33 – 0,95, *p*0,032 and HR 0,306, 95% CI 0,172 – 0,544, *p*<0,001, respectively). According to the KM curves, these differences were observed in pathological stage I (*p*=0,001) and III (*p*=0,032), but not in pathological stage II (**Figure 7**). When adjusted to the type of cancer (**Table 3**), the OS curves were statistically significant in the group with ≥30 LN retrieved (HR 0,401, 95% CI 0,229 – 0,699,

p=0,001), when compared to the <15 LN group. According to the KM curves (**Figure 8**), these differences were observed in early tumors (*p*=0,001). The OS curves adjusted to the N stage (**Table 3**) were statistically significant (HR 0,42, 95% CI 0,24 – 0,736, *p*=0,002) in the group of patients with ≥30 LN retrieved when compared to the <15 LN group. According to the KM curves (**Figure 8**), these differences were observed in pN- tumors (*p*=0,003).

Disease specific survival (DSS)

Analysis of the effect of the number of LN retrieved

There was not an association (crude HR 1,005, 95% CI 0,986 – 1,025, *p*=0,585) between the number of LN retrieved and the DSS. When DSS was adjusted to pathological stage the difference was statistically significant (adjusted HR 0,977, 95% CI 0,956 – 0,999, *p*=0,039). However, when DSS was adjusted to the type of cancer and N stage there was no significant differences (*p*=0,479 and *p*=0,712) (**Table 3**).

Comparative analysis < 15 LN *versus* ≥15 LN retrieved

The DSS of patients with ≥15 LN retrieved was not significantly different (*p*=0,824) than that of patients with <15 LN retrieved (**Table 3**). When DSS curves were adjusted to pathological stage the differences were statistically significant in pathological stage II (*p*=0,012) (**Figure 9**). When adjusted to the type of cancer and N stage (**Table 3**), the DSS curves were not statistically significant (*p*=0,462 and *p*=0,341) (**Figure 10**).

Comparative analysis < 15 LN *versus* 15 - 29 LN *versus* ≥30 LN retrieved

The DSS of patients with 15 to 29 LN retrieved and with ≥30 LN retrieved was not statistically better than that of patients with < 15 LN retrieved (*p*=0,416 and *p*=0,227) (**Table 3**). Although, when adjusted to pathological stage the difference was significant (*p*=0,027) in the group with ≥30 LN retrieved, when compared to the <15 LN group. According to the KM curves (**Figure 11**), these differences were observed in pathological stage II (*p*=0,027). When adjusted to

the type of cancer and N stage (**Table 3**), the DSS curves were not statistically significant in both groups (**Figure 12**).

Disease free survival (DFS)

Analysis of the effect of the number of LN retrieved

There was not an association (crude HR 1,005, 95% CI 0,988 – 1,023, *p*=0,566) between the number of LN retrieved and the DFS. When DFS was adjusted to pathological stage the difference was statistically significant (*p*=0,043). Nevertheless, when DFS was adjusted to the type of cancer and N stage there was no significant differences (*p*=0,451 and *p*=0,61) (**Table 3**).

Comparative analysis < 15 LN *versus* ≥15 LN retrieved

The DFS of patients with ≥15 LN retrieved was not significantly different (*p*=0,683) than that of patients with <15 LN retrieved (**Table 3**). When DFS curves were adjusted to pathological stage the differences were statistically significant in pathological stage II (p=0,002) (**Figure 13**). When adjusted to the type of cancer and N stage (**Table 3**), the DFS curves (**Figure 14**) were not statistically significant (*p*=0,488 and *p*=0,344).

Comparative analysis < 15 LN *versus* 15 - 29 LN *versus* ≥30 LN retrieved

The DFS of patients with 15 to 29 LN retrieved and with ≥30 LN retrieved was not statistically better than that of patients with less than 15 LN retrieved (*p*=0,649 and *p*=0,657) (**Table 3**). Although, when adjusted to pathological stage the difference was significant in the group with ≥30 LN retrieved in pathological stage II (*p*=0,002) (**Figure 15**). When adjusted to the type of cancer and N stage (**Table 3**), the DFS curves were not statistically significant in both groups (**Figure 16**).

Impact of the number of LN retrieved in surgery duration and morbidity

There was a significant association between the number of LN retrieved and the

surgery duration (standardized beta coefficient 0,148, 95% CI 0,141 – 1,134, *p*=0,012), but the number of LN retrieved only explains 2,2% of the surgery duration (R^2=0,022). In fact, each LN retrieved increased the surgery duration in 0,148 minutes (**Figure 17**).

In terms of morbidity, the number of LN harvested was not significantly (*p*=0,053) associated with the presence of complications (**Table 4**).

Predictors of the number of LN retrieved

In univariate analysis (**Table 5**), the following variables were predictors of the number of LN harvested: age (p<0,001), type of resection (p<0,001), type of lymphadenectomy (p<0,001), tumor location (p<0,001), tumor size (p=0,003), type of tumor [EGC vs advanced] (p=0,015), pN (p=0,037), number of LN invaded (p<0,001) and pStage (p<0,001). According to the multivariate analysis (R^2 0,261 for this model), the independent predictors were: type of resection (p<0,001), type of lymphadenectomy (p<0,001), tumor location (p=0,013) and number of LN invaded (p<0,001) (**Table 5**).

Discussion

Gastric cancer is still a global and important concern nowadays, being the second leading cause of cancer-related death in the world. The notion that the number of lymph nodes harvested acts as an important prognostic factor in gastric cancer is not recent and has been previously described in multiple studies [7, 11-14]. It is also known that various factors can influence the number of lymph nodes retrieved in gastric cancer patients, such as surgical technique, extent of surgery, extent of pathologic examination of the specimen and individual characteristics like fat volume and innate number of lymph nodes [5, 15].

Currently the cut-off established by AJCC for adequate staging is 15 lymph nodes, however this seems not always appropriate to increase survival, reduce recurrence and prevent stage migration. Numerous studies have suggested different cut-off values, but the ideal one remains unclear.

Data et al [16] observed that harvesting less than 15 LN was independently related to worst OS. Ling et al [17] demonstrated that patients with more than or equal to 20 LN retrieved had an improvement on the survival rate when compared with patients with less than 20 lymph nodes. Shen et al [13] showed that patients with more than 30 lymph nodes resected had significantly better OS and progression free survival (PFS) than those with less than or equal to 14 lymph nodes retrieved. However, there was no significant difference in OS and PFS between patients with less than 14 and 15 to 29 lymph nodes retrieved. This study also revealed that harvesting more than 30 lymph nodes was associated with better OS than harvesting 15 to 29 lymph nodes, suggesting that dissecting a superior number of lymph nodes probably contributes to improve survival in gastric cancer patients. Our data showed that, when the number of lymph nodes retrieved was analyzed as a continuous variable and adjusted to pathological stage, patients with a superior number of lymph nodes harvested presented with a better survival and reduce disease recurrence. Harvesting more than or equal to 15 lymph nodes had positive effects on the OS of gastric cancer patients with pathological stage I, early gastric cancer and negative N stage (pN-), but not in more advanced stage patients. Moreover,

retrieving more than or equal to 15 lymph nodes contributed to improvements in DSS and DFS of patients in pathological stage II. This information is congruent with other studies. Son et al [17] concluded that the outcomes of T1N0 stage patients in whom the number of examined lymph nodes was less than 15 were worse than patients in whom more than 15 lymph nodes were examined. Zhao et al [6] revealed that a lymphadenectomy with dissection of more than 15 lymph nodes might improve the long-term survival of patients with pT1N0 gastric cancer. All together this data suggests that patients with less advanced stages of gastric cancer may benefit from a more extensive lymphadenectomy in terms of survival. The cause for this association is not clear but it can be due to superior loco-regional disease control. As the number of lymph nodes harvested increases, the probability of retrieving positive lymph nodes or lymph nodes with micrometastases rises, resulting in a more accurate N stage classification and higher survival rates [18, 19]. However, these findings were not showed in other studies. Shen et al [13] concluded that the number of lymph nodes harvested had no effect on both OS and PFS of gastric cancer patients with pathological stage I and II, N0, T1 and T2. Instead they find that the higher the number of lymph nodes retrieved, the better was the OS and PFS in T3 to T4 gastric cancer, N+ and III to IV stages and, specifically when more than or equal to 15 lymph nodes were harvested, the OS and PFS were significantly better in N+ patients. Deng et al [12] also demonstrated that harvesting more than or equal to 15 lymph nodes was associated with better prognosis of N+ gastric cancer. Gholami et al [7] showed that survival after gastrectomy was improved when 16 lymph nodes or more were removed in all pathological stages, except advanced stage (III-B e III-C or N3).

In this study, we also showed that when more than 30 lymph nodes were retrieved the OS of gastric cancer patients was significantly better not only in stage I, early gastric cancer and N-, but also in stage III. Furthermore, harvesting more than 30 lymph nodes was associated with better DSS and DFS in pathological stage II gastric cancer patients. The findings are similar to those of other studies. Siewert et al [20] determined that harvesting more than 25 lymph nodes had a significant and independent effect on survival in patients with stage II tumors. Shen et al [13] demonstrated that retrieving more than 30

lymph nodes was associated with better OS than harvesting 15 to 29 lymph nodes and is highly recommended for patients with advanced stages (T3 to T4, N+ and III to IV stages). Based on our results it seems that in pathological stage III tumors the established cut-off of 15 lymph nodes may not be appropriate. Our data suggests that lymphadenectomy should be individually designed for each patient according to tumor stage.

In the past, many western studies reported that D2 lymphadenectomy was associated with higher rates of perioperative mortality and surgical complication [21-23]. However, more recent western studies demonstrated that D1 and D2 lymphadenectomy have similar perioperative mortality [20, 24, 25]. Furthermore Gholami et al [7] revealed that perioperative mortality between patients with 7 to 15 lymph nodes and more than 16 lymph nodes retrieved was similar (4,7% versus 3,1% respectively). These results support our findings, since we showed that harvesting more lymph nodes was not associated with higher rates of post-surgical complications.

In conclusion, our results suggest that the number of lymph nodes harvested has prognostic implication on gastric cancer patients survival and that retrieving a higher number of lymph nodes is not associated with more complications. Therefore, lymphadenectomy extension should be individually designed for each patient according to tumor stage.

Disclosure

The authors have declared no conflicts of interest.

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Comparative analysis <15 LN versus ≥15 LN retrieved: Baseline characteristics				
	< 15 LN retrieved (n=40)	**≥15 LN retrieved (n=248)**	**Total**	**P value**
Demographics				
Age at surgery (years), mean ± SD	69,93 ± 10,885	66,46 ± 12,39	66,94 ± 12,234	ns (0,105)*
Gender, n (%)				ns (0,864)
Male	24 (60)	143 (57,7)	167 (58)	
Female	16(40)	105 (42,3)	121 (42)	
BMI, mean ± SD	26,196 ± 3,0304	25,805 ± 3,9092	25,852 ± 3,8102	ns (0,509)*
Comorbidities presence, n (%)	38 (95)	199 (80,2)	237 (82,3)	**0,024**
ASA score, n (%)				ns (0,077)
I	2 (5)	38 (15,3)	40 (13,9)	
II	19 (47,5)	138 (55,6)	157 (54,5)	
III	18 (45)	69 (27,8)	87 (30,2)	
IV	1 (2,5)	3 (1,2)	4 (1,4)	
Clinico-pathological profile				
Tumor location, n (%)				**0,004**
Antrum	37 (92,5)	158 (63,7)	195 (67,7)	
Body	2 (5)	77 (31)	79 (27,4)	
Fundus	0 (0)	4 (1,6)	4 (1,4)	
Extensive	1 (2,5)	9 (3,6)	10 (3,5)	
Tumor size (cm), mean ± SD	3,338 ± 3,2825	4,611 ± 2,8768	4,444 ± 2,9584	**0,001****
Mascroscopic appearance, n (%)				ns (0,095)
Fungating	11 (33,3)	34 (14,5)	45 (16,9)	
Ulcerated	9 (27,3)	67 (28,6)	76 (28,5)	
Infiltrative	2 (6,1)	29 (12,4)	31 (11,6)	
Ulcero-fungating	3 (9,1)	29 (12,4)	32 (12)	
Ulcero-infiltrative	8 (24,2)	75 (32,1)	83 (31,1)	
Histologic type (Laurén), n (%)				ns (0,121)
Intestinal	24 (63,2)	102 (42,1)	126 (45)	
Diffuse	2 (5,3)	35 (14,5)	37 (13,2)	
Unclassified of solid structure	0 (0)	8 (3,3)	8 (2,9)	
Unclassified of mixed structure	9 (23,7)	68 (28,1)	77 (27,5)	
Unclassified SOE	3 (7,9)	29 (12)	32 (11,4)	
Growth pattern (Ming), n (%)				ns (0,931)
Expansive	7 (18,9)	51 (21,1)	58 (20,8)	
Infiltrative	28 (75,7)	176 (72,7)	204 (73,1)	
Unclassified	2 (5,4)	15 (6,2)	17 (6,1)	
pT (7th ed, 2010), n (%)				ns (0,120)
pTis	1 (2,5)	5 (2)	6 (2,1)	
pT1a	10 (25)	42 (16,9)	52 (18,1)	
pT1b	11 (27,5)	41 (16,5)	52 (18,1)	
pT2	6 (15)	31 (12,5)	37 (12,8)	
pT3	5 (12,5)	82 (33,1)	87 (30,2)	
pT4a	6 (15)	42 (16,9)	48 (16,7)	
pT4b	0 (0)	4 (1,6)	4 (1,4)	
pTx	1 (2,5)	1 (0,4)	2 (0,7)	
Type of tumor, n (%)				**0,014**
Early gastric cancer	23 (57,5)	89 (35,9)	112 (38,9)	
Advanced gastric cancer	17 (42,5)	159 (64,1)	176 (61,1)	
pN (7th ed, 2010), n (%)				**0,031**
N0	29 (72,5)	123 (49,6)	152 (52,8)	
N1	7 (17,5)	39 (15,7)	46 (16)	
N2	2 (5)	28 (11,3)	30 (10,4)	
N3a	2 (5)	39 (15,7)	41 (14,2)	
N3b	0 (0)	19 (7,7)	19 (6,6)	
LN ratio, n (%)				ns (0,074)
≤ 0,2	35 (87,5)	184 (74,2)	219 (76,0)	
> 0,2	5 (12,5)	64 (25,8)	69 (24)	
Pathological stage, n (%)				**0,003**
I	28 (70)	102 (41,1)	130 (45,1)	
II	6 (15)	61 (24,6)	67 (23,3)	
III	6 (15)	85 (34,3)	91 (31,6)	
Lymphatic permeation, n (%)	13 (34,2)	144 (59,5)	157 (56,1)	**0,005**
Venous invasion, n (%)	14 (36,8)	113 (46,5)	127 (45,2)	ns (0,297)
Perineural invasion, n (%)	8 (21,1)	97 (39,9)	105 (37,4)	**0,03**

Therapeutic approach				
Surgery approach				ns (0,651)
Open	23 (57,5)	134 (54)	157 (54,5)	
Laparoscopic	15 (37,5)	107 (43,1)	122 (42,4)	
Converted	2 (5)	7 (2,8)	9 (3,1)	
Type of resection, n (%)				**<0,001**
Total gastrectomy	5 (12,5)	105 (42,3)	110 (38,2)	
Billroth II Distal Gastrectomy	34 (85)	124 (50)	158 (54,9)	
Roux-en-Y Distal Gastrectomy	1 (2,5)	19 (7,7)	20 (6,9)	
Type of lymphadenectomy, n (%)				**0,016**
D1	17 (42,5)	55 (22,2)	72 (25)	
D1+	14 (35)	98 (39,5)	112 (38,9)	
D2	9 (22,5)	95 (38,3)	104 (36,1)	
Neoadjuvant therapy, n (%)	5 (12,5)	21 (8,5)	26 (9,0)	ns (0,38)

Table 1: Comparative analysis <15 LN *versus* ≥15 LN retrieved: Baseline characteristics (n=288).
SD, *standard deviation*; ns, *non-significant*; BMI, *body mass index*; ASA, *American Society of Anesthesiologists*.
*Student's t-test; ** Non-parametric test Mann Whitney U.
Significant p values (<0,05) and strong trend p values (<0,1) are highlighted in bold.

Outcomes				
	< 15 LN retrieved (n=40)	**≥15 LN retrieved (n=248)**	**Total**	***P* value**
Short-term complications				
Morbidity, n(%)	11 (27,5)	50 (20,2)	61 (21,2)	ns (0,3)
Anastomotic leakage	3 (7,5)	5 (2)	8 (2,8)	ns (0,085)
Clavien classification, n(%)				ns (0,448)
I	0 (0)	1(2)	1 (1,6)	
II	7 (58,3)	20 (40)	27 (43,5)	
IIIa	0 (0)	7 (14)	7 (11,3)	
IIIb	1 (8,3)	12 (24)	13 (21)	
IVa	0 (0)	1 (2)	1 (1,6)	
IVb	0 (0)	1 (2)	1 (1,6)	
V	4 (33,3)	8 (16)	12 (19,4)	
Clavien ≥ IIIa (need for intervention)	5 (45,5)	30 (58,8)	35 (56,5)	
Post-operative mortality, n(%)	4 (10)	8 (3,2)	12 (4,2)	ns (0,069)
Reintervention, n(%)	4 (10)	21 (8,5)	25 (8,7)	ns (0,762)
Readmission, n(%)	2 (5)	17 (6,9)	19 (6,6)	ns (1)
Transfusion presence, n(%)	4 (11,1)	33 (15,9)	37 (15,2)	ns (0,617)
Long-term complications				
Long-term complications, n(%)	0 (0)	16 (6,5)	16 (5,6)	ns (0,14)
Reintervention, n(%)	0 (0)	5 (2)	5 (1,7)	ns (1)
Perioperative outcomes				
Surgery duration (minutes), mean ± SD	208,8 ± 72,006	229,52 ± 51,991	226,64 ± 55,520	ns (0,087)**
Post-operative LOS (days), mean ± SD	13,25 ± 17,137	10,92 ± 16,408	11,24 ± 16,501	ns (0,414)*
Oncological related outcomes				
R classification, n (%)				ns (1)
R0	39 (97,5)	239 (96,4)	278 (96,5)	
R1	1 (2,5)	9 (3,6)	10 (3,5)	
Proximal margin distance (cm), mean ± SD	4,568 ± 2,6585	5,410 ± 3,0761	5,296 ± 3,0322	ns (0,124)*
Distal margin distance (cm), mean ± SD	3,689 ± 2,7389	4,4 ± 6,996	4,304 ± 3,1457	ns (0,238)*
Lymph nodes invaded, mean ± SD	0,75 ± 1,765	4,04 ± 6,996	3,59 ± 6,622	**0,001***
Recurrence presence, n (%)	7 (17,5)	62 (25)	69 (24)	ns (0,424)
Recurrence type, n (%)				ns (0,38)
Local	0 (0)	9 (16,1)	9 (14,5)	
Ganglionar	0 (0)	5 (8,9)	5 (8,1)	
Distant metastases	6 (100)	42 (75)	48 (77,4)	

Table 2: Comparative analysis <15 LN *versus* ≥15 LN retrieved: Outcomes (n=288).

SD, *standard deviation*; ns, *non-significant*; LOS, *length of stay*; LN, *lymph node*.

*Non-parametric test Mann Whitney U; **Welch's t-test.

Significant p values (<0,05) and strong trend p values (<0,1) are highlighted in bold.

I. Survival analysis: Cox regression, crude and adjusted HR for pathological stage

	Beta coefficient	Crude HR	95% CI	*P* value	Beta coefficient	pStage Adjusted HR	95% CI	*P* value
Overall survival (OS)								
Number of LN retrieved	-0,014	0,986	0,971 - 1,001	ns (0,072)	-0,031	0,97	0,954 - 0,986	**<0,001**
Minimum number of LN for adequate staging (cut off 15 LN)	-0,386	0,68	0,415 - 1,112	ns (0,124)	-0,83	0,436	0,262 - 0.727	**0,001**
LN retrieved <15		Ref				Ref		
LN retrieved 15-29	-0,115	0.891	0,543 - 1,464	ns (0,65)	-0,579	0,56	0,33 - 0,95	**0,032**
LN retrieved ≥ 30	-0,33	0,719	0,430 - 1,204	ns (0,21)	-1,185	0,306	0,172 - 0,544	**<0,001**
Disease specific survival (DSS)								
Number of LN retrieved	0,005	1,005	0,986 - 1,025	ns (0,585)	-0,023	0,977	0,956 - 0,999	**0,039**
Minimum number of LN for adequate staging (cut off 15 LN)	0,096	1,101	0,472 - 2,567	ns(0,824)	-0,835	0,44	0,183 - 1,028	ns (0,058)
LN retrieved <15		Ref						
LN retrieved 15-29	0,36	1,433	0,602 - 3,408	ns (0,416)	-0,628	0,533	0,217 - 1,311	ns (0,171)
LN retrieved ≥ 30	0,531	1,7	0,718 - 4,022	ns (0,227)	-1,027	0,358	0,144 - 0,889	**0,027**
Disease free survival (DFS)								
Number of LN retrieved	0,005	1,005	0,988 - 1,023	ns (0,566)	-0,021	0,98	0,96 - 0,999	**0,043**
Minimum number of LN for adequate staging (cut off 15 LN)	0,188	1,207	0,552 - 2,637	ns (0,638)	-0,602	0,548	0,248 - 1,209	ns (0,136)
LN retrieved <15		Ref				Ref		
LN retrieved 15-29	0,189	1,208	0,534 - 2,732	ns (0,649)	-0,409	0,664	0,292 - 1,513	ns (0,330)
LN retrieved ≥ 30	0,186	1,205	0,529 - 2,743	ns (0,657)	-0,793	0,452	0,196 - 1,044	ns (0,063)

II. Survival analysis: Cox regression, crude and adjusted HR for EGC and pN

	Beta coefficient	EGC Adjusted HR	95% CI	*P* value	Beta coefficient	pN Adjusted HR	95% CI	*P* value
Overall survival (OS)								
Number of LN retrieved	-0,024	0,976	0,961 - 0,992	**0,003**	-0,019	0,981	0,966 - 0,996	**0,015**
Minimum number of LN for adequate staging (cut off 15 LN)	-0,63	0,532	0,324 - 0,875	**0,013**	-0,625	0,535	0,324 - 0,883	**0,014**
LN retrieved <15		Ref				Ref		
LN retrieved 15-29	-0,41	0,664	0,395 - 1,116	ns (0,122)	-0,442	0,643	0,381 - 1,085	ns (0,098)
LN retrieved ≥ 30	-0,915	0,401	0,229 - 0,699	**0,001**	-0,867	0,42	0,24 - 0,736	**0,002**
Disease specific survival (DSS)								
Number of LN retrieved	-0,008	0,992	0,972 - 1,014	ns (0,479)	-0,004	0,996	0,976 - 1,017	ns (0,712)
Minimum number of LN for adequate staging (cut off 15 LN)	-0,318	0,728	0,312 - 1,698	ns (0,462)	-0,415	0,66	0,281 - 1,553	ns (0,341)
LN retrieved <15		Ref				Ref		
LN retrieved 15-29	-0,232	0,793	0,326 - 1,928	ns (0,608)	-0,384	0,681	0,278 - 1,670	ns (0,402)
LN retrieved ≥ 30	-0,4	0,67	0,274 - 1,636	ns (0,380)	-0,448	0,639	0,259 - 1,573	ns (0,330)
Disease free survival (DFS)								
Number of LN retrieved	-0,007	0,993	0,974 - 1,012	ns (0,451)	-0,005	0,995	0,977 - 1,014	ns (0,61)
Minimum number of LN for adequate staging (cut off 15 LN)	-0,278	0,758	0,346 - 1,661	ns (0,488)	-0,382	0,683	0,310 - 1,504	ns (0,344)
LN retrieved <15		Ref				Ref		
LN retrieved 15-29	-0,191	0,826	0,364 - 1,877	ns (0,649)	-0,335	0,716	0,314 - 1,632	ns (0,426)
LN retrieved ≥ 30	-0,365	0,694	0,304 - 1,588	ns (0,387)	-0,431	0,65	0,283 - 1,494	ns (0,31)

Table 3: Survival analysis: Cox regression
I. Crude and adjusted HR for pathological stage
II. Adjusted HR for EGC and pN
HR, *hazard ratio*; CI, *confidence interval*; EGC, *early gastric cancer*.

Impact of the number of LN retrieved in morbidity: Logistic regression				
	Beta coefficient	**Crude OR**	**95% CI**	***P* value**
Complications				
Number of LN retrieved	-0,024	0,976	0,953 - 1	ns (0,053)

Table 4: Impact of the number of LN retrieved in morbidity: Logistic regression OR, *odds ratio*; CI, *confidence interval*.

Predictors of number of LN retrieved: Linear regression			
	R square	***P* value**	***P* value**
	Univariate analysis		**Multivariate analysis**
Age	0,053	<0,001	
Type of resection	0,11	<0,001	<0,001
Type of lymphadenectomy	0,097	<0,001	<0,001
Tumor location	0,053	<0,001	0,013
Tumor size	0,032	0,003	
Type of tumor (EGC vs Advanc	0,021	0,015	
pN (7th ed, 2010)	0,015	0,037	
Type of tumor (EGC vs Advanc	0,021	0,015	
Number LN invaded	0,083	<0,001	<0,001
Pathological stage	0,042	<0,001	

R square for the model of the multivariate analysis: 0,261

Table 5: Predictors of number of LN retrieved: Uni and multivariate analysis by linear regression

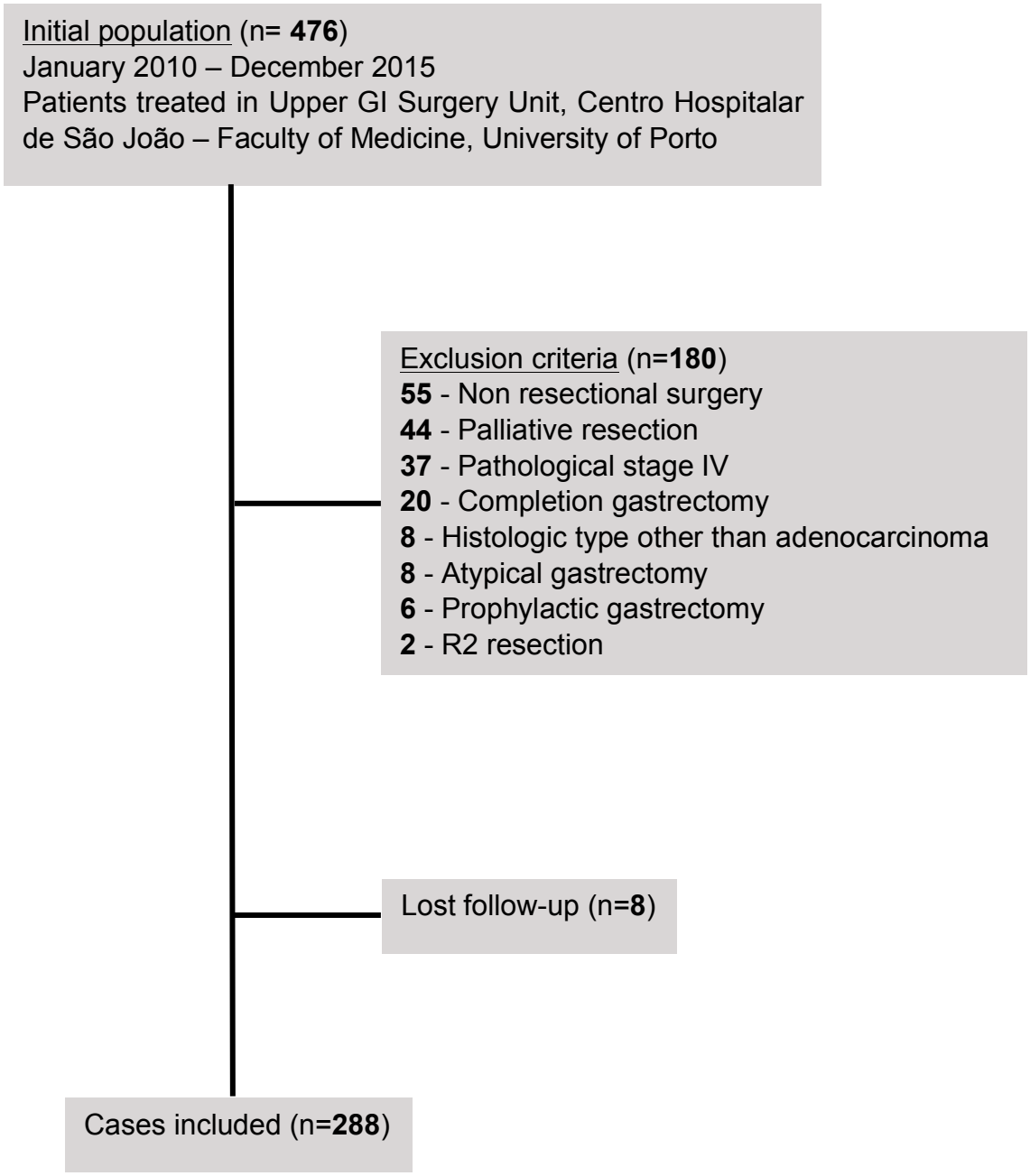

Figure 1: Flow chart of the study design

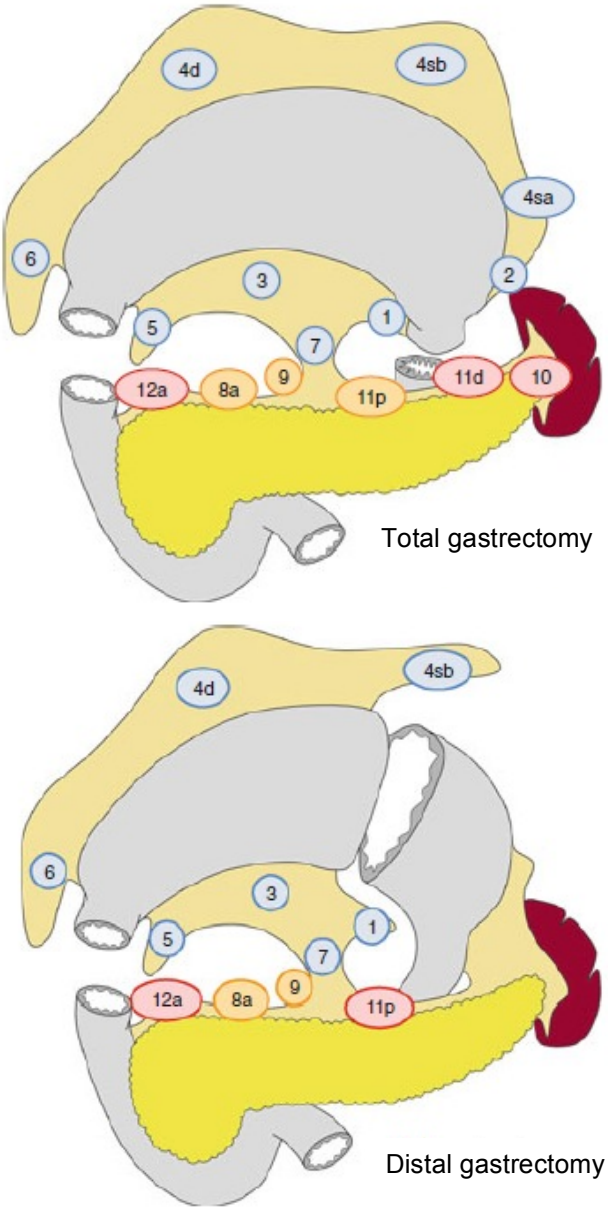

Figure 2: Type of lymphadenectomy according to the type of resection (total or distal gastrectomy) in Japanese Gastric Cancer Association. Japanese gastric cancer treatment guidelines (ver.3). Gastric Cancer- 2011 Jun; 14(2): 113-23.

Total gastrectomy:
D0: Lymphadenectomy less than D1;
D1: Nos. 1–7 (*highlighted in blue*);
D1+: D1 plus Nos. 8a, 9, 11p (*highlighted in yellow*);
D2: D1+ plus Nos. 10, 11d, 12a (*highlighted in red*).

Distal gastrectomy:
D0: Lymphadenectomy less than D1;
D1: Nos. 1, 3, 4sb, 4d, 5, 6, 7 (*highlighted in blue*);
D1+: D1 plus Nos. 8a, 9 (*highlighted in yellow*);
D2: D1+ plus Nos. 11p, 12a (*highlighted in red*).

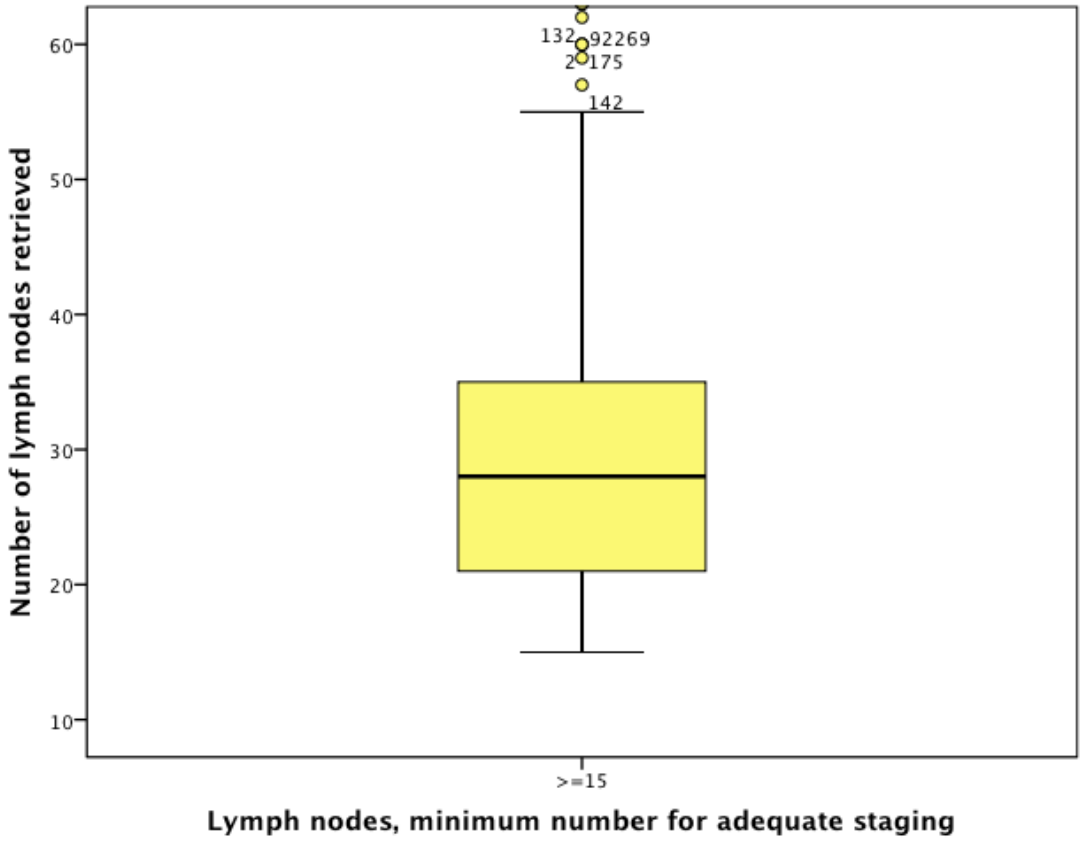

Figure 3: Box plot of the number of lymph nodes retrieved in patients with minimum number for adequate staging (≥15 LN)

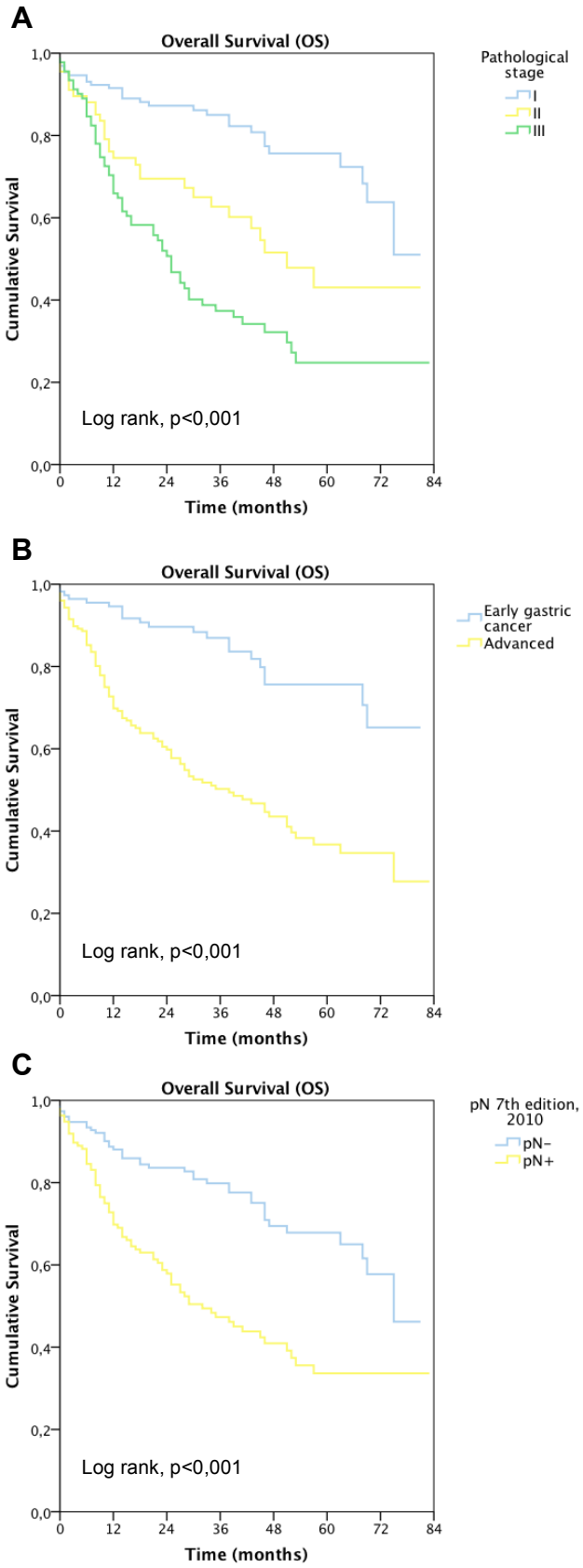

Figure 4: OS of gastric cancer patients according to (A) pathological stage, (B) type of gastric cancer (EGC *versus* AGC) and (C) pN stage.

OS, *overall survival*; EGC, *early gastric cancer*; AGC, *advanced gastric cancer*.

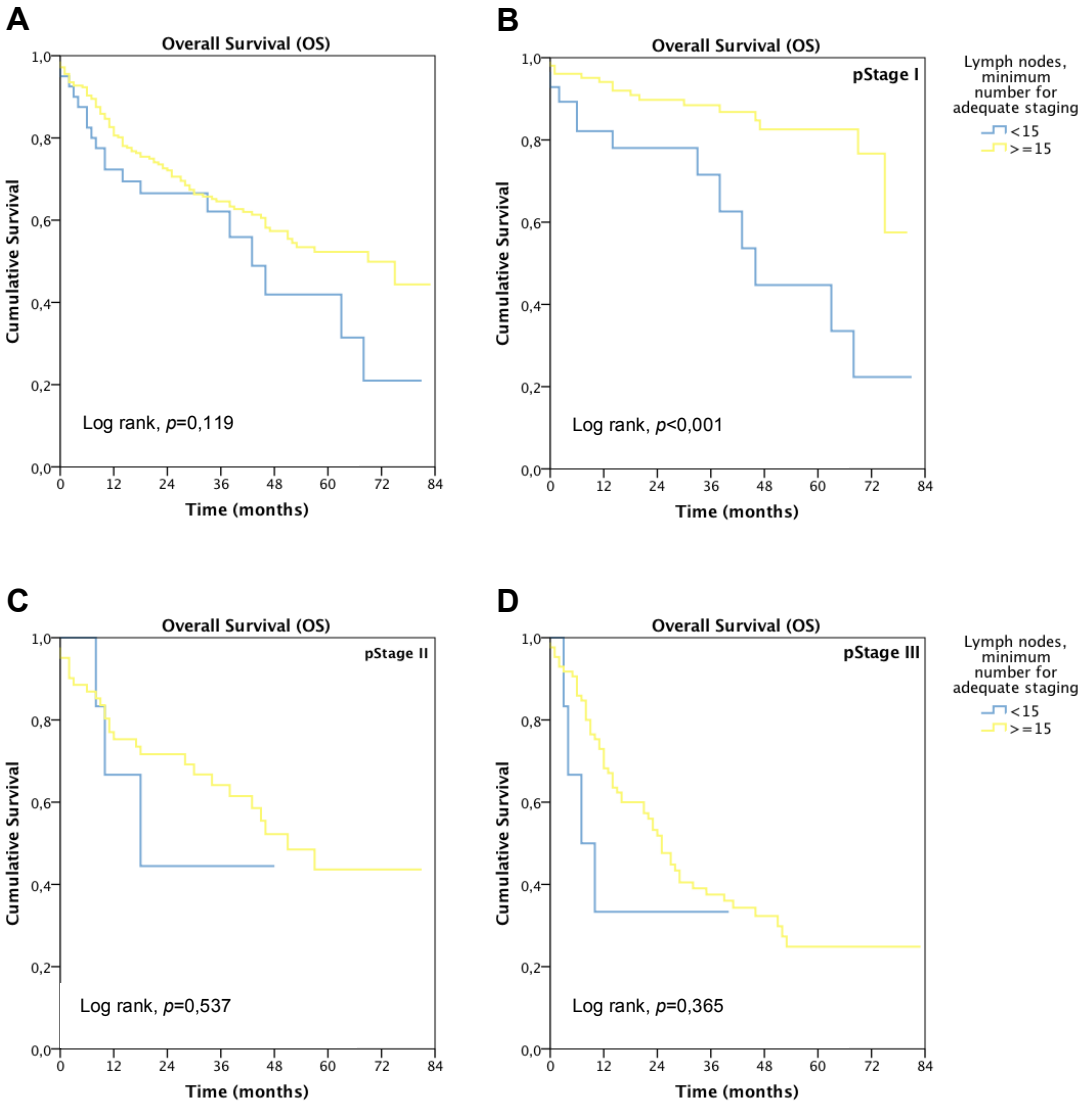

Figure 5: Impact of the number of lymph nodes retrieved in OS of gastric cancer patients (A) and adjusted to tumor stage. (B) OS of stage I, (C) OS of stage II and (D) OS of stage III.

OS, *overall survival*.

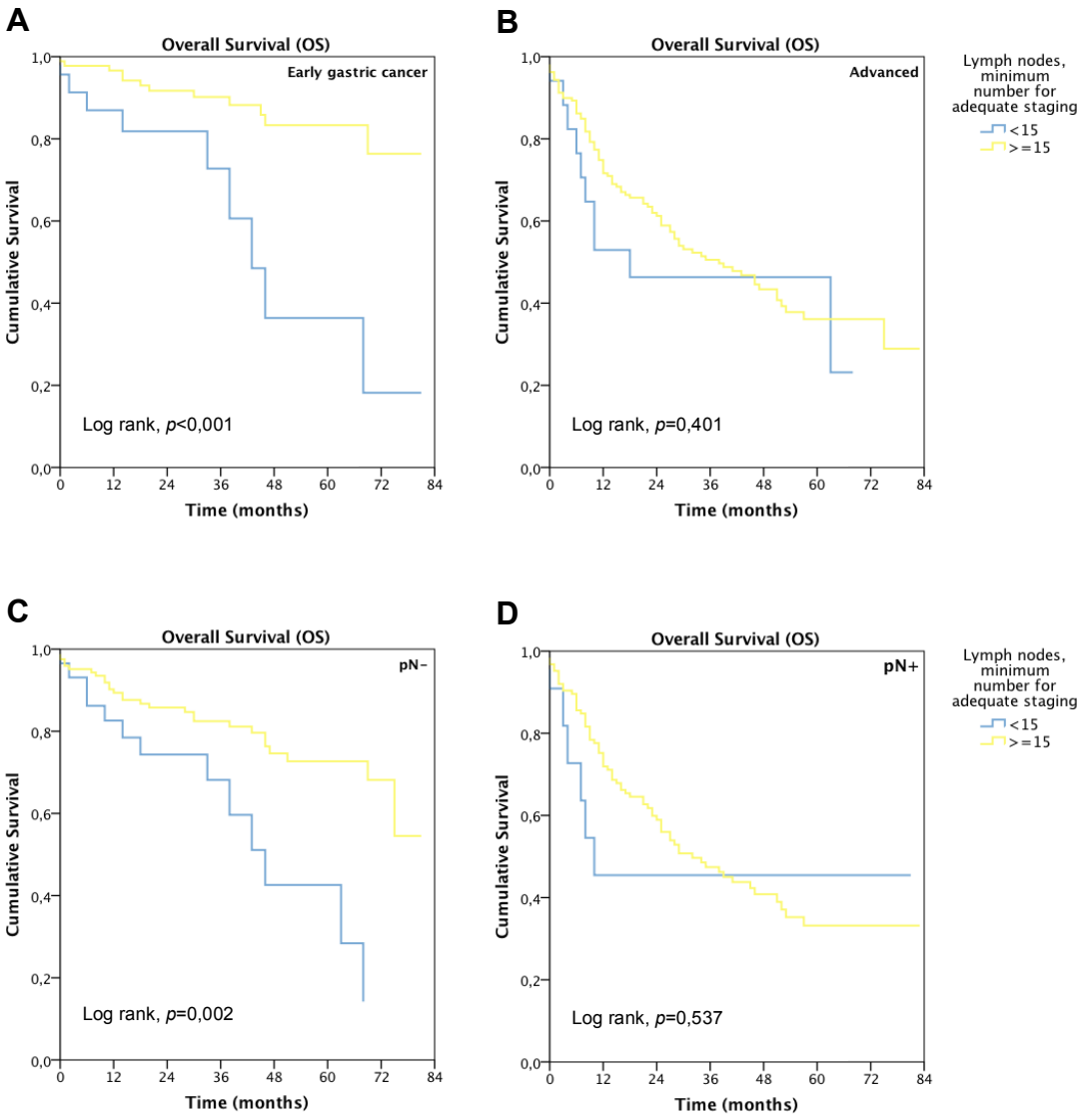

Figure 6: Impact of the number of lymph nodes retrieved in OS of gastric cancer patients according to the type of tumor and N stage. (A) OS of EGC, (B) OS of AGC, (C) OS of N- and (D) OS of N+.

OS, *overall survival*, EGC, *early gastric cancer*, AGC, *advanced gastric cancer*.

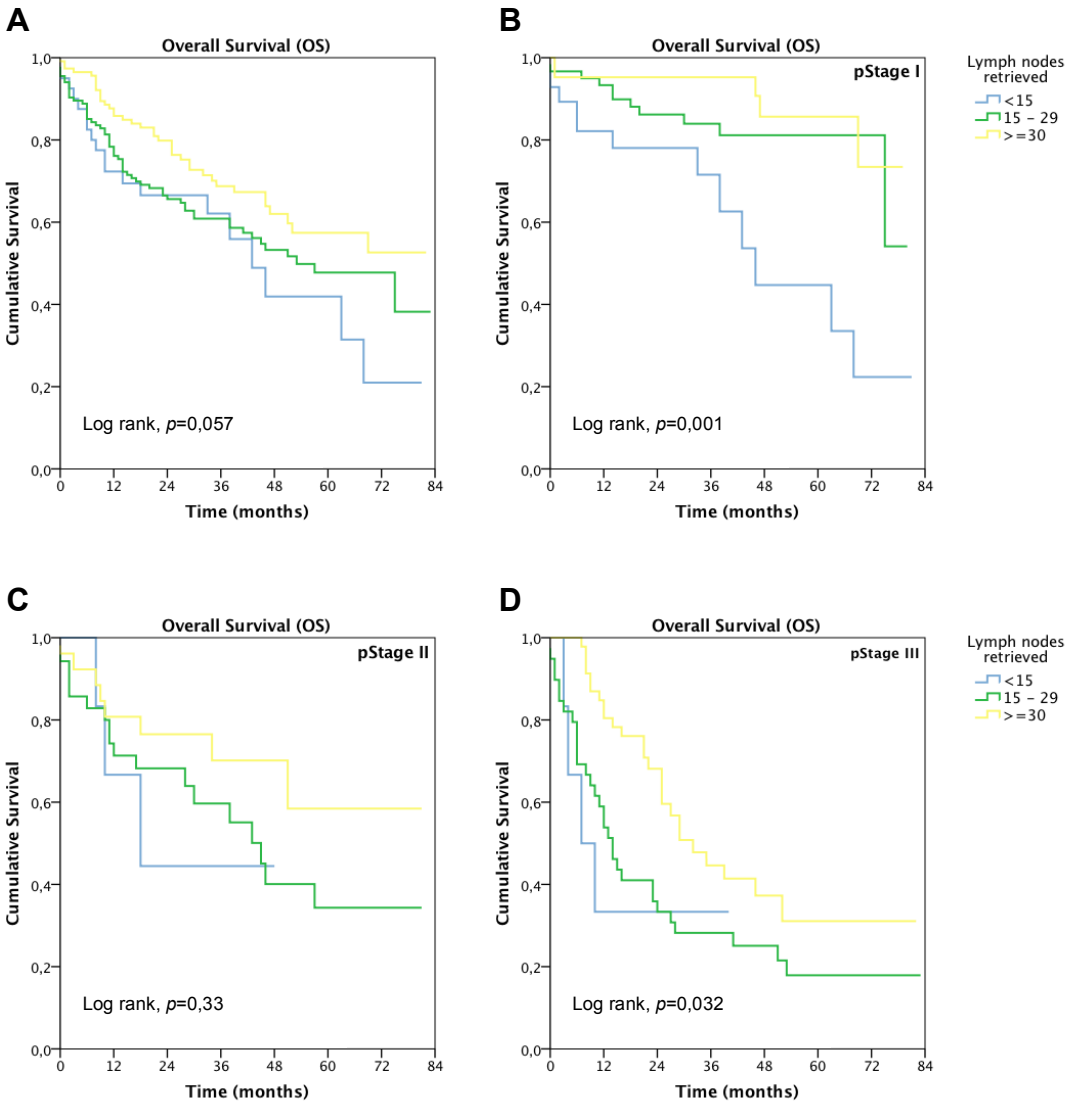

Figure 7: Impact of the number of lymph nodes retrieved in OS of gastric cancer patients (A) and adjusted to tumor stage. (B) OS of stage I, (C) OS of stage II and (D) OS of stage III.

OS, *overall survival*.

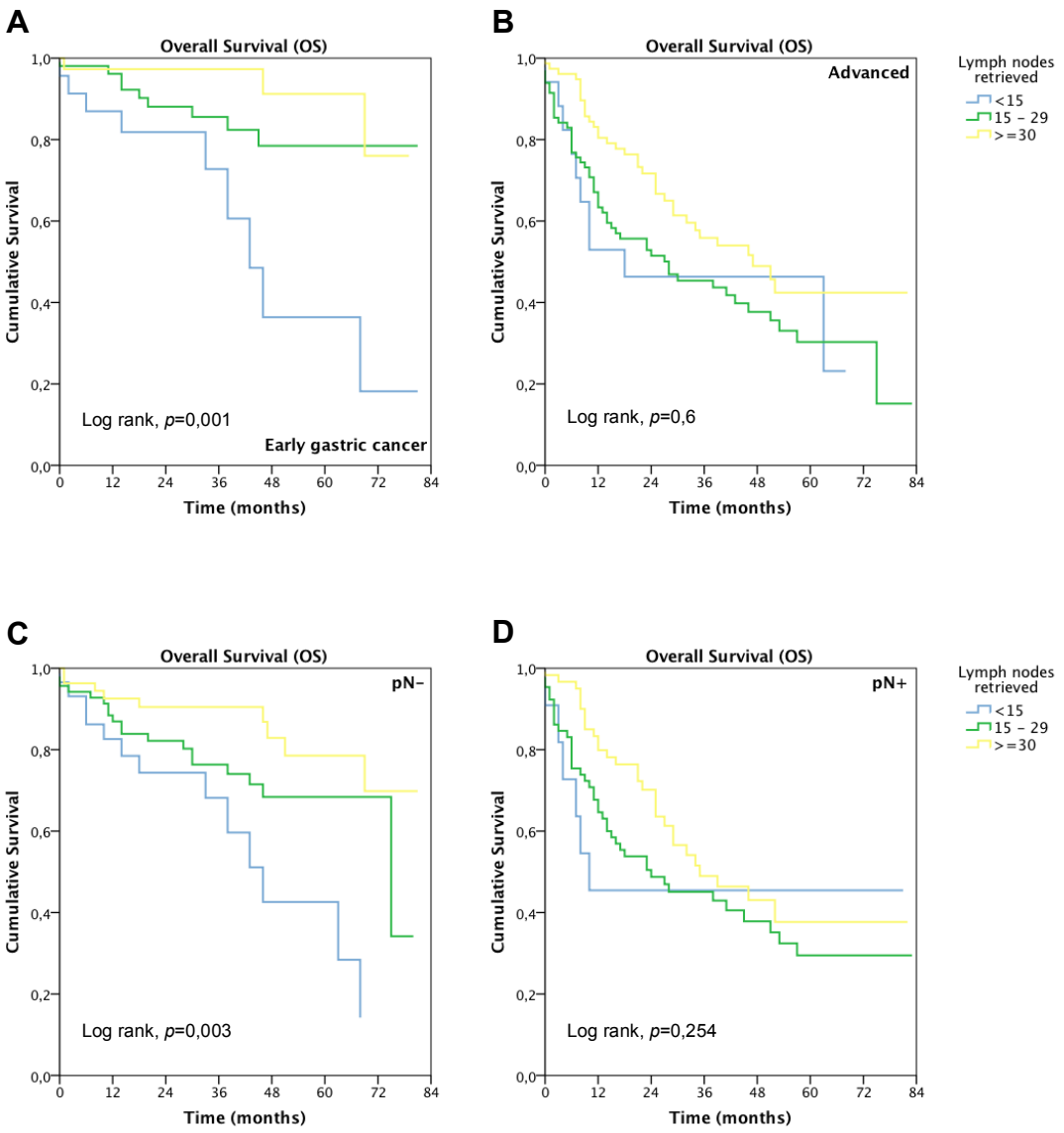

Figure 8: Impact of the number of lymph nodes retrieved in OS of gastric cancer patients according to the type of tumor and N stage. (A) OS of EGC, (B) OS of AGC, (C) OS of N- and (D) OS of N+.

OS, *overall survival*, EGC, *early gastric cancer*, AGC, *advanced gastric cancer*.

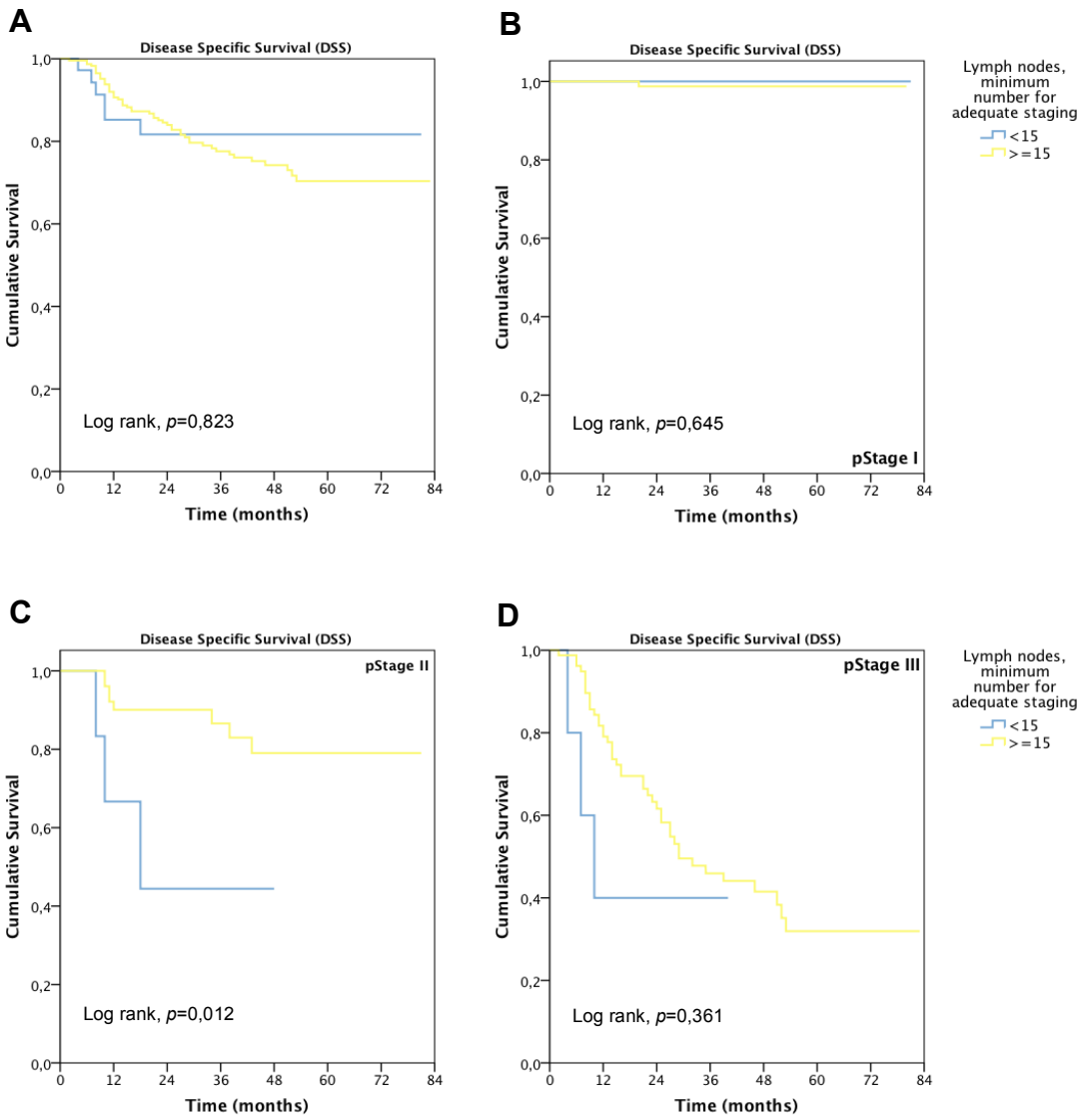

Figure 9: Impact of the number of lymph nodes retrieved in DSS of gastric cancer patients (A) and adjusted to tumor stage. (B) DSS of stage I, (C) DSS of stage II and (D) DSS of stage III.

DSS, *disease specific survival*.

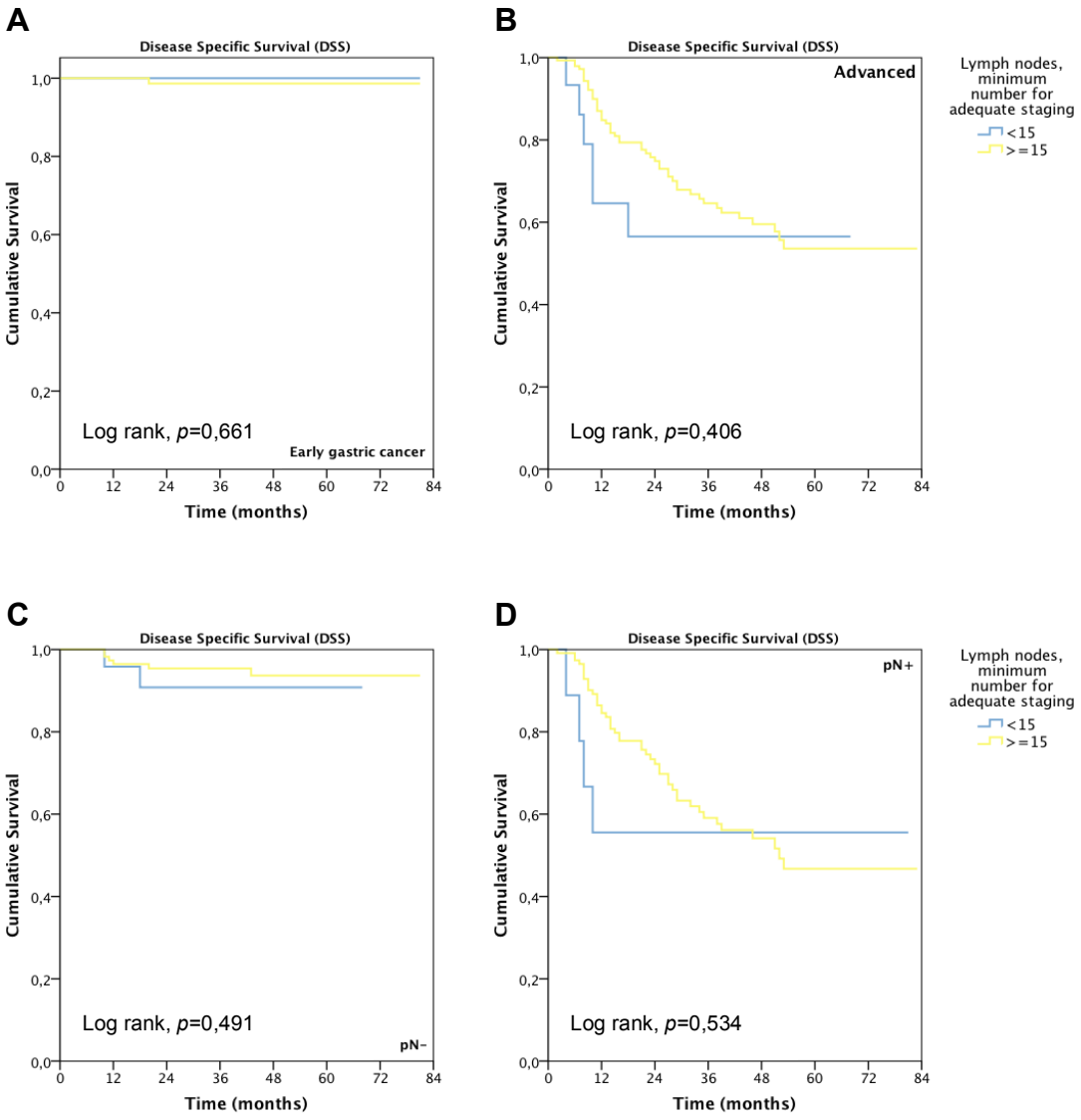

Figure 10: Impact of the number of lymph nodes retrieved in DSS of gastric cancer patients according to the type of tumor and N stage. (A) DSS of EGC, (B) DSS of AGC, (C) DSS of N- and (D) DSS of N+.

DSS, *disease specific survival*, EGC, *early gastric cancer*, AGC, *advanced gastric cancer*.

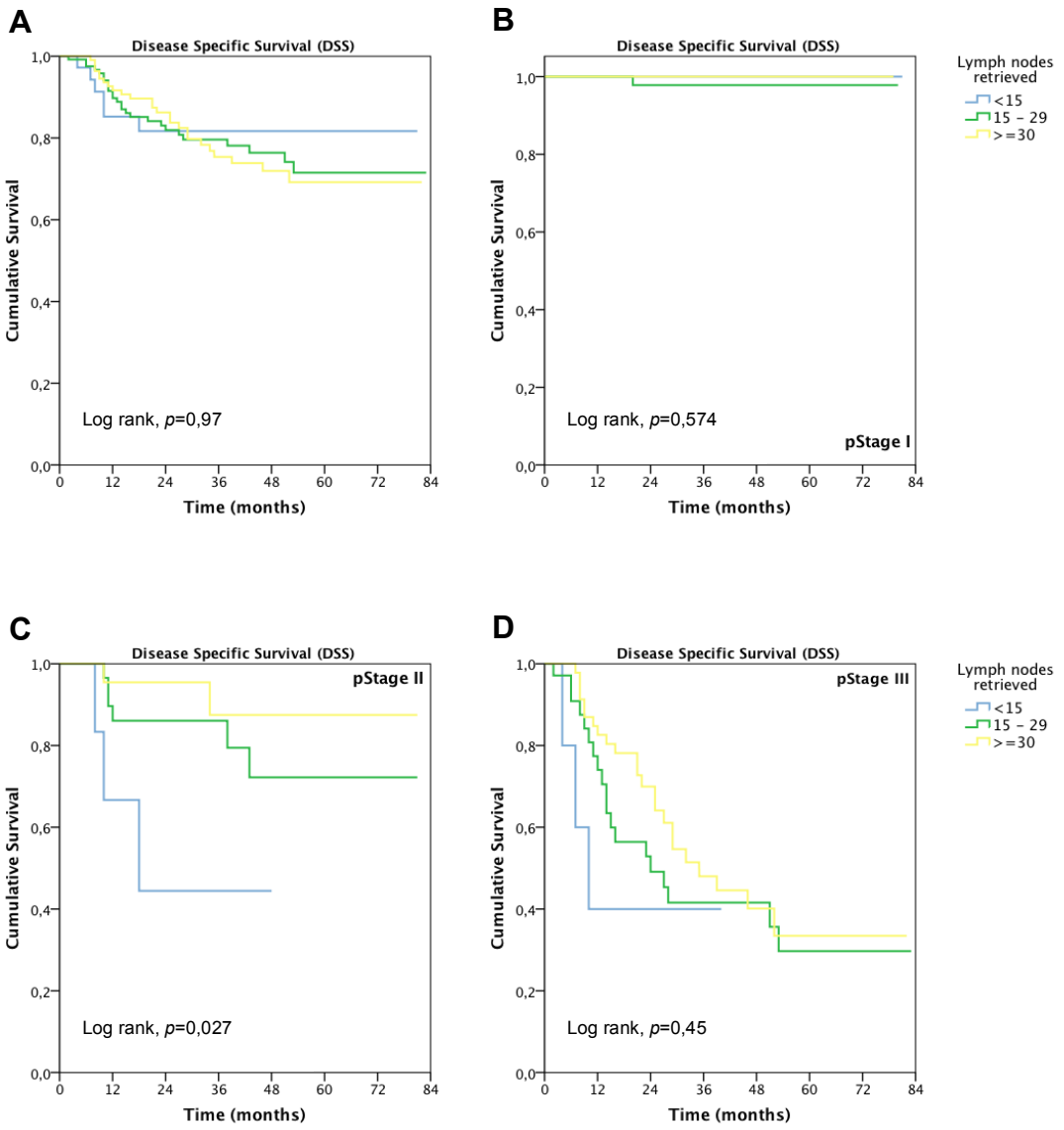

Figure 11: Impact of the number of lymph nodes retrieved in DSS of gastric cancer patients (A) and adjusted to tumor stage. (B) DSS of stage I, (C) DSS of stage II and (D) DSS of stage III.

DSS, *disease specific survival*.

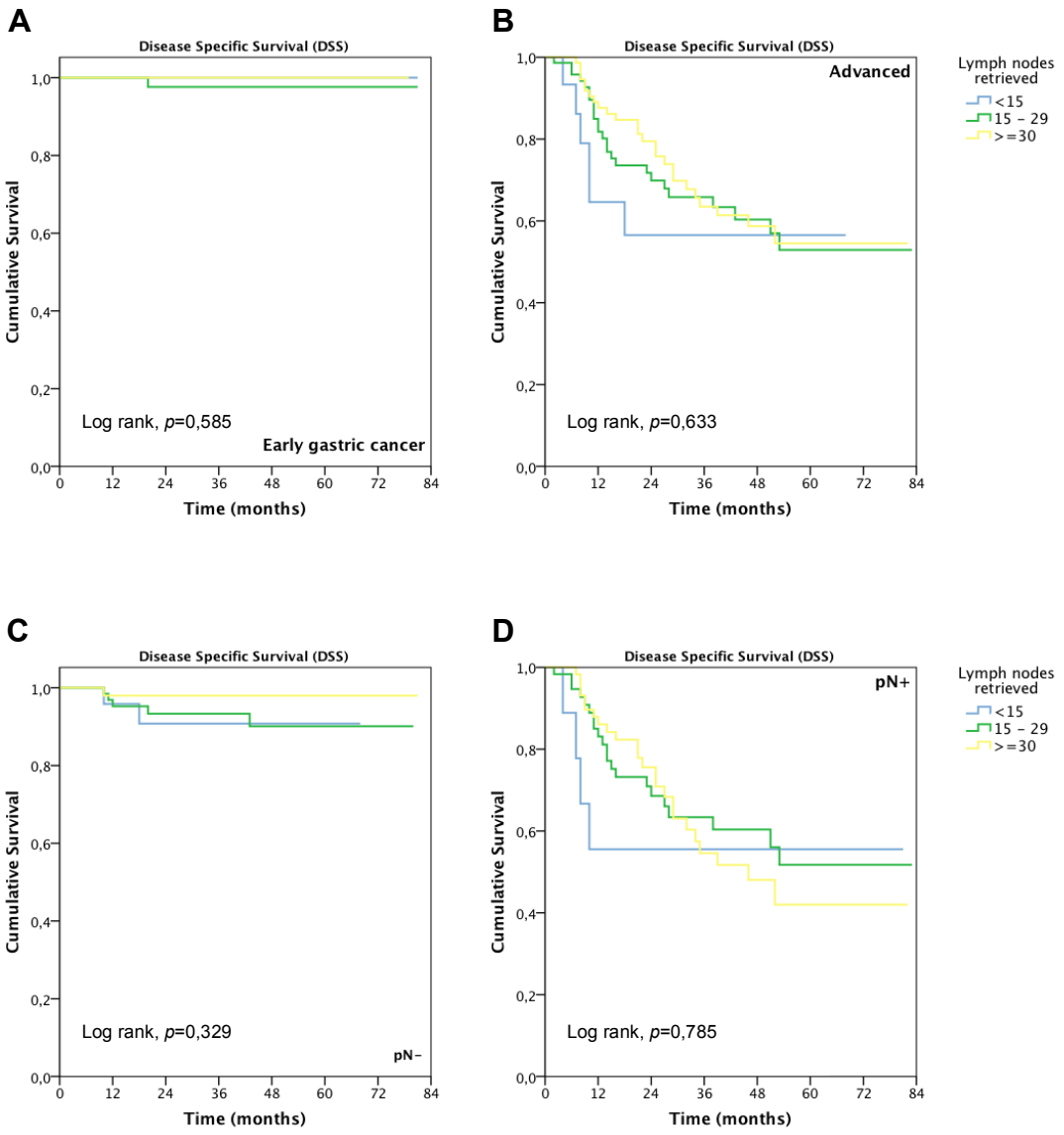

Figure 12: Impact of the number of lymph nodes retrieved in DSS of gastric cancer patients according to the type of tumor and N stage. (A) DSS of EGC, (B) DSS of AGC, (C) DSS of N- and (D) DSS of N+.

DSS, *disease specific survival*, EGC, *early gastric cancer*, AGC, *advanced gastric cancer*.

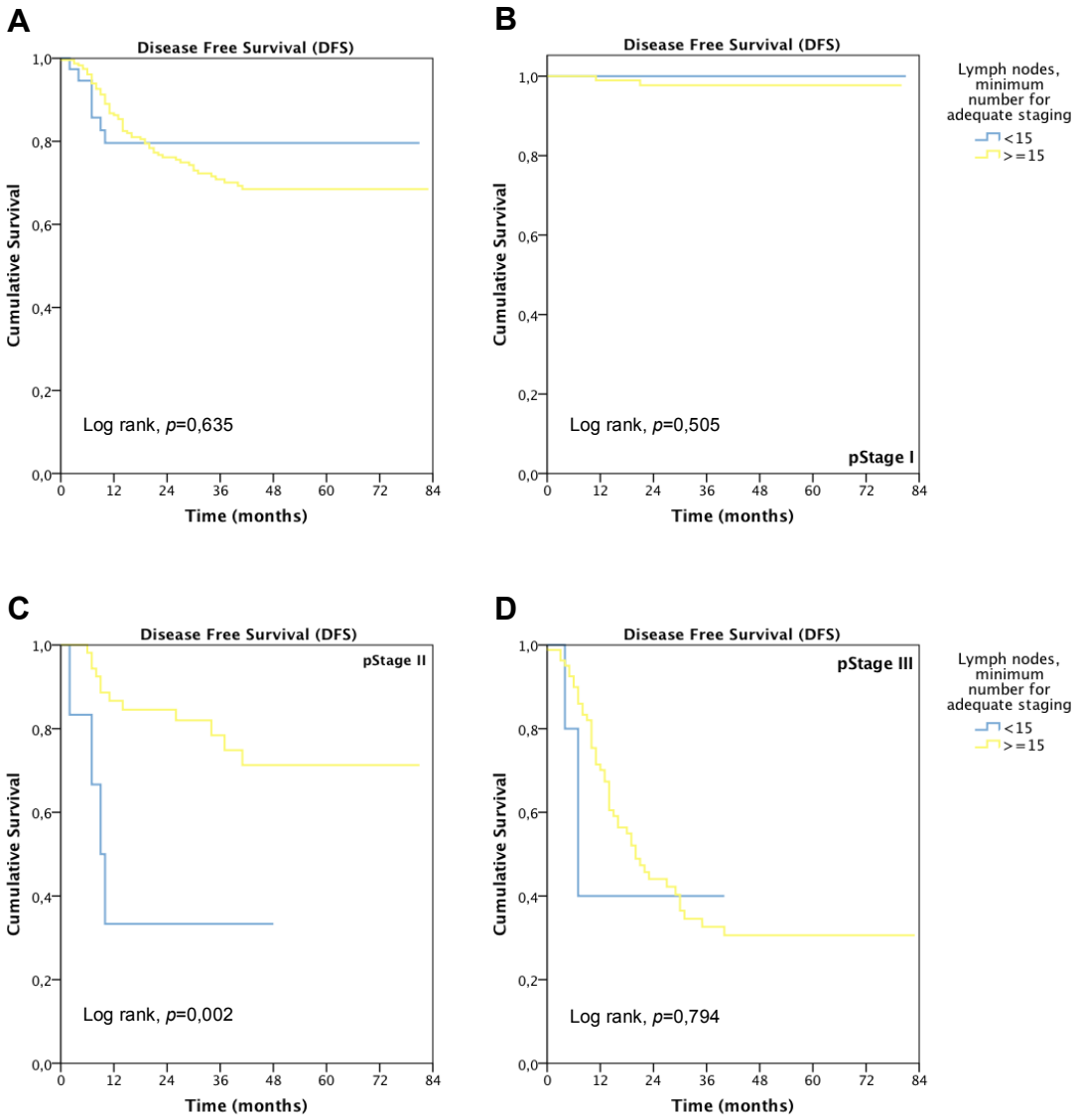

Figure 13: Impact of the number of lymph nodes retrieved in DFS of gastric cancer patients (A) and adjusted to tumor stage. (B) DFS of stage I, (C) DFS of stage II and (D) DFS of stage III.

DFS, *disease free survival*.

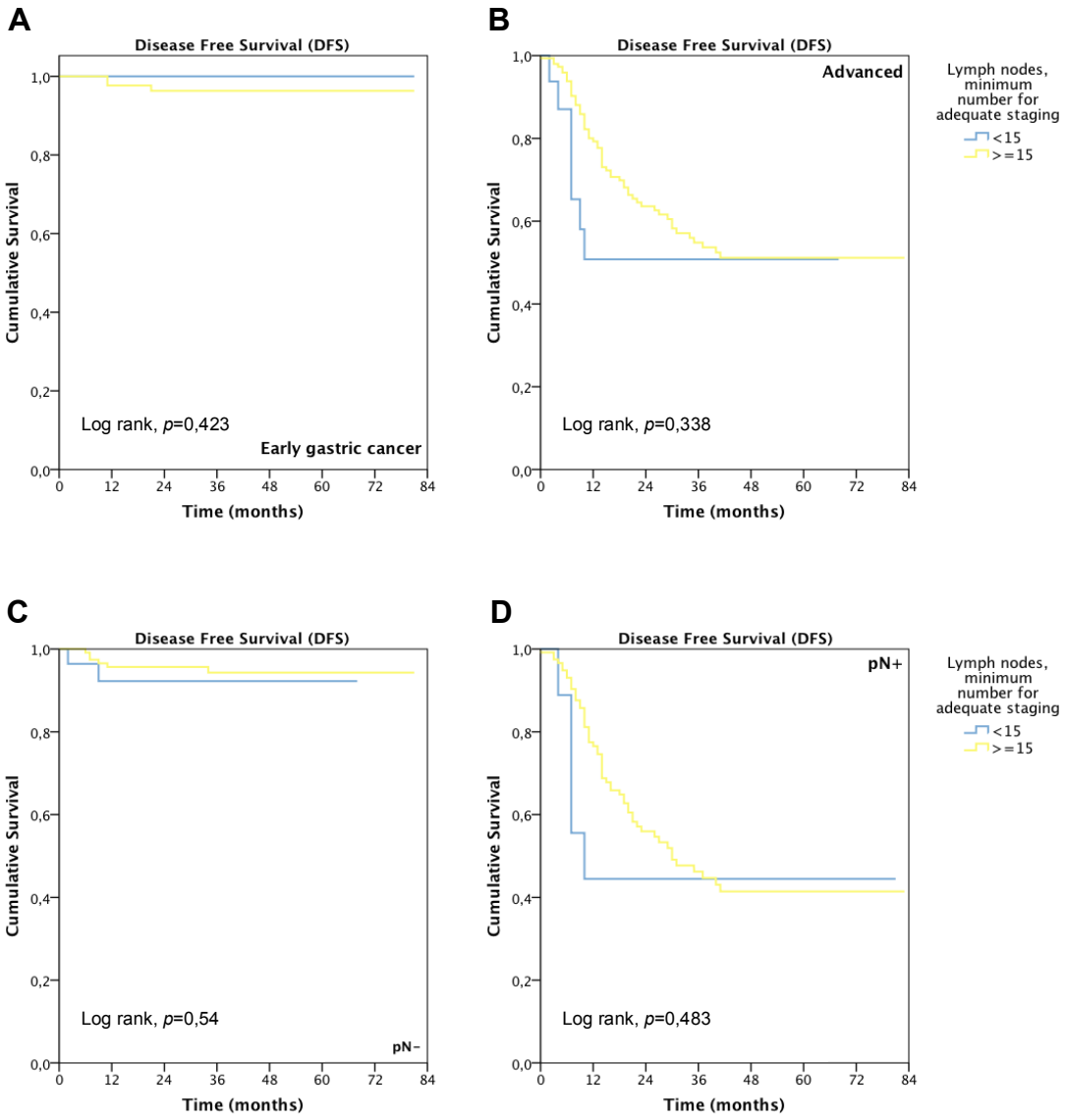

Figure 14: Impact of the number of lymph nodes retrieved in DFS of gastric cancer patients according to the type of tumor and N stage. (A) DFS of EGC, (B) DFS of AGC, (C) DFS of N- and (D) DFS of N+.

DFS, *disease free survival*, EGC, *early gastric cancer*, AGC, *advanced gastric cancer*.

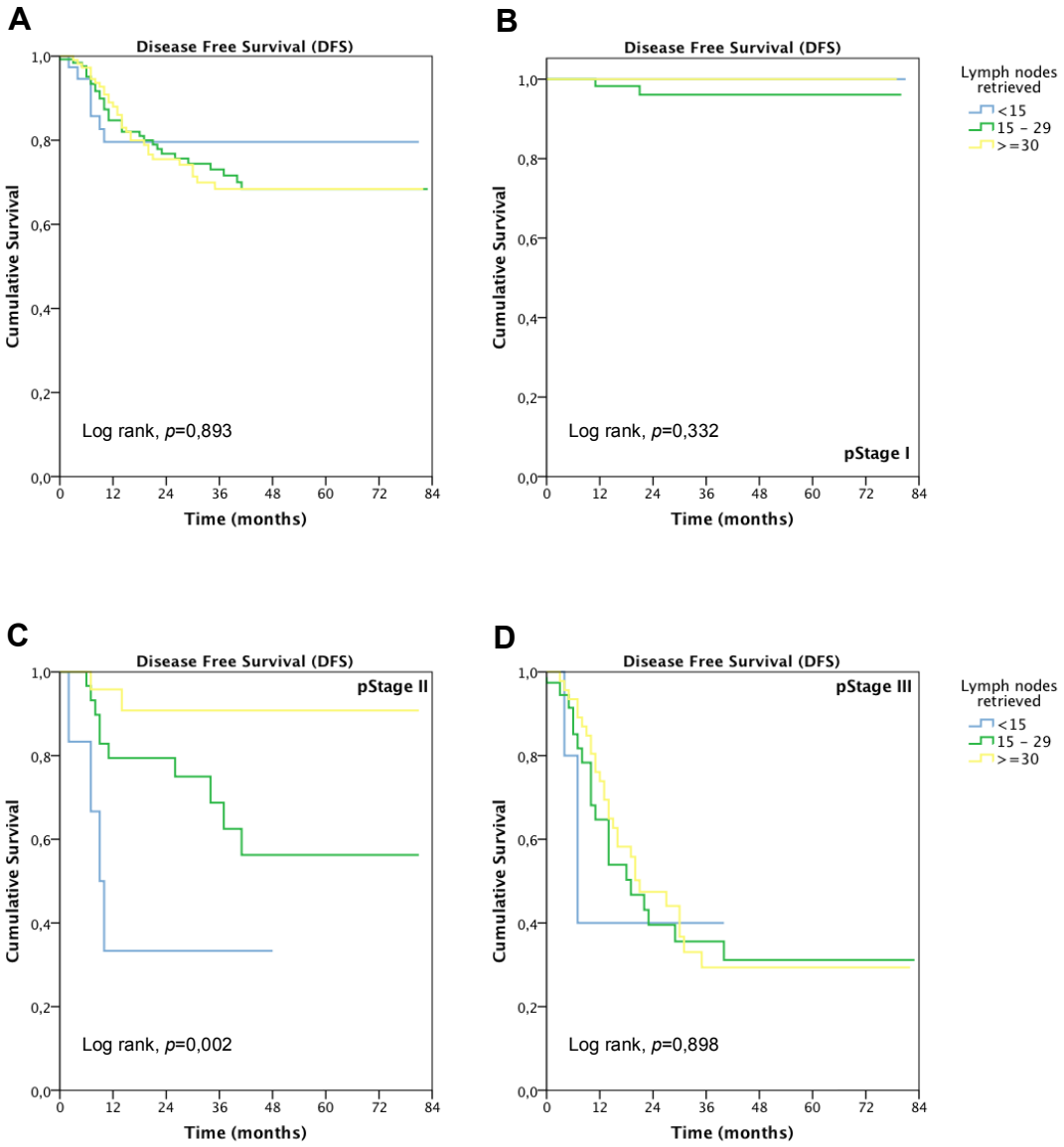

Figure 15: Impact of the number of lymph nodes retrieved in DFS of gastric cancer patients (A) and adjusted to tumor stage. (B) DFS of stage I, (C) DFS of stage II and (D) DFS of stage III.

DFS, *disease free survival*.

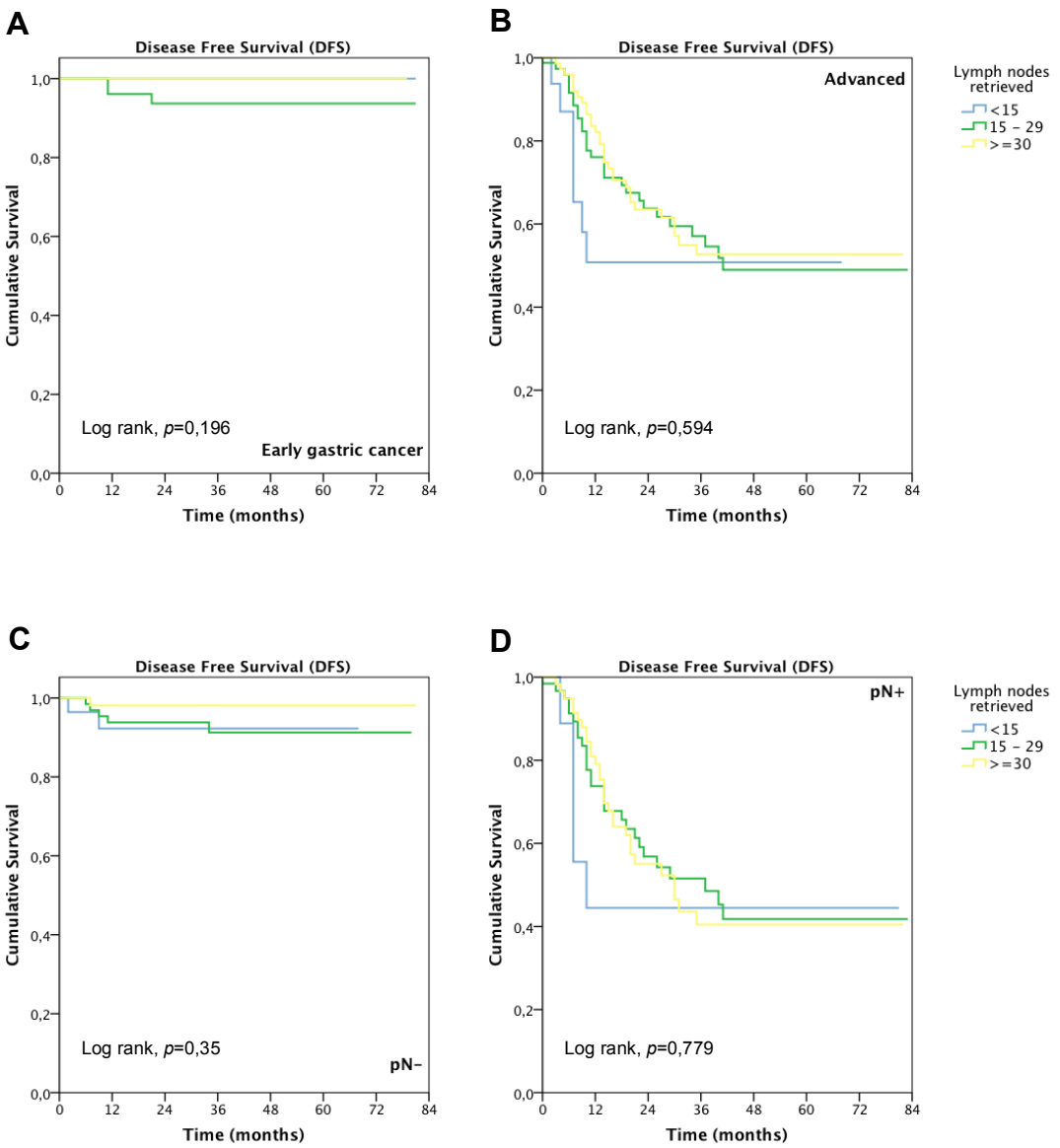

Figure 16: Impact of the number of lymph nodes retrieved in DFS of gastric cancer patients according to the type of tumor and N stage. (A) DFS of EGC, (B) DFS of AGC, (C) DFS of N- and (D) DFS of N+.

DFS, *disease free survival*, EGC, *early gastric cancer*, AGC, *advanced gastric cancer*.

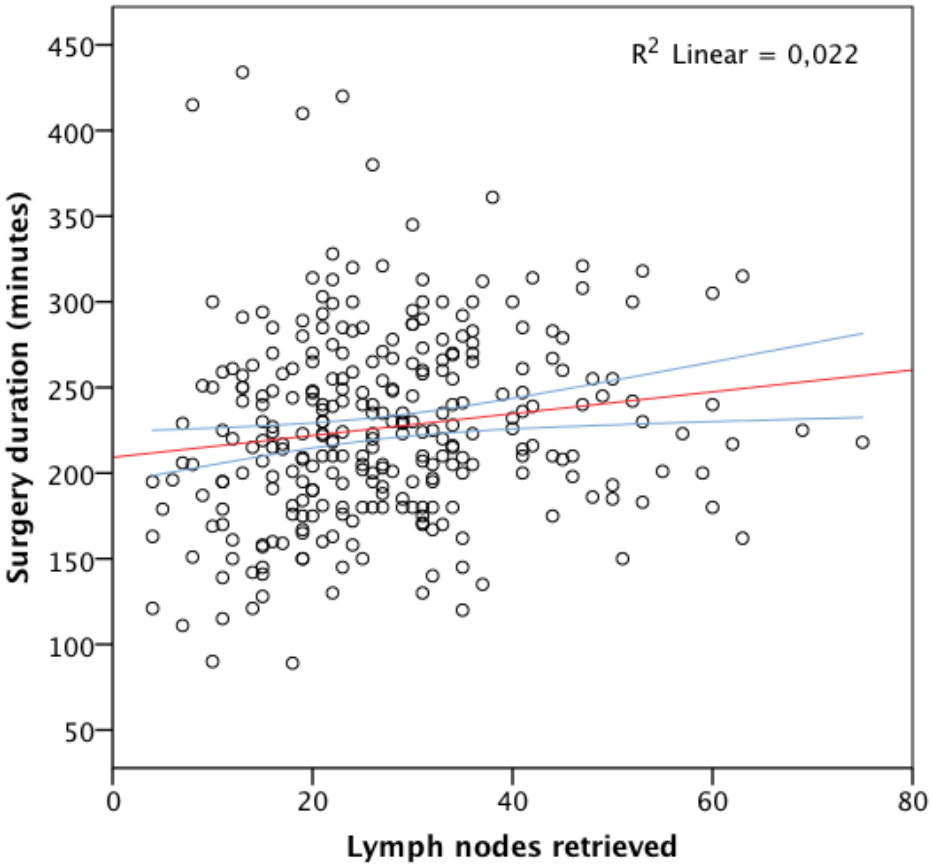

Figure 17: Impact of the number of LN retrieved in *s*urgery duration
Red line, fit line of linear regression model
Blue lines, 95% CI for the mean of surgery duration

Agradecimentos

Em primeiro lugar gostaria de agradecer ao Dr Hugo por toda a disponibilidade, enorme interesse e empenho dedicado à orientação deste projeto. Gostaria também de agradecer à Drª Beatriz Caldeira, ao Dr Vítor Devezas e à Drª Bárbara Castro pelo contributo essencial no preenchimento da base de dados.

Aos meus pais e à minha irmã agradeço do fundo do coração todo o carinho e apoio demonstrado em todas as etapas da minha vida. Sem eles os meus objetivos nunca teriam sido atingidos.

Agradeço ao Ricardo por todo o amor, carinho, dedicação, apoio e por nunca duvidar de mim. Agradeço-lhe ainda pela ajuda tremenda ao longo de todo este projeto, foi sem dúvida um elemento fundamental para a sua realização.

Aos meus amigos por estarem sempre presentes, me fazerem rir e nunca me deixarem desanimar.

Anexos

1 – Parecer da Comissão de Ética

2 – Normas da Revista

Unidade de Investigação

Tomei conhecimento. Nada a opor.

28 de Março de 2016

A Coordenadora da Unidade de Investigação

(Prof.ª Doutora Ana Azevedo)

DIRECÇÃO CLÍNICA
31/3/2016
A CA dá parecer favorável do DC -

60-1a

AUTORIZADO

CONSELHO DE ADMINISTRAÇÃO REUNIÃO DE 07 ABR 2016

Presidente do Conselho de Administração

(Dr. António Oliveira e Silva)

Diretor Clínico | Enfermeira Diretora | Vogal Executivo | Vogal Executivo

(Dr. José Artur Paiva) | (Enf.ª Fernanda Cabrita) | (Dr. Luís Pinto Gomes) | (Dr. Renato G. Matos)

Exmo. Senhor

Presidente do Conselho de Administração do

Centro Hospitalar de S. João – EPE

Assunto: Pedido de autorização para realização de estudo/projecto de investigação

Nome do Investigador Principal: Hugo Miguel Teixeira Ferraz dos Santos Sousa

Título do projecto de investigação: Impact of the number of lymph nodes harvested in gastric cancer prognosis.

Pretendendo realizar no(s) Serviço(s) de Cirurgia Geral do Centro Hospitalar de S. João – EPE o estudo/projecto de investigação em epígrafe, solicito a V. Exa., na qualidade de Investigador/Promotor, autorização para a sua efectivação.

Para o efeito, anexa toda a documentação referida no dossier da Comissão de Ética do Centro Hospitalar de S. João respeitante a estudos/projectos de investigação, à qual endereçou pedido de apreciação e parecer.

Com os melhores cumprimentos.

Porto, 3 / Dezembro / 2015

O INVESTIGADOR/PROMOTOR

SÃO JOÃO

Comissão de Ética para a Saúde – Centro Hospitalar São João

Parecer

Título do Projecto: Impact of the nymber of lymph nodes harvested in gastric cancer prognosis.

Nome do Investigador Principal: Hugo Miguel Teixeira Ferraz dos Santos Sousa

Local onde sera realizado o estudo: Serviço de Cirurgia Geral do Centro Hospitalar de São João e está incluído autorização da respectiva Directora de Serviço.

Objectivo do estudo: Avaliação do impacto do número de gânglios linfáticos colhidos, na cirurgia de ressecção por cancro gástrico, no prognóstico dos doentes.

Período previsto de conclusão: 2017

Benefício: N/A

Risco: N/A

Respeito pela liberdade e autonomia do sujeito do ensaio: N/A

Confidencialidade dos dados: está garantida a confidencialidade dos dados e esta informação será restrita ao investigador principal e respectivo grupo de investigação.

SÃO JOÃO

O Investigador Principal dispõe de competência técnica e científica para a realização do estudo.

Não prevê a realização de questionários.

Custos: O estudo não prevê custos acrescidos para os participantes nem para a instituição.

Parecer: Em face da análise do protocolo de estudo, proponho a sua aprovação pela CES do CHSJ.

Porto, CHSJ, 23 de fevereiro de 2016

O Relator

Dr. John Preto

7. SEGURO

a. *Este estudo/projecto de investigação prevê intervenção clínica que implique a existência de um seguro para os participantes?*

SIM ☐ (Se sim, junte, por favor, cópia da Apólice de Seguro respectiva)
NÃO ☑
NÃO APLICÁVEL ☐

8. TERMO DE RESPONSABILIDADE

Eu, Hugo Miguel Teixeira Ferraz dos Santos Sousa,
abaixo-assinado, na qualidade de Investigador Principal, declaro por minha honra que as informações prestadas neste questionário são verdadeiras. Mais declaro que, durante o estudo, serão respeitadas as recomendações constantes da Declaração de Helsínquia (com as emendas de Tóquio 1975, Veneza 1983, Hong-Kong 1989, Somerset West 1996 e Edimburgo 2000) e da Organização Mundial da Saúde, no que se refere à experimentação que envolve seres humanos. Aceito, também, a recomendação da CES de que o recrutamento para este estudo se fará junto de doentes que não tenham participado em outro estudo no decurso do actual internamento ou da mesma consulta.

Porto, 3 / Dezembro / 2015

O Investigador Principal

PARECER DA COMISSÃO DE ÉTICA PARA A SAÚDE DO CENTRO HOSPITALAR DE S. JOÃO

emitido na reunião plenária da CES de 26 / Janeiro / 2016

A Comissão de Ética para a Saúde APROVA por unanimidade o parecer do Relator, pelo que nada tem a opor à realização deste projecto de investigação.

Instructions to authors (updated June 2016)

Editor-in-chief

Professor J.-C. Soria, Institut Gustave Roussy, Chairman of Drug Development Department, 114 rue Edouard Vaillant, Villejuif, 94805, France

Aims and scope

Annals of Oncology publishes manuscripts that describe new findings of particular significance in any area related to clinical oncology and clinically oriented basic cancer research. The criteria for acceptance are originality and high scientific quality. Manuscripts should be submitted with a letter specifying that the report is not under consideration for publication elsewhere and that all named authors have agreed to its submission. Papers reporting clinical studies should, where appropriate, contain a statement that they have been carried out with ethics committee approval. Papers disregarding the welfare of experimental animals will be rejected. Studies should be carried out in accordance with the relevant national and local guidelines.

If you plan to submit a manuscript to *Annals of Oncology* please read the editorials *Annals of Oncology: an editorial perspective* (Ann Oncol 2014; 25: 5-6) by J.-C. Soria and *Annals of Oncology: a statement of editorial intent* (Ann Oncol 2012; 23: 1931-1932) by J. B. Vermorken, which describes in some detail the kinds of manuscripts the journal will, and will not, now consider for publication.

The editorial office will rapidly review the manuscripts in order that new findings may appear with minimum delay. The editorial office will return to authors within 3 weeks, whenever possible, all papers that are found to be of insufficient priority for further consideration. Papers of high interest will be sent out for external review.

Authors will normally be notified of acceptance, rejection, or need for revision within 6 weeks of submission. Contributors will be provided with an electronic pdf proof, and corrections must be returned within three working days.

Ethics

When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) or with the Helsinki Declaration (1964, amended in 1975, 1983, 1989, 1996 and 2000) of the World Medical Association. Do not use patients' names, initials, or hospital numbers, especially in any illustrative material. When reporting experiments on animals, indicate whether the institution's or the National Research Council's guide for, or any national law on, the care and use of laboratory animals was followed.

Manuscript presentation

The journal's language is English. British English or American English spelling and terminology may be used, but either one should be followed consistently throughout the article. Number the pages consecutively with the first page containing the following headings:

- article type
- title
- author(s) list: first name(s) written with initials only, and followed by the last name - e.g. J. E. Smith

  There is no restriction on the number of authors; manuscripts can have as many authors as needed
- affiliation(s) list: the affiliation list should be written as follows: Department/Division Name (in English), Affiliation/Institution, City, Country

- full address for correspondence
  - Only one Corresponding Author should be designated, multiple names are not permitted.
  - For Original Article and Review:
    this should be written as follows: title of corresponding author (Mr/Mrs/Ms/Dr/Prof) without academic title (MD, PhD, etc.), author name (written with first name, middle initial, then last name) Department/Division/Unit Name (in English),
    Affiliation/Institution, street address, city, postal code, country, country code and telephone number, email address
  - For Editorial and Letter to the Editor:
    Corresponding Author e-mail address in brackets, e.g. (*E-mail:……..@…..)

Abstracts

Please provide a short summary of 300 words or less. The summary should not contain any undefined abbreviations or unspecified references. Summaries should be organized and formatted according to the following headings: (1) *Background*, (2) *Patients and methods*, (3) *Results* and (4) *Conclusion(s)*. Authors may substitute 'Design' or 'Materials and methods' for 'Patients and methods' in summaries of Review articles or of papers dealing with basic research.

Key words

Please provide a maximum of six key words, suitable for indexing.

Abbreviations

Abbreviations should be explained at first occurrence. Non-standard abbreviations should be avoided.

Decimal numerals

To enhance readability and clarity of the text as well as tables and figures, decimal numerals should - with the obvious exception of *P* -values - be rounded to the unit whenever possible (i.e. in all cases in which the rounding procedure does not change the meaning). Value " *N* " and " *P* " should always be written in italic.

Manuscript File

Most text formats are acceptable, however Microsoft Word documents (.doc/.docx) are strongly recommended for submission.

xls/ppt/latex/pdf are NOT acceptable formats.

File should be saved with a brief name, such as “ms.doc” or “manuscript.doc”; long names can create uploading problems.

Key Message

Please provide a short description, 400 characters maximum including spaces, of the key message of your article. If the article is accepted, this description will be published online as part of the journal’s table of contents.

Article types

Annals of Oncology publishes material in the form of editorials, original articles, letters, reviews, industry corner, and special articles.

Editorials. Editorials are solicited by the editor and are generally related to a paper published in the same issue. Length and format of the editorial will be agreed upon between editor and author.

Original articles. Full articles should generally be no longer than 3500 words, excluding manuscript heading, abstract, acknowledgements, and funding. References will be included in the word count and should not generally exceed 40 in number. Tables and figures are not limited in number but each will count as 150 words towards the total word count of 3500; tables with excessive word counts will have the total words included in the final manuscript word count, however, extended material may be published as Supplementary Material.

In the case of Supplementary Material, please indicate if it can be published online only. Online-only Supplementary Material should be uploaded in separate file(s), and described in the manuscript, in order to allow proper linking.

Figures, tables and references must be prepared according to specific instructions (see below).There is no limit on the number of figures or tables, but please consider that the journal is limited for space and that it may be possible to present some figures and tables as online only Supplementary Data. For further information click here. Supplementary tables or figures should be named and numbered accordingly (S1, S2 etc.) in the manuscript and in the file.

Similarly, it may be possible to present an extended bibliography for online-only presentation.

Pre-submission queries are welcome, but for original articles direct online submission should get a rapid response.

Letters to the editor. Letters to the editor are for correspondence relating to previously published articles, and only then within an appropriate time frame, or interesting practice points, e.g. emerging side-effects of new drugs, rare diseases where there is a real practice issue.

Letters are welcome and will be published if appropriate. They should be no longer than 500 words and a maximum of five references; one table or figure is acceptable if absolutely necessary. No abstract is required.

Reviews

Meeting reports can only be considered for publication as reviews under exceptional circumstances; in such cases the report should not simply be a report of new data presented but an attempt to synthesise the state of the art in a particular field.

Consensus documents based on the views of ad hoc expert panels are no longer acceptable; the panel must have been convened under the auspices of a widely recognised body or meeting and be identified as such in the title.

The journal places no restriction on the style of review: narrative reviews, systematic reviews, and meta-analyses will all be considered.

Reviews are generally solicited by the editor. Unsolicited contributions will also be considered, and should be submitted to the journal directly online for a rapid response.

Review manuscripts summarize the state-of-the-art in a particular field. Reviews should be no longer than 4000 words, on first submission, excluding manuscript heading, abstract, references (which are unrestricted in number), acknowledgements, funding, tables and figures. In the case of Supplementary Material, please indicate if it can be published online only. If so, please upload it in separate file(s) (see appendices section). There is no limit on the number of figures or tables, but please consider that the journal is limited for space and that it may be possible to present some figures and tables as online only. Similarly, it may be possible to present an extended bibliography for online-only presentation.

Industry corner: perspectives and controversies. These articles are by invitation only. The Editors will, however, consider presubmission queries submitted to the Editorial Office.

Special articles. Special articles are by invitation only.

Case reports. Case reports cannot be considered for publication unless they are a direct response to a previously published article in Annals of Oncology, in which cases they should be submitted as a “Letter to the Editor”.

Word counts

Manuscripts that marginally exceed the stated word counts (not more than 10%) will not be automatically rejected on the grounds of length alone, although immediate rejection remains a possibility, if the editors deem it necessary on the grounds of insufficient interest. If an overlong manuscript is submitted to peer review, shortening of the manuscript may be required if the manuscript is returned

for revision.

When providing word counts please indicate which word processing software and which version you are using.

Clinical trials

Authors reporting clinical trials may find the guidelines given in the report of Simon and Wittes useful. (Simon R, Wittes RE. Methodologic guidelines for reports of clinical trials. Cancer Treat Rep 1985; 69: 1-3.) Particularly critical is the correct application and presentation of survival analyses: useful guidelines can be found in the appendix of the report by D. G. Altman et al. (Altman DG, De Stavola BL, Love SB, Stepniewska KA. Review of survival analyses published in cancer journals. Br J Cancer 1995; 72: 511-518).

The quality of data reporting on randomized clinical trials will be evaluated following the rules and checklist of the CONSORT statement (CONSORT 2010 Statement: Updated Guidelines for Reporting Parallel Group Randomized Trials. Schulz KF, Altman DG, Moher D et al. Ann Intern Med 2010; 152: 1-7); if required, material concerning this statement will be forwarded to the authors. Randomized clinical trials, which have begun after 1 January 1997, must be monitored and carried out in a manner permitting an absolute adherence to the rules of the CONSORT statement, as regards publication of their results. Potentially acceptable manuscripts will be submitted for statistical review. Any registered clinical trial number should be indicated after the abstract.

Phase I trials

Reports of phase I studies can only be considered where there are additional translational research components. In exceptional cases, specifically where a remarkable response rate was observed, translational research is not required. The reporting of response rates for rare tumors is in any case encouraged.

Phase II trials

Reports of phase II studies should be testing novel and innovative ideas and producing data that form the basis for important RCTs, or data that clearly suggest the lack of potential for such RCTs, i.e. there is no objection to negative phase II studies, provided they give clear guidance for future work. Single arm phase II studies with combination schedules that include established drugs, but without additional translational research cannot be considered. Phase II studies should use recognised statistical designs.

Phase III trials

Submission of reports of prospective, randomised phase III studies is encouraged. Fast-track facilities for editorial handling and, potentially, publication (to print) are available subject to agreement via a pre-submission query. Please contact the Editorial office.

Longer-term follow up reports of previously reported phase III trials are welcomed.

Studies of "prognostic" markers of no real future clinical utility and single biomarkers studies cannot be considered. These studies should be prospective and have a clear view of the practical clinical applications of the results. Retrospective analysis of biomarkers will be considered if done within the framework of data collected from a prospective trial, with appropriate statistics and with multivariate analysis that includes established predictive/prognostic markers.

Reports of tumor registry studies need to have clear clinical relevance; pre submission queries are encouraged.

The journal is committed to translational research for the development of oncology, including basic, i.e. wholly preclinical, cancer research where clinical potential is clear.

Figures and tables

For preparation of figures for online submission and peer review please use the submission instructions web site. Supplementary Table & Figures, must be uploaded as separate files and numbered accordingly (S1, S2 etc.) in the manuscript.

Submission of tables

Tables should be provided in editable format as separate Word/text documents. Value " *N* " and " *P* " should always be written in italic, throughout tables, figures and manuscript. Please note that PDF is not a permissible format for tables. Footnotes are preferable to long explanatory texts in either the heading or body of the table. Footnotes should be identified by superscript letters and be placed immediately below the table.

Submission of electronic figures

Figures should be saved in the following formats: TIFF/JPEG or EPS. Figures should be saved in separate files without their captions, which should be included with the text of the article. Files should be named in sequence, e.g. 'fig1.tif ' /'fig2.tif'/etc. For vector graphics, EPS is the preferred format. Lines should not be thinner than 0.25 pts and in-fill patterns and screens should have a density of at least 10%. Font-related problems can be avoided by using standard fonts such as Times Roman and Helvetica. For bitmapped graphics, TIFF is the preferred format but EPS is also acceptable.

The following resolutions are optimal: black-and-white line figures, 600-1200 dpi; line figures with some grey or coloured lines, 600 dpi; photographs, 300 dpi; screen dumps, leave as is. Higher resolutions will not improve output quality but will only increase file size, which may cause problems with printing; lower resolutions (<300 dpi) may compromise output quality. Please try to provide artwork that approximately fits within the typeset area of the journal. Especially screened originals, i.e. originals with grey areas, may suffer badly from reduction by more than 10-15%.

Each figure and table should be numbered and mentioned in the text accompanied by an explanatory legend. The figure legends should be grouped and placed on a

separate page. All Figures will be relabelled and coloured to the journal-specific standard colour palette. In addition, a small number of Figures in Reviews will be redrawn by a medical illustrator. The Figures will be published in colour in the online version for no charge. Authors will be charged for reproducing figures in colour in the print version, with the exception of Reviews. During the online submission authors will be asked to agree any necessary colour figure payments, and this will be checked at proof stage. Reviews will be printed in colour for no charge.

In tables, footnotes are preferable to long explanatory material in either the heading or body of the table. Such explanatory footnotes, identified by superscript letters, should be placed immediately below the table.

Section headings

First-, second-, third- and fourth-order headings should be clearly distinguishable but not numbered. Generally, an original article should be structured as follows: introduction; methods-patients and methods-materials and methods-etc; results; discussion (conclusion may be used as a subheading in the discussion); acknowledgements; funding; disclosure; references.

Appendices

Supplementary material, such as a long list of collaborators who cooperated/contributed in the study, should be collected in an Appendix and placed after the Reference section. Material can alternatively be published online-only as Supplementary Data. If, however, co-authors listed in an appendix to the main article should appear as co-authors in Medline, please make sure that the appendix of names is included at the end of the main manuscript file, rather than as a supplementary file. Names published as Supplementary Material cannot be tagged as co-authors and will not appear in Medline. Multimedia formats, such as audio and video, can only be presented as online-only supplementary material.

Cross-referencing

In the text, a reference identified by means of an author's name should be followed by the reference number in square brackets. When there are more than two authors, only the first author's name should be mentioned, followed by et al.

Examples.

Winograd [1]

Bullen and Bennett [2]

Wilson et al. [3]

Acknowledgements

Acknowledgements of people, grants, funds, etc. should be placed in a separate section before the References.

Funding

Details of all funding sources for the work in question are mandatory, please include a funding statement before the disclosure, or add 'none declared'.

The following rules should be followed.

- The sentence should begin: 'This work was supported by …
- The full official funding agency name should be given, i.e. 'the National Cancer Institute at the National Institutes of Health' or simply 'National Institutes of Health' not 'NCI' (one of the 27 subinstitutions) or 'NCI at NIH' (full RIN-approved list of UK funding agencies). Grant numbers should be given in brackets as follows: '[grant number xxxx]'
- Multiple grant numbers should be separated by a comma as follows: '[grant numbers xxxx, yyyy]'

- Agencies should be separated by a semi-colon (plus 'and' before the last funding agency) or grant number 'to [author initials]'.
- Where individuals need to be specified for certain sources of funding the following text should be added after the relevant agency or grant number 'to [author initials]'.
- Please state if no grant number is applicable.

An example is given here: 'This work was supported by the National Institutes of Health [AA123456 to C.S., BB765432 to M.H.]; and the Alcohol & Education Research Council [hfygr667789].'

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Crossref Funding Data Registry: In order to meet your funding requirements authors are required to name their funding sources, or state if there are none, during the submission process. For further information on this process or to find out more about the CHORUS initiative please click here.

Disclosure

Any financial interests or connections, direct or indirect, or other situations that might raise the question of bias in the work reported or the conclusions, implications or opinions stated – including pertinent commercial or other sources of funding for the individual author(s) or for the associated department(s) or organization(s), personal relationships, or direct academic competition – should be disclosed. For further information see FAQ.

If there are none, the disclosure should say: 'The authors have declared no conflicts of interest.'

If one or a few authors have a conflict to disclose, further to that statement, there

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References

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