Prognostic value of local invasive patterns in upper gastrointestinal cancers

Ph.D. thesis

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1. Introduction

Despite their declining incidence, upper gastrointestinal cancers are still common and rank high among the cancers causing death. Lymph node metastases (LNMs) are especially important for the planning of surgical approach, since early stage diseases in which lymphatic spreading of the tumor has not yet occured are manageable with endoscopic modalities, while in more advanced cases with developed LNMs, gastrectomy / esophagectomy with lymph node dissection is preferred. Thus, there is an increasing need for biomarkers that are able to predict the development of LNMs in upper gastrointestinal cancers. Local invasive patterns are histological phenomena whose evaluation can be quickly performed on routine haematoxylin-eosin (HE) slides.

Tumor buds (TBs) and poorly differentiated clusters (PDCs) are part of the same biological phenomenon, the epithelial-mesenchymal transition (EMT). TBs are comprised of up to four tumor cells, while PDCs consist of more than five cells. They are the cross-sections of the invasive branches of the tumor visible on 3D spatial reconstructions, observed as cell clusters on a conventional histological slide. Their assessment according to the recommendations of the International Tumor Budding Consensus Conference (ITBCC) has been incorporated into the UICC guidelines in the routine evaluation of colorectal cancers (CRCs) in 2017, influencing the therapeutic approach.

Stromal AReactive Invasion Front Area (SARIFA) is a recently identified histological phenomenon where tumor cells come into direct contact with adipocytes, without the presence of interposing stromal or inflammatory cells. It has been proven that it is an independent adverse prognostic factor in gastric cancers.

2. Objectives

1. We aimed to assess the relationship between the phenomenon of Stroma AReactive Invasion Front Area (SARIFA) and tumor budding (tumor buds /TBs/ and poorly differentiated clusters /PDCs/) in esophageal cancers, as well as their relationship to the established clinicopathological factors.
2. We aimed to examine the prognostic role of SARIFA in esophageal squamous cell carcinomas (ESQCCs), prevously only studied on adenocarcinomas, by assessing SARIFA on ESQCCs beside esophageal adenocarcinomas (EACs).
3. We aimed to identify histologic markers that are able to predict LNMs in ESQCCs and EACs by the analysis of the invasion front.
4. We aimed to assess the prognostic impact of TBs, PDCs and SARIFA on the overall survival of ESQCC and EAC patients.
5. We aimed to evaluate whether the TB- and PDC-count assessed according to the protocols of the International Tumor Budding Consensus Conference (ITBCC) described in colorectal cancers is able to predict the occurence of lymph node metastases (LNMs) in gastric adenocarcinomas (GACs).

3. Methods

3.1. Case selection, gathering of data, ethical approval

Two separate cohorts were created, one cohort containing 100 esophageal cancer cases who underwent surgery in Semmelweis University between 2008 and 2021 (ethical approval: SE-RKEB 242-1/2021), excluding patients who died perioperatively and patients whose tumor showed complete remission after neoadjuvant therapy, the other cohort containing 290 gastric adenocarcinoma cases who underwent gastrectomy between 2008 and 2019 in Semmelweis University (ethical approval: SE-RKEB 245/2019), excluding patients who received neoadjuvant therapy. In the latter cohort, patients who died perioperatively were excluded from the survival analyses. The pathological assessment of the cases of both cohorts were performed in the Department of Pathology, Forensic and Insurance Medicine of Semmelweis University. The clinical histories of patients were gathered from the electronic registry of Semmelweis University (MedSol software), and the pathological data were obtained from the Department's own registry (MedRec software). Additional follow-up data was collected from the National Cancer Registry. In both cohorts, patients whose clinical data were incomplete were excluded.

3.2. Histological work-up and assessment

The esophageal cancer cohort contained cases of esophageal squamous cell cancer (ESQCC) and esophageal adenocarcinoma (EAC), defined by the UICC TNM's 8th edition which also gave base for the pathological staging of the cases. The gastric cancer cohort was comprised of intestinal, diffuse and mixed gastric adenocarcinoma cases as defined by the Lauren classification, whereas the pathological staging was performed according to the TNM. Diagnostic glass slides of the invasion front were scanned and digitized (Pannoramic 1000 scanner). TBs and PDCs were

evaluated following the hot-spot method as described by the ITBCC (in an area of 0.785 mm2 where budding activity is the highest). TBs were defined as tumor cell clusters containing up to 4 cells, whereas PDCs contain more than 5 cells, with the criterium of neither entity containing a lumen. SARIFA, which is defined as a tumor cell cluster of at least 5 cells making direct contact with adipocytes without stromal or inflammatory reaction, was evaluated on the same slide as TBs and PDCs, based on the presence of SARIFA (SARIFA+ or SARIFA−). Two independent researchers performed the evaluation in both cohorts, joined by a supervisor in cases of discrepancies to reach a consensus. Based on the total number of TBs and PDCs counted, cases were classified into TB and PDC grades (Bd/PDC 0: 0, Bd/PDC 1: 1-4, Bd/PDC 2: 5-9, Bd/PDC 3: ≥10).

3.3 Statistical analysis

Calculations and plotting were carried out in the R software environment. In order to preserve statistical power, dichotomization of the original groups in the evaluation were performed (Bd0/PDC0, Bd1/PDC1, Bd2/PDC2 into Bd/PDC low, Bd3/PDC3 into Bd/PDC high; pN0 into pN-, pN1/2/3 into pN+; pT1/pT2 into pT low, pT3/4 into pT high). Non-parametric tests were performed on the cohorts (Fisher's exact test, Mann-Whitney-U test by combining the TB and PDC grades onto one spectrum, Wilcoxon test, Kruskal-Wallis test). Survival analysis were performed using the Kaplan-Meier/log-rank test. The factors influencing the overall survival were investigated using the age-adjusted Cox proportional hazards model with backwards selection. Prognostic factors of LNMs were identified by logistic regression with backwards stepwise selection. In multiple analyses the Benjamini-Hochberg method was applied to adjust the p value. All *p*-values were calculated as two-tailed, and considered significant when $p < 0.05$.

4. Results

4.1 Cohort characteristics of esophageal cancer cases

Based on our results, SARIFA occurs significantly less frequently in ESQCCs than in EACs (p=0.0046). In the case of TBs and PDCs, no significant difference could be observed.

4.2 Relationship of the SARIFA status and the size of the tumor cell clusters in the invasion front of esophageal cancers

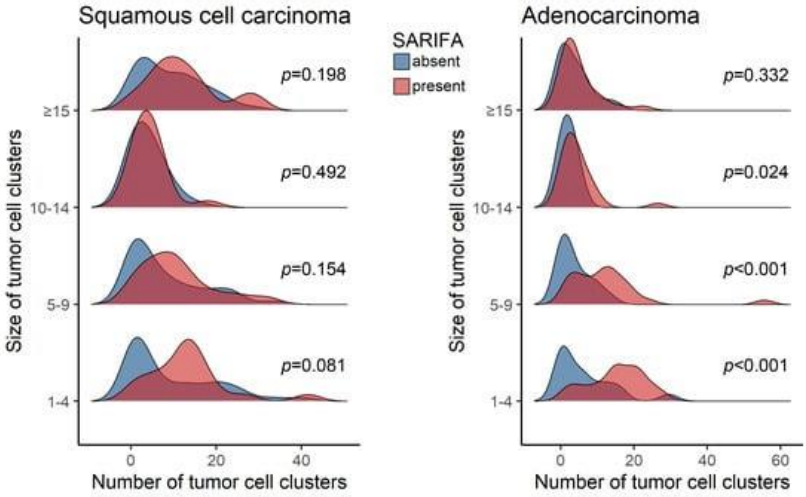

Figure 2. Tumor cell clusters of different sizes in SARIFA-positive and negative cases. Abbreviation: SARIFA—Stroma AReactive Invasion Front Area. (Jakab et al 2024.)

TBs and PDCs were combined into one spectrum for this analysis. The number of small tumor cell clusters (TBs and PDCs of up to 14 cells) is significantly higher in the invasive front of SARIFA+ EACs than in SARIFA− EACS. No such correlation was observable in ESQCCs.

4.3 Association between the TB/PDC/SARIFA status and the extent of the tumor in esophageal cancers

In EACs, high TB status and the presence of SARIFA were associated with the pT high status, i.e. the greater extent of the tumor (p=0.0162 and p=0.0012, respectively). However, the PDC status showed no significant correlation with the tumor extent. In the case of ESQCCs, no significant correlation could be observed.

4.4 LNM prediction in esophageal cancers

Based on logistic regression analyses, there is a significant positive correlation between the pN+ status and the low/high TB category in ESQCCs (p=0.0006 for the univariable and p=0.0006 for the multivariable analysis). In EACs, pN+ status was correlated with the SARIFA+ status (p=0.0054 for the univariable and p=0.0111 for the multivariable analysis). According to these results, TB is an independent prognostic factor for LNMs in ESQCCs, while in EACs, LNMs are predicted independently by SARIFA.

4.5 Analysis of the overall survival of esophageal cancer patients

Based on the Kaplan-Meier curves and the log-rank test, neither the TB-, PDC- and SARIFA-status was significantly associated with the OS in ESQCCs, nor in EACs. The Cox proportional hazards model yielded significant results when the TB and PDC counts were used as continous variables, i.e. not classified into Bd and PDC grades, using their absolute counts for the analysis instead. In ESQCCs, both the absolute TB and PDC counts showed a statistically significant negative association with the OS (p=0.0269 and p=0.0377, respectively). In EACs, a similar trend not reaching the threshold of significance was observable in the case of SARIFA (p=0.0658).

4.6 Association of TBs and PDCs with clinicopathological parameters in gastric cancers

Besides a number of factors (Lauren phenotype, R1 resection, perineural-and lymphovascular invasion, pT status, grade, UICC stage), high TB was positively correlated with the presence of LNMs ($p<0.0001$), while in the case of high PDC, this correlation was not observable.

4.7 Effects of TBs/PDCs on the LNMs of different gastric cancer subtypes

Based on the results of the multivariable logistic regression analysis, high TB is an independent prognostic factor for LNMs in both the total gastric cancer cohort ($p=0.019$) and the intestinal gastric cancer subcohort ($p=0.038$). In the case of high PDC, a trend not reaching significance was observable ($p=0.057$ for the total cohort and $p=0.075$ for the intestinal subcohort). Furthermore, the lymph node ratio (LNR, the rate of metastatic lymph nodes among the examined lymph nodes) was significantly correlated with the TB grade in both the total cohort ($p<0.0001$) and the intestinal ($p=0.012$) and diffuse ($p=0.03$) subcohorts. The PDC grade showed a significant correlation with the LNR only in the total cohort ($p=0.02$) and the diffuse subcohort ($p=0.011$) (Figure 6).

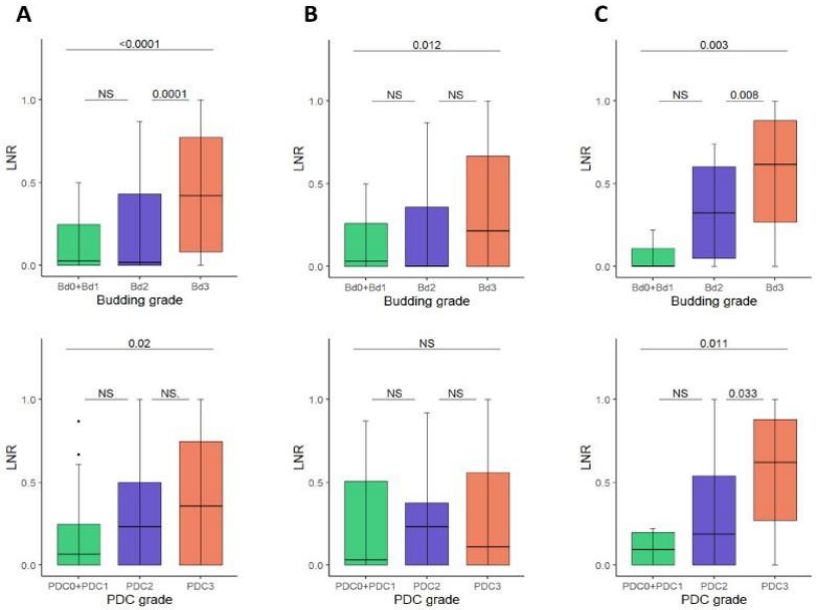

Figure 6. Association of the the lymph node ratio (LNR) and tumor budding/PDC grades. (**A**) Total cohort, (**B**) Intestinal type gastric cancers, (**C**) Diffuse type gastric cancers. PDC: poorly differentiated clusters. (Szalai et al. 2022.)

5. Conclusions

1. Tumor budding is an independent predictor of the development of lymph node metastases (LNMs) in esophageal squamous cell carcinomas (ESQCCs), while in esophageal adenocarcinomas (EACs) LNMs are predicted by the presence of Stroma AReactive Invasion Front Area (SARIFA).
2. Our study group was the first to assess the phenomenon of SARIFA on squamous cell cancers, previously only studied on gastrointestinal adenocarcinomas. We found that SARIFA occurs significantly less frequently in ESQCCs than in EACs.
3. Examining the associations of invasive patterns and clinicopathological factors in EACs, we found that SARIFA positivity is associated with a greater number of small cell clusters on the invasion front (TBs and PDCs up to 14 tumor cells) and a higher extent of the tumor (T3 and T4), the latter also being associated with a higher degree of tumor budding. No such correlation was found in ESQCCs.
4. Results of the survival analyses revealed that while the TB and PDC grades are not predictive of overall survival (OS), the absolute number of cell clusters in the categories of TBs and PDCs proved to be an independent prognostic factor of the OS of ESQCC patients. Therefore, the ITBCC criteria derived from colorectal cancers need to be re-evaluated and adapted to esophageal cancers before incorporating the assesment of tumor budding into the routine diagnostic protocols.
5. In the total gastric cancer (GC) cohort and the intestinal GC subcohort, the lymph node ratio (LNR) showed a significant positive correlation with both the TB and PDC grade, while in diffuse GCs, LNR was correlated only with the PDC grade. Moreover, based on the multivariable analysis, TB

grade proved to be an independent prognostic factor for LNM development in intestinal GCs, outperforming the PDC grade in this manner.

6. Bibliography of the candidate's publications

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