cancers

# Supplementary Materials: Prognostic Role of High-Grade Tumor Budding in Pancreatic Ductal Adenocarcinoma: A Systematic Review and Meta-Analysis with a Focus on Epithelial to Mesenchymal Transition

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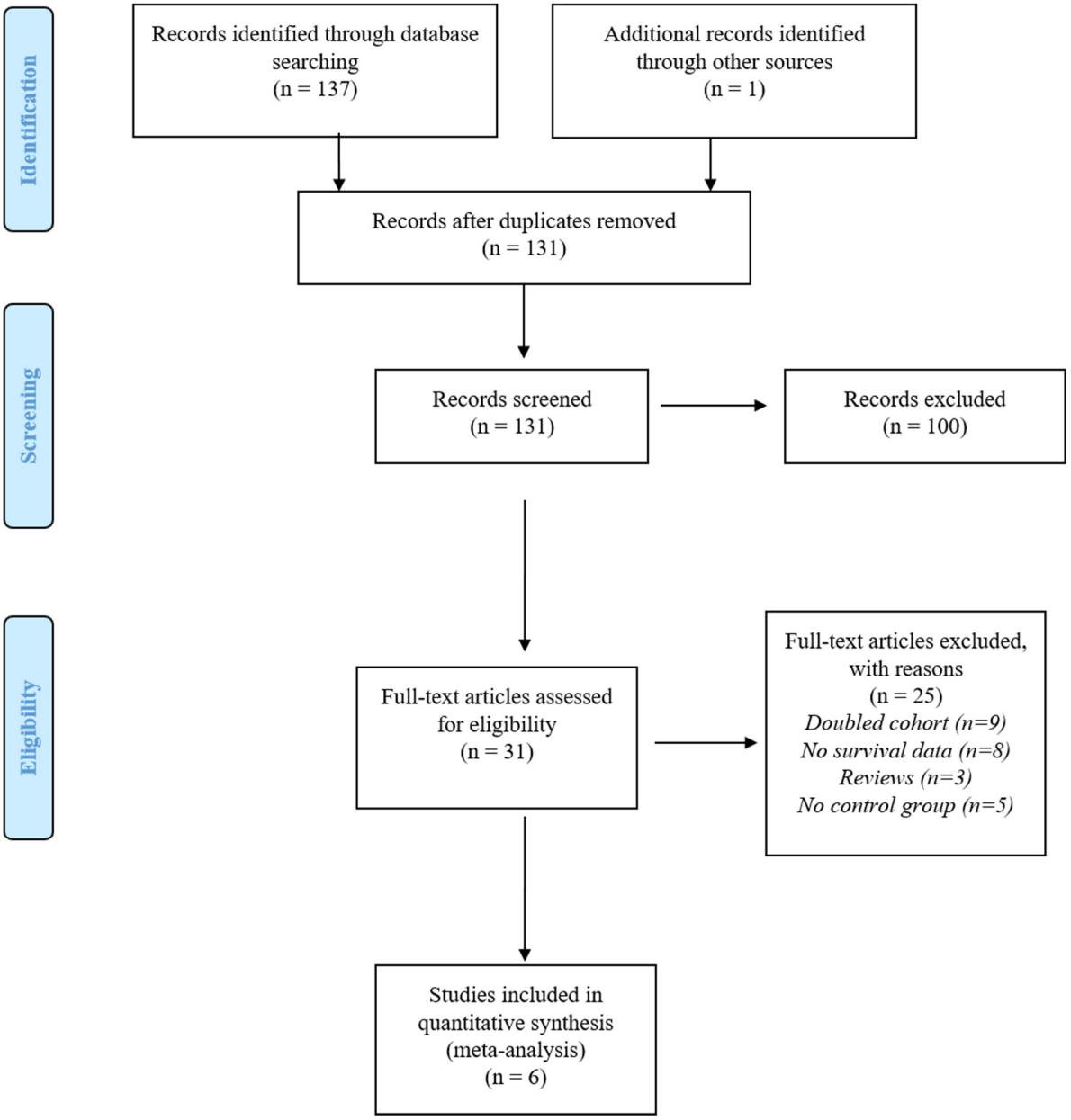


**Figure 1.** PRISMA checklist for this meta-analysis.

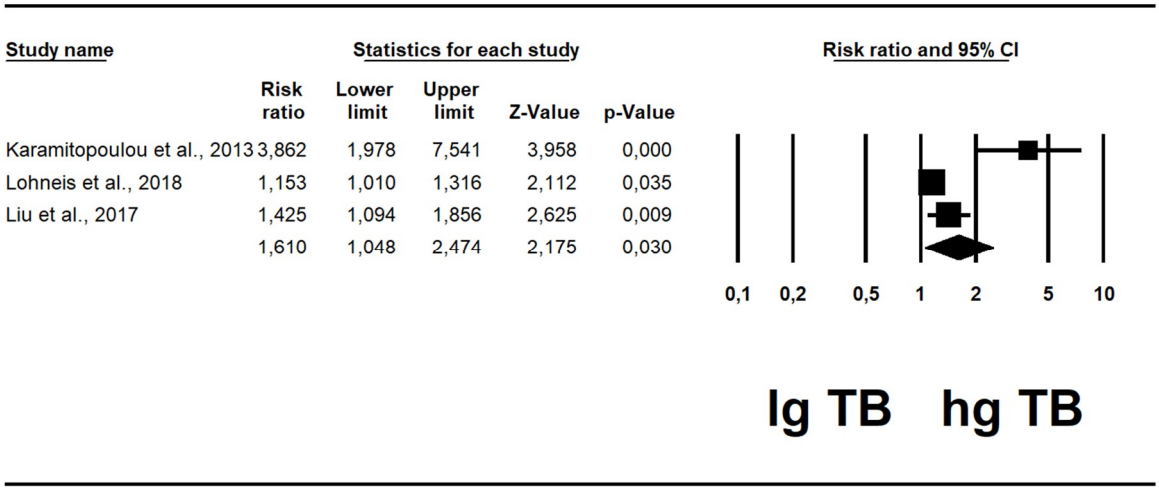


**Figure S2.** Forrest plot indicating pancreatic ductal adenocarcinoma risk ratio for recurrence in patients with high-grade tumor budding vs. low-grade tumor budding.

**Table S1.** Characteristics of the studies according to tumor budding, high-grade (Hg-TB) vs. low grade (Lg-TB).

| | | | | Hg-TB | | | | | Lg-TB | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Author, Year (Country)** | **Exclusion Criteria** | **Methods of TB Assessment** | **Analysis of EMT-associated variables** | **Number of Participants** | **N. of Females (%)** | **Mean Age ± SD** | **pT** | **LNM** | **Number of Participants** | **N. of Females (%)** | **Mean Age ± SD** | **pT** | **LNM** | **Number of Adjustments** | **NOS** | **Mean Follow-Up Period (Months)** |
| Chouat, 2018 (Tunisia) [1] | M1 | 1 | cytokeratin, vimentin | 28 | NA | NA | NA | NA | 22 | NA | NA | NA | NA | 4 | 8 | 34 |
| Karamitopoulou, 2013 (Greece) [2] | NS | 2 | NA | 83 | 45.8% | 68 (44-84) | pT1–2: 6.4%; pT3–4: 93.6% | No: 17.1%; Yes: 82.9% | 34 | 44.1% | 65 (range: 34-83) | pT1–2: 18.2%; pT3–4: 81.8% | No: 21.1%; Yes: 78.8% | 4 | 8 | >36 |
| Liu, 2017 (China) [3] | R2 resection, M1, DPC | 1 | Expression of cytokeratin | 20 | 35.3% | 60.8±11.7 | pT1–2: 17.6%; pT3–4: 82.4% | No: 35.3%; Yes: 64.7% | 26 | 37.9% | 60.9±11.2 | pT1–2: 10.3%; pT3–4: 89.7% | No: 37.9%; Yes: 62.1% | 0 | 6 | NS |
| Lohneis, 2018 (Germany) * [4] | Lack of follow-up information | 3 | NA | 57 | 67% | <65 y: 58%; ≥65 y: 42% | pT1–2: 14%; pT3–4: 86% | No: 25%; Yes: 75% | 116 | 55% | <65 y: 66%; ≥65 y: 34% | pT1–2: 10%; pT3–4: 90% | No: 24%; Yes: 76% | 7 | 8 | >36 |
| O'Connor, 2015 (Canada) [5] | NS | 2 | NA | 32 | NA | NA | NA | NA | 136 | NA | NA | NA | NA | 0 | 6 | >36 |
| Zhang, 2016 (China) [6] | No diagnosis of PDAC | 4 | NA | 31 | NA | NA | NA | NA | 28 | NA | NA | NA | NA | 2 | 6 | 15 |
| **Total Studies (weighted values)** | **-** | **1, 2: 2 studies; other: 1 study each** | **Vimentin: 1 study** | **251** | **51.9%** | **66.6 y** | **pT1–2: 10.7%; pT3–4: 89.3%** | **No: 21.9%; Yes: 78.1%** | **362** | **50.6%** | **63.2 y** | **pT1-2: 11.9%; pT3-4: 88.1%** | **No: 25.6%; Yes: 74.4%** | **Range: 0–7** | **Median: 7** | **Mean: 31.4** |

Abbreviations: EMT: epithelial.to-mesenchymal transition; M1: metastatic disease; NS: not specified; NA: not available/ not assessed; DPC: death for post-operative complications; PDAC: pancreatic ductal adenocarcinoma; pT pathologic stage; LNM: lymph node metastasis.

Methods of tumor budding assessment:

(1) Tumor budding was defined according to the consensus definition as the presence of de-differentiated single cells or small clusters of up to 5 cells in the tumor stroma (at the center or the periphery of tumor). More than 10 high-power field (HPF) were examined. The HPF surface was 0.19 $mm^2$. Tumor budding were categorized into low-grade (if 1 HPF comprised between 1 and 9 buds) and high-grade (if 1 HPF contained > 10 buds);

(2) Tumor budding was defined according to the consensus definition as the presence of de-differentiated single cells or small clusters of up to 5 cells in the tumor stroma (at the center or the periphery of tumor). The number of buds was counted using the 40× magnification (surface 0.49 $mm^2$) in 10HPFs (400×) and the scoring was performed based to the average number of buds. Cases with an average of 0–10 buds across 10 HPFs were defined as low-grade budding, while cases with an average of >10 buds across 10 HPFs were defined as high-grade budding;

(3) The quantification approach published by ITBCC for reporting tumor budding in colorectal cancer was used. Therefore, tumor buds were counted in one field of view (20× objective, 22 mm field of view ocular) at the "hotspot" of budding and the number of tumor buds per 0.785 $mm^2$ was determined using a normalization factor. Budding was grouped according to ITBCC into Bd 1 (0–4 buds), Bd 2 (5–9 buds) and Bd 3 (10 or more buds). Bd 1 and Bd 2 formed the category of low-grade tumor budding, while Bd 1 was considered as high-grade tumor budding. Additionally, in areas of maximal tumor budding, detected at scanning magnification, the number of tumor buds was counted in 10 high-power fields (1 HPF 0.238 $mm^2$, 40× objective; 22mm field of view ocular). Tumor budding was not classified into peritumoral and intratumoral;

(4) Tumor budding was defined according to the consensus definition as the presence of de-differentiated single cells or small clusters of up to 5 cells in the tumor stroma (at the center or the periphery of tumor). More than 10 high-power field (HPF) were examined. The HPF surface was 0.19 $mm^2$. Tumor budding were categorized into low-grade (if 1 HPF comprised between 1 and 17 buds) and high-grade (if 1 HPF contained > 17 buds).

**Table 2.** Methodological quality of cohort studies included in the meta-analysis*.

| First Author, Publication Year | Representativeness of the Exposed Cohort | Selection of the Unexposed Cohort | Ascertainment of Exposure † | Outcome of Interest Not Present at Start of Study †† | Control for Important Factor or Additional Factor ††† | Assessment of Outcome | Follow-Up Long Enough for Outcomes to Occur †††† | Adequacy of Follow-Up of Cohorts | Total Quality Scores |
|---|---|---|---|---|---|---|---|---|---|
| Chouat, 2018 [1] | * | * | * | * | ** | * | - | * | 8 |
| Karamitopoulou, 2013 [2] | * | * | * | * | * | * | * | * | 8 |
| Liu, 2017 [3] | * | * | * | * | - | * | - | * | 6 |
| Lohneis, 2018 [4] | * | * | * | * | * | * | * | * | 8 |
| O'Connor, 2015 [5] | * | * | * | - | - | * | * | * | 6 |
| Zhang, 2016 [6] | * | * | * | - | * | * | - | * | 6 |

Original studies were analyzed in the quality assessment.

* A study could be awarded a maximum of one star for each item except for the item Control for important factor or additional factor. The definition/explanation of each column of the Newcastle-Ottawa Scale is available at http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm.

† For this index, one star was given if in Method section tumor budding assessment was clearly defined (e.g.: microscopic definition and method of count of buds)

†† Being outcome of interest mortality, we took as outcome of interest for assessment of quality if the risk of recurrence was assessed.

††† A maximum of 2 stars could be awarded for this item. Studies that controlled their survival analyses for at least two confounders received one star, whereas studies that investigated also epithelial-to-mesenchymal transition markers, an additional star.

†††† A cohort study with a mean/median follow-up time ≥3 y (36 months) takes one star.

**Table 3.** Type and number of adjustments (in addiction of tumor budding) for each study.

| First author, Publication Year | Adjustments | Maximum Number of Adjustments |
|---|---|---|
| Chouat, 2018 [1] | Age, tumor size, R status, vimentin expression | 4 |
| Karamitopoulou, 2013 [2] | R status, pN, L status, chemotherapy | 4 |
| Liu, 2017 [3] | - | 0 |
| Lohneis, 2018 [4] | Treatment, pT, pN, G, R status, age, sex | 7 |
| O'Connor, 2015 [5] | - | 0 |
| Zhang, 2016 [6] | AJCC, parasympathetic neurogenesis | 2 |

Abbreviations: R status: status of resection margins, pT: pathologic tumor stage; pN: pathologic status of lymph nodes; G: tumor grading, AJCC: comprehensive stage according to the American Joint Commission on Cancer staging system.

## Reference


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