Peer Review File

Article information: http://dx.doi.org/10.21037/atm-20-7953

Reviewer A

Comment: The aim of this report was to "analyze the association of Lauren classification and prognosis of gastric adenocarcinoma using comprehensive statistical analyses". Appropriate statistical analyzes were used for the research.

Response: *We appreciated very much the insightful comments on our work, which made our manuscript much strengthened.*

Comment: I have no comments on the description of the methodology based mainly on statistical analyzes and material – study group description, variables and definition, endpoints, etc. The results of the work are also presented correctly, legibly and do not raise any objections.

Response: *We appreciated the positive comments on our work.*

Comment: The aim of the work has been achieved and the work limits are marked. However, please consider adding a paragraph on Mixed-Type histology, as we know that this subtype may present even worse prognosis than Diffuse type. It is also a predictive factor for postoperative complications, like esophagojejunostomy leak

(doi.org/10.3390/cancers12061701)

Response: *We thank the reviewer for the professional comments on our work.*

As is mentioned by the reviewer, mixed type of gastric adenocarcinoma has presented a worse prognosis than diffuse type and intestinal type according to several reports (1-3). These reports emphasized the importance of mixed type in the analysis of prognosis of gastric cancer. We intended to analyze the characteristics and clinical value of Lauren's classification, including mixed type. ***However, mixed type information was not available in the SEER database.*** *This restrained us to from performing further analysis concerning mixed type.*

Therefore, we tried to solve this problem by referring to several previous studies that have also used SEER database to investigate the clinical value of Lauren's classification in gastric adenocarcinoma. ***We found that none of these reports analyzed the characteristics and clinical significance of mixed type. Instead, they clarified the limitations of unavailability of mixed-type gastric adenocarcinoma in the SEER database in their discussions.*** *For example, Tang et al. revealed that diffuse-type early gastric cancer (EGC) had a better prognosis than intestinal-type EGC by using propensity score matching (PSM) for adjusting confounders (4). The authors also divided gastric adenocarcinoma into intestinal type (M8140, M8211, M8010, and M8144) and diffused type (M8145, M8490, and M8142). The authors have mentioned that limited information is provided in the SEER database, which may affect their results. Another study by Li et al. analyzed the characteristics and clinical value of Lauren's classification in early gastric cancer (EGC) (5). This study defined diffused type as histologically confirmed SRC (M8490), diffuse carcinoma (M8145), and linitis plastica (M8142) and intestinal type as*

carcinoma (not otherwise specified; M8010), adenocarcinoma (not otherwise specified; M8140), tubular (M8211), and intestinal type (M8144) using the International Classification of Disease for Oncology, third edition. This classification is consistent with our study. In their limitations, they also mentioned that information about the percentage of diffuse type in Lauren's mixed type and the proportion of mixed cell type was not available in the SEER database.

Therefore, we did not analyze the prognosis of mixed-type gastric adenocarcinoma here. But considering the importance of mixed type in gastric adenocarcinoma, we will investigate the characteristics and clinical value of mixed type histology in more studies in our databases in the near future. ***We have clarified this limitation in the Discussion section in our manuscript (line 409~410; line 413~417).***

Comment: 75% of the patients were >T1, which means they presented advanced GC. In the West, this would result in multimodal treatment, based on perioperative chemotherapy and surgery. Additionally, diagnostic laparoscopy is of great value in terms of appropriate staging and tailored treatment. There is a paper on the Lauren classification in SL in GC patients (doi.org/10.1002/jso.25711) and this could be added in the Discussion.

Response: *We thank the reviewer for this insightful comment. As is suggested by the reviewer, the diffuse type was an independent predictor of peritoneal metastases using staging laparoscopy (6). Our data showed that the diffuse type of gastric adenocarcinoma was*

*significantly associated with advanced stages compared with the intestinal type, which is consistent with this report. We have added the description in the Discussion section (**line 338~342, …Our results also showed that the diffuse type of gastric adenocarcinoma was significantly associated with advanced stages compared with the intestinal type. This is also consistent with a previous report that revealed that diffuse type was an independent predictor of peritoneal metastases using staging laparoscopy (22)**).*

Comment: Recommend publication after minor revision.

Response: *We appreciated for the positive comments on our work.*

Reviewer B

Comment: In this retrospective cohort study the authors compared the prognosis for gastric carcinomas classified as intestinal and diffuse according to Lauren. In the original description by Lauren about 15% of the gastric carcinomas could not be classified as either, representing an intermediate group. The authors started with about 78 000 carcinomas and after exclusions including nearly 8000 with neither intestinal or diffuse types, probably representing the intermediate type according to Lauren, they ended up with 20 218 among which 14 374 were of the intestinal and 5 844 of the diffuse type. This distribution is similar to previous studies. Moreover, like previously found, the diffuse

type of gastric cancer was relatively more common in women. By subgroup analyses they found that diffuse gastric carcinomas had a worse prognosis except for small tumours at an early stage (T1).

This is large study where the authors have tried to reduce confounding factors by different statistical methods. The conclusions seem sound. I have the following remarks.

Response: *We thank the reviewer for the insightful comments on our work, which made our manuscript much strengthened.*

Comment: Lauren´s classification seem to represent an important biological difference as the types seldom or never transform into the other. The authors have localized the tumours to eight parts of the stomach with a ninth as overlapping. I would have preferred that they tried to localize them to the three mucosa in the stomach (Cardiac, which may just be a metaplasia, the oxyntic and the antral, although it has become evident during the last decade that oxyntic glands may be found in parts of the antral mucosa). Curiously, few have discussed the impact of the mucosal origin of gastric cancers (Waldum, Mjønes: Cancers 2020).

Response: *We appreciated the professional comments on our work very much. We followed the reviewer's comment and performed a sensitivity analysis by classifying the primary site into five categories: cardia, oxyntic mucosa, antral mucosa, stomach NOS and overlapping lesions. The results of univariable Cox regression analysis showed that lesions from cardia, oxyntic*

mucosa and antral mucosal showed a marginally different impact on cancer-specific survival of gastric adenocarcinoma (sTable5, Cardia, HR, reference; Oxyntic mucosa, HR, 0.99, 95% CI, 0.94–1.05; Antral mucosa, HR, 1.05, 95% CI, 1.00–1.11). The results of multivariable Cox regression models showed that the association of Lauren's classification and cancer-specific survival was stable (sTable6, HR, 1.19; 95% CI, 1.13–1.24, P<0.001) with adjusting age of diagnosis, primary site (five sites), grade of differentiation, T stage, N stage, M stage and tumor size (Model III), which was comparable to nine sites in the original version of our manuscript (HR, 1.19; 95%CI, 1.14–1.25, P<0.001). After adjusting all the potential confounding variables (five sites, Model IV), the diffuse-type group also showed a significantly higher risk of cancer-specific death than the intestinal-type group (HR, 1.20; 95%CI, 1.15–1.26, P<0.001). These results showed that mucosal origin had marginal effects on the association between Lauren's classification and cancer specific survival.

sTable 5 Univariable Cox regression analysis for cancer-specific survival in patients with gastric cancer

Variable	HR (95% CI)	P-value
Primary site		
Cardia	Reference	
Oxyntic mucosa	0.99 (0.94, 1.05)	0.7774
Antral mucosa	1.05 (1.00, 1.11)	0.0493
Stomach, NOS	1.42 (1.32, 1.53)	<0.0001
Overlapping lesions	1.72 (1.59, 1.85)	<0.0001

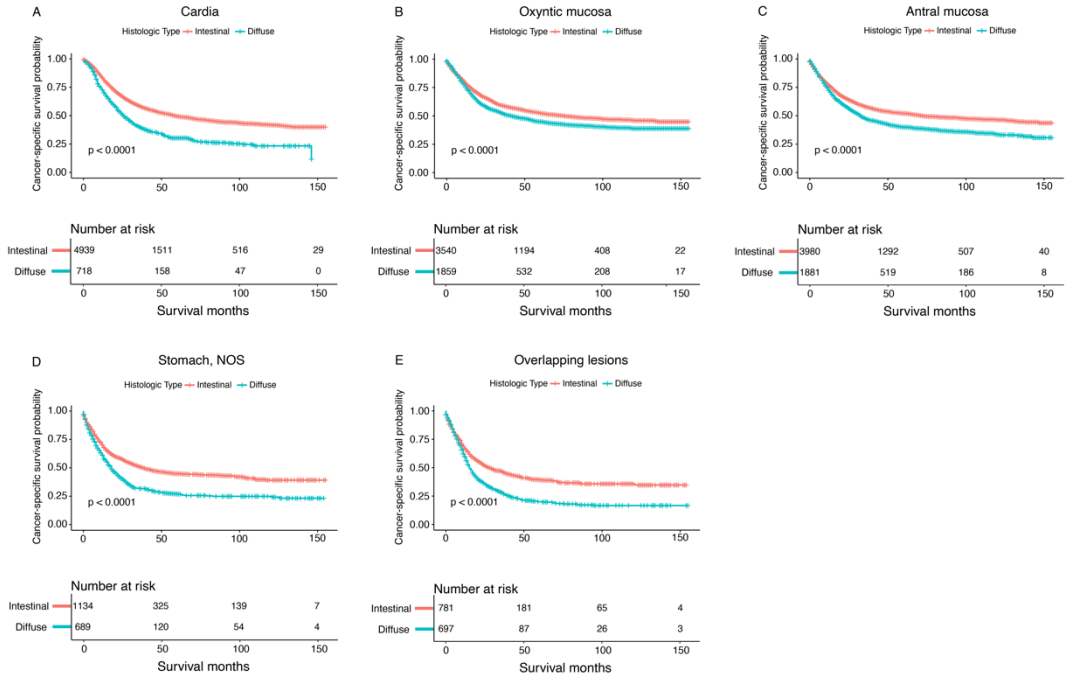
sTable 6 Multivariable Cox regression models evaluating the association between Lauren's classification and cancer-specific survival

Lauren's Classification	Crude		Model III		Model IV	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Intestinal	Reference		Reference		Reference	

Diffuse	1.44	(1.38, 1.50)	<0.001	1.19	(1.13, 1.24)	<0.001	1.20	(1.15, 1.26)	<0.001

Adjustment III: Age; Primary Site (5 sites); Grade; T stage; N stage; M stage; Tumor size.
Adjustment IV: Age; Sex; Year; Primary Site (5 sites); Grade; T stage; N stage; M stage; Operation; Chemotherapy; Tumor size; Race; Insurance; Marital status.

*To further validate our results, we conducted a survival analysis in subgroups of five primary sites. The results showed that CSS rate was significantly higher in intestinal-type gastric adenocarcinoma in each site (sFig. 2). Besides, the subgroup analysis revealed a highly consistent pattern with these results. In the subgroups with different mucosal origins, the diffuse-type group showed more inferior CSS (Fig 3, HR > 1.00; P for interaction, 0.299) compared with the intestinal type. These results demonstrated that mucosal origin had marginal effects on the association between Lauren's classification and cancer-specific survival. We have added the impact of the mucosal origins on the association between Lauren's classification and cancer-specific survival in the Discussion (**line 371~377, Our previous reports have suggested that different stomach parts express different glands and exhibit various clinicopathological features (7). However, few studies reported the impact of the mucosal origin on Lauren's classification in gastric cancers. Here, the results based on the mucosal origins showed that the diffuse-type group showed a significantly higher risk of cancer-specific death than the intestinal-type group in different mucosa origins.**).*



sFig. 2 Kaplan–Meier analysis of cancer-specific survival based on Lauren classification in subgroups of primate sites.

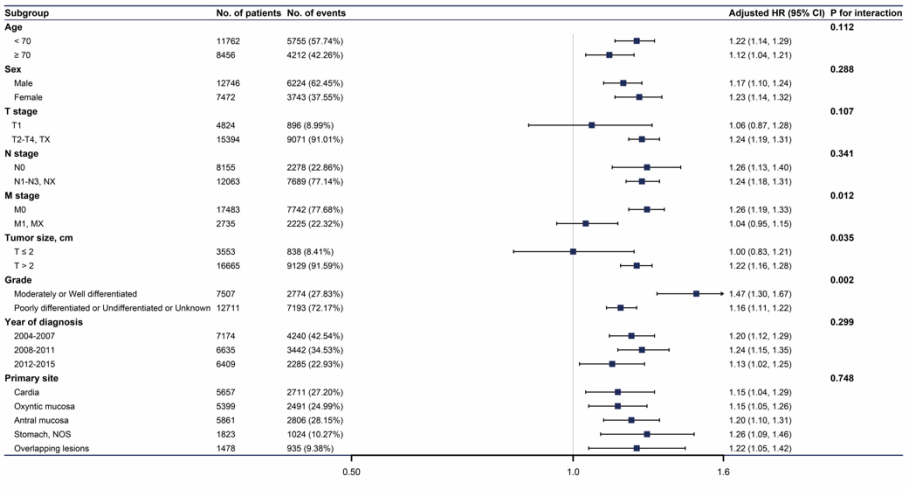


Fig. 3 Subgroup analyses of the effect of Lauren classification on cancer-specific survival. Adjusted for Age; Sex; Year; Primary Site; Grade; T stage; N stage; M stage; Operation; Chemotherapy; Tumor size; Race; Insurance and Marital status, if not be stratified.

Comment: On lines 142-143 the authors may seem to suggest that Helicobacter pylori does not cause gastric carcinomas of diffuse type. Helicobacter pylori is the dominating cause of both types although the mechanisms seem to be different.

Response: *We thank the reviewer very much for the professional comments on our work. We apologize for the inappropriate expressions here. We have followed the kind comments and modified the manuscript.*

*Both intestinal-type and diffuse-type gastric adenocarcinoma show a comparable strong association with Helicobacter pylori (H. pylori) infection compared with intestinal type via different mechanisms (8). Intestinal-type lesions derive from premalignant lesions through an initial H. pylori-induced chronic gastritis and subsequent atrophic and metaplastic gastritis (9). Diffuse-type lesions also originate from H. pylori-induced chronic inflammation with overpassing multiple steps including atrophic gastritis and intestinal metaplasia (10, 11). (**line 82~89, Previous reports have shown that diffuse-type gastric adenocarcinoma exhibits a comparable strong association with Helicobacter pylori (H. pylori) infection compared with intestinal type via different mechanisms (8). Intestinal-type lesions derive from premalignant lesions through an initial H. pylori-induced chronic gastritis and subsequent atrophic and metaplastic gastritis (9). Diffuse-type lesions also originate from H. pylori-induced chronic inflammation with overpassing multiple steps including atrophic gastritis and intestinal metaplasia (10, 11).)*