Research Square

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Tumor budding in relation to vascular invasion patterns and patient survival in breast cancer

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Abstract

Background

Tumor budding (TB) represents an important mechanism for metastases where epithelial cancer cells detach from the primary tumor as individual cells or small groups of tumor cells and gain mesenchymal-like features that allow them to migrate.

Methods

Here, we looked at TB counts in breast cancer, in relation to lymphatic vessel involvement (LVI) and blood vessel invasion (BVI), different clinico-pathological features, most frequent site-specific metastases and patient prognosis. We studied a population based, retrospective cohort with 247 invasive breast cancers. Full sections from surgical specimens were prioritized with dichotomization for TB as high (upper quartile) and low counts.

Results

High TB values were strongly associated with traditional histopathological features like large tumor diameter, higher grades, and positive lymph nodes ($p \leq 0.003$). A logistic regression analysis showed that high TB counts predicted LVI (OR 2.5 CI 1.3–4.9, $p = 0.008$). In contrast, BVI was predicted by higher grades (OR 2.0 CI 1.3–16.2, $p = 0.015$). LVI showed strong association with bone and lung metastases. High TB and BVI both demonstrated strong relations to bone, lung and liver metastases, although only BVI was significantly associated with brain involvement. In a multivariate analysis, high TB count displayed reduced disease specific survival (HR 2.1, $P = 0.033$) together with positive lymph nodes (HR 4.9, $P < 0.001$).

Conclusions

The present study points to a relation between high TB and LVI, and highlights TB as an important biomarker for tumor spread and patient survival. BVI is also related to breast cancer spread in tumors with aggressive features like higher grade or a basal-like phenotype.

Introduction

Tumor budding (TB) has been defined as single cells or small clusters of tumor cells that have detached from the invasive front of the tumor (1). TB may be an expression of epithelial mesenchymal transition (EMT) and has been perceived as a favorable prognostic factor for severe prognosis in several types of neoplasms (2). Notably, there have been many studies of TB in colorectal carcinoma, and TB has been reported as an independent prognostic predictor for lymph node metastasis and survival in pT1 and

stage II colorectal cancer, respectively. This feature has been included as a prognostic biomarker in the AJCC-TNM classification(2).

Many studies have previously reported a prognostic impact of vascular invasion based on H&E staining, but these reports have rarely distinguished between the types of vessel invasion, whether lymphatic or blood vessels. In a prior study using endothelial markers CD31 and D2-40, we identified lymphatic vessel invasion (LVI) in 25% and blood vessel invasion (BVI) in 15% of breast cancer cases, and BVI was especially related to aggressive tumor features (3).

The impact of TB has been investigated in some studies of breast cancer, and an association with lymph node metastasis and vessel invasion have been demonstrated, but with diverse relations to basic prognostic factors as histologic grade or tumor diameter (4–9). However, in most of these studies, no attempt has been made to discriminate between LVI or BVI. The aim of this study was to examine TB counts in a breast cancer cohort and search for correlations to lymph vessel invasion (LVI) and blood vessel involvement (BVI). Furthermore, we have looked at the relation to clinico-pathologic features and the most common site-specific metastases as well as patient survival.

Materials and Methods

Patient series

We recruited female patients from Vestfold County (230,000 females) in Eastern Norway for this study. The Norwegian Breast Cancer Screening Program offers mammography. Between 2004–2009, a total of 37,977 women took part in the program. During this time, we found 204 invasive cancers by screening, and 85 invasive interval breast tumors.

Data regarding completeness were obtained from the Cancer Registry of Norway. Patients (age 50–69 years) were Caucasian women living in Vestfold County. We decided to exclude 35 invasive lobular cases, due to often dyscohesive or single cell growth and cause a morphological challenge with respect to TB counts.

Three individuals were excluded for the following reasons: one case with no remaining tumor tissue for further analysis, one tumor was classified as a malignant phyllodes tumor, and one patient had widespread metastases at diagnosis. Four women in this cohort showed bilateral tumors, and the Nottingham Prognostic Index (NPI) was used to determine which tumor had the poorest prognosis in three of the patients. For the last patient, both tumors had identical NPI scores, and the tumor with the highest Ki67 level was included. In total, 247 cases (177 screen-detected and 70 interval cancers) were included in the final analysis. We collected clinical information from medical records with the most recent follow-up recorded in November 2025. The median follow-up time was 136 months (range 6-240 months).

Histopathological data

Histological grading was done according to the Nottingham criteria. Tumor diameter was measured microscopically in mm, and lymph node status was included from the pathology report.

Evaluation of TB and immunohistochemistry

We used one full section from each surgical specimen for evaluation in this study. Due to poor fixation, or limited/no remaining tumor in the main specimen, diagnostic biopsies were used for TB assessment in some cases (10%). We decided to include biopsies for TB assessment since 3–4 needle biopsies were taken from each patient, representing satisfactory tumor volume with the tumor periphery visualized in the sections. Furthermore, it was important to include these cases to avoid selection bias in the patient series.

Evaluation of TB counts. TB were detected as 1 to ≤ 5 adhesive tumor cells in the tumor periphery. After careful inspection of the tumor boundary, we marked a hot-spot area with the largest number of buds. We used an eye-piece graticule (10x10 gridlines; 0.31x0.31 mm; total 0.096 mm2) and the number of buds was evaluated separately in each case. The area with the highest number of buds was defined as the hotspot tumor count (HTB count) **(**Fig. 1**)**. HTB counts from all cases were calculated and dichotomized as high TB counts (upper quartile) or low levels.

For evaluation of inter-observer agreement, TB counts in 50 cases were evaluated by two pathologists (YC and TAK), showing good agreement with a kappa value of 0.61.

Immunohistochemistry and evaluation of staining for LVI (D2–40) and BVI (CD31) have been described previously (10) using whole sections. Localization of VI was predominantly peritumoral. Similarly, evaluation of ER, PR, and HER2 status has been recorded and described (11). In addition, approaches for assessing CD163 and TILs have also been presented by a quantitative method applied on tissue microarray slides (12, 13). For definition of the basal-like phenotype, expression of CK5/6 and/or P-cadherin was employed (14) Surrogate markers representing molecular subtypes of breast cancer were used in line with the St Gallen consensus from 2013 (15). Threshold for ER and PR was 1% for this study.

Statistical methods

All statistical analyses were carried out with IBM SPSS Statistics, version 25.0 (Armonk, NY: IBM Corp.). A two-tailed p-value below 0.05 was regarded as statistically significant. Relations for categorical variables were evaluated using Pearson's chi-square (χ^2) test. Kaplan-Meier curves were generated for survival analyses (log-rank test for differences), death from breast cancer was used as endpoint (disease specific survival, DSS). Entry date was the time of diagnosis. Patients who died from other causes were censored at the date of death. The Cox´ proportional hazards method was used for multivariate survival analyses. Variables were visually examined by log-minus-log plots to check

proportionality assumptions. A multivariate analysis was conducted for TB counts together with standard prognostic variables. A multivariable logistic regression analysis was applied to assess the ability of high TB counts to predict LVI or BVI. For statistical analysis, TB counts were dichotomized as high TB counts (upper quartile) and low TB counts. After exclusion of some cases (see above), a total of 247 patients were available for survival analyses in the current study

Results

TB counts and clinico-pathological factors

Table 1 shows a strong correlation between high HTB counts and basic clinico-pathological factors such as high tumor grade, larger tumors and lymphatic spread. Moreover, there is a clear connection between HTB counts and both LVI and BVI. No other significant relations were found, although cases with high Ki67 and the presence of basal-like features showed a trend to higher budding counts. Additionally, HTB counts were tested in relation to upper quartile counts of TIL subsets, CD3, CD4, CD8, FOXP3, CD45 and CD163 macrophages without any significant associations for high TB counts. Association analyses did not demonstrate any significant relation between high TB counts and molecular BC subtypes in this series.

TB counts and different categories of vessel invasion

High HTB counts showed a strong association with BVI and LVI (Table 1). We found 79 patients with LVI and/or BVI based on immunohistochemistry (D2-40 or CD31) in our patient cohort. More specifically, 39 patients (16%) had only LVI, 16 patients (6%) had only BVI, and 24 patients (10%) had combined BVI/LVI.

Cases with high HTB counts showed a strong association with all combinations of vessel invasion (38-42%) compared with cases without vessel invasion (15%) (Table 2). In a multivariate logistic regression model with HTB counts, tumor diameter, histologic grade and lymph node status as explanatory variables, HTB counts were a strong predictor for LVI (OR 2.5 CI 1.3-4.9, p=0.008) together with higher grades (2-3) (OR 2.3 CI 1.1-5.1 p=0.033) and lymph node metastases (OR 2.0 CI 1.1-3.9 p=0.033). In contrast, with the same explanatory variables, higher grades (2-3) were the only significant predictor for BVI (OR 2.0 CI 1.3-16.2, p=0.015).

TB counts, BVI, LVI and distant metastases

We recorded distant metastases in 38 patients (15%), with more than one metastasis in 28 patients, and only local recurrences in 8 patients (3%). The most common distant metastases were recorded in bone (26 cases), liver (20 cases), lungs (19 cases) and brain (11 cases). High HTB counts showed a significant correlation to metastases in bone, liver and lungs (P>0,011), whereas LVI showed significant correlation to bone and lung metastases (P>0.003). In contrast, BVI was strongly related to metastases in all four most common distant metastatic sites (p<0.007). High HTB counts together with BVI were

strongly associated with liver metastases (p<0.001) in contrast to LVI (p= 0.12), whereas only patients with BVI showed a strong relation to brain metastases (p=0.007).

Survival analysis

This series had a median follow-up time of 135 months (range 2-240 months), and 37 patients died of breast cancer in the study period.

HTB counts demonstrated a strong relation to decreased DSS (p= 0.001), as well as the presence of LVI (p=0.016) and BVI (p< 0.001)in univariate analysis **(Figure 2)**.

We performed a multivariate Cox' regression model for DSS with traditional histopathological variables and HTB counts. We found that high HTB counts showed a significant association with reduced DSS (HR 2.1, p= 0.033) together with lymphatic spread (HR 4.9, p<0.001) (Table 4). With inclusion of molecular subtypes (Table S1) and subtypes together with LVI and BVI (Table S2), in two other models, high HTB counts and lymph node invasion were related to reduced DSS in the first, while positive lymph nodes and BVI were related to reduced DSS in the second model.

Discussion

Several studies have demonstrated a significant relation between TB counts and lymphovascular invasion, without discrimination between LVI and BVI. Here, we found significant associations between high TB counts and LVI as well as BVI. Whereas high TB counts predicted LVI in a multivariable logistic model, histologic grade was the only predictor for BVI, indicating some differences between these two processes. This finding may suggest that concurrent LVI and BVI in some patients may contribute to an association between high TB counts and BVI. We have only found one previous study of breast cancer with a distinction between LVI and BVI (16), where an association with high TB counts and LVI was found, but not with BVI. The authors speculated that the tumor buds found their way to metastases through lymphatic spread instead of blood vessel invasion.

However, other tumor properties in addition to TB counts may support direct capillary blood invasion and early systemic disease in some cases of breast cancer. Angiogenesis is one of the hallmarks of cancer and has been linked to an angiogenic breast cancer phenotype with glomeruloid microvascular proliferation (17). These tumors often show a basal-like differentiation with high histologic grade, TP53 mutations and a positive hypoxia signature (17, 18). In a previous study of this series, we demonstrated that BVI independently predicted a basal-like phenotype, but LVI did not show this prediction (3). This may indicate that other mechanisms as angiogenesis are more important than TB counts in some aggressive breast tumors.

High TB counts showed a strong relation to the basic histologic factors like larger tumor diameter, higher histologic grade and positive lymph node status. In line with this, other studies (6, 19, 20) have found associations with grade and size aligning with our findings. TB is connected to an EMT-like phenotype in

breast cancer, and tumors with high TB counts show reduced cell adhesion, increased migration with invasive properties, and reduced survival (21). Similarly, the Nottingham prognostic index (NPI) with graded weighting of the basic factors predicts reduced survival, and our findings of an association between high TB counts and all three traditional prognostic markers are well compatible with this.

According to the ‘seed and soil’ hypothesis, primary breast tumor cells act as seeds, whereas site specific metastatic tissue microenvironment act as the soil (22). Breast cancer cells exhibit an organ-specific dissemination and metastasize primarily to bone, liver, lung and brain with decreasing frequencies. The relation between high TB counts and the three most frequent metastatic sites (bone, lungs and liver) highlights TB as an important biomarker for tumor spread.

However, our findings of no correlation between high TB counts and brain metastases could possibly indicate different mechanisms for tumor spread. Although EMT may be pivotal for breast cancer brain metastases, an interaction between several factors like angiogenesis, EMT, metabolic reprogramming and remodeling of the extracellular matrix are of importance in breast cancer metastases (23). Furthermore, certain molecular subtypes, like the HER2 type and basal-like breast cancer have been linked to a greater risk of brain metastases (24, 25). In line with this, we have demonstrated a strong correlation between BVI and basal-like or HER2 positive tumors compared with luminal/HER2 negative cases in a prior study (3). This may indicate that BVI is of particular importance for patients with brain metastases.

High TB counts were related to reduced survival in a multivariate setting in this study. This is in line with a meta-analysis concluding that high tumor budding is correlated to decreased overall survival and progression free survival, underscoring TB as pivotal actor in breast cancer prognosis (26). Based on this, TB counts could possibly assist in further treatment decisions in some cases of breast cancer.

The method used in this study may represent a limitation, as there is currently no standardized approach for assessing tumor budding in breast cancer. In support of the method used, it is very similar to the standardized evaluation used for budding in colon cancer within a similar tumor area (a selected hotspot examined at 20 x objective) at the invasive tumor front. Another potential limitation is the use of routine histological staining rather than immunohistochemical staining with an epithelial marker. Immunohistochemistry may be useful in cases with extensive inflammation, glandular fragmentation or many reactive cells in the tumor periphery. Nevertheless, meta-analyses have demonstrated comparable prognostic value between routine staining and immunohistochemical methods (27). We could also have considered the use of a digitalized approach with automated bud counting, which could potentially provide a more objective quantification. Although several algorithms for tumor budding assessment have been evaluated, substantial methodological differences remain among previous, and further standardization and validation is needed (2).

Conclusions

In summary, this study indicates that high TB counts were strongly associated with traditional histopathological features like large tumor diameter, higher grade and positive lymph nodes. High TB count predicted LVI, but not BVI in a multivariate regression model. For BVI, there are probably other factors like angiogenesis that stimulate intravasation in tumors with aggressive features, such as activated EMT. This study showed a relation between high TB and the three most common metastatic sites (bone, lungs and liver), which highlights TB as an essential determinant for tumor spread and metastases. However, for brain metastases, BVI is possibly more crucial compared with high TB counts. Furthermore, high TB counts were related to reduced survival analyses.

Declarations

Acknowledgements

Not applicable

Author Contributions

Tor Audun Klingen: Writing original draft, Review and editing, Project administration, Methodology, Investigation, Formal analysis and Data curation. **Ying Chen**: Writing original draft, Methodology, Data curation **Hans Aas**: Methodology, Data Curation **Lars A. Akslen**: Writing original draft, Supervision, Methodology, Formal analysis, Data curation.

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Data availability

The data used to support the results of this study can be obtained from the authors.

Ethics approval and consent to participate:

The study adheres to the Declaration of Helsinki. The study was approved by the Regional Ethics Committee for medical and health research of Eastern Norway: Regional Etisk Komite, Gullhaugveien 1, 0484 Oslo (reference #2018/1102). The Regional Ethics Committee waived the requirement for informed consent. Data used were deidentified in this study.

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests

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Tables

Tables 1 to 4 are available in the supplementary files section

Figures

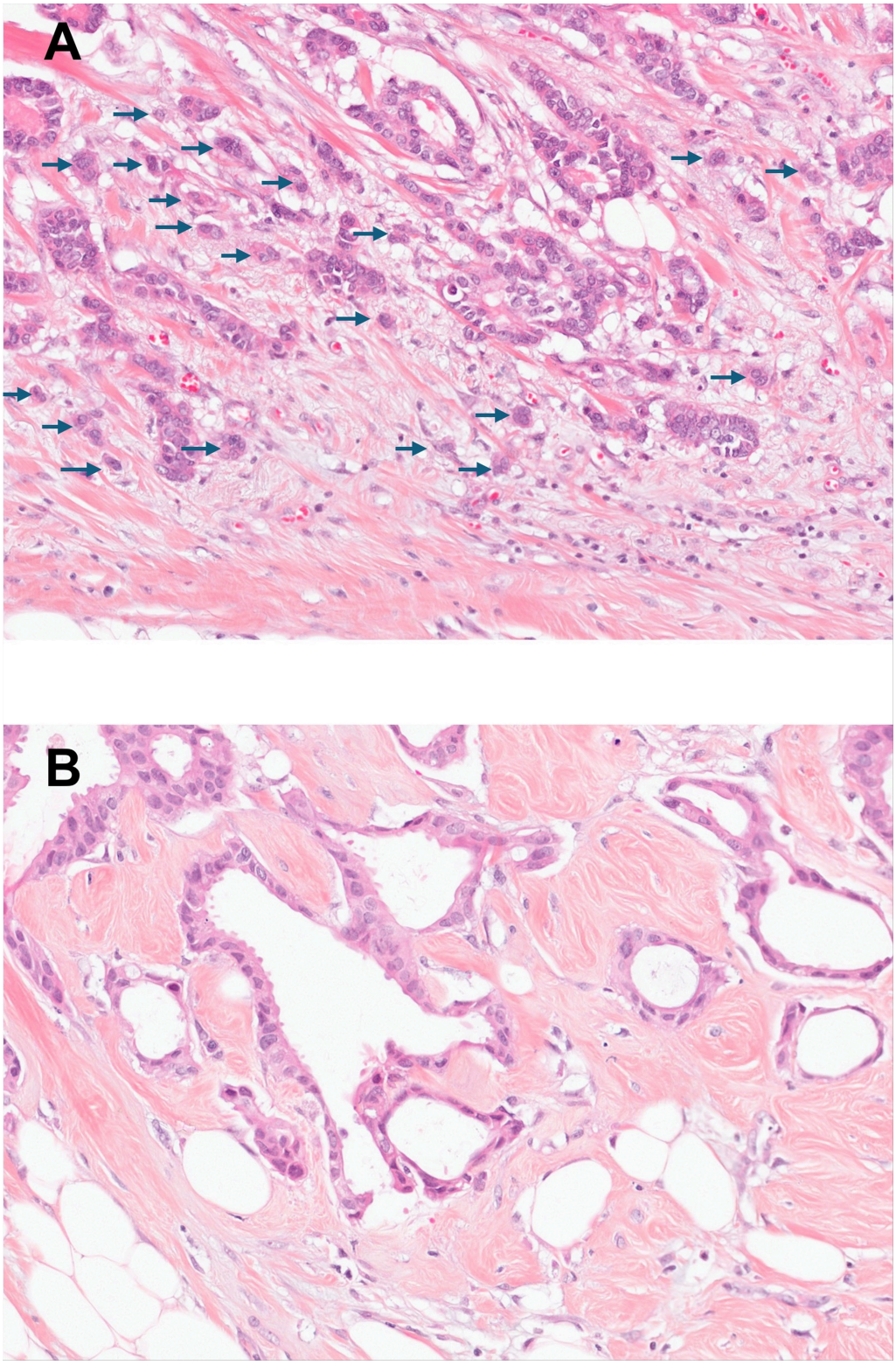


Figure 1

Histological images with many tumor buds marked with arrows (A) and no tumor buds (B). Magnification x 200.

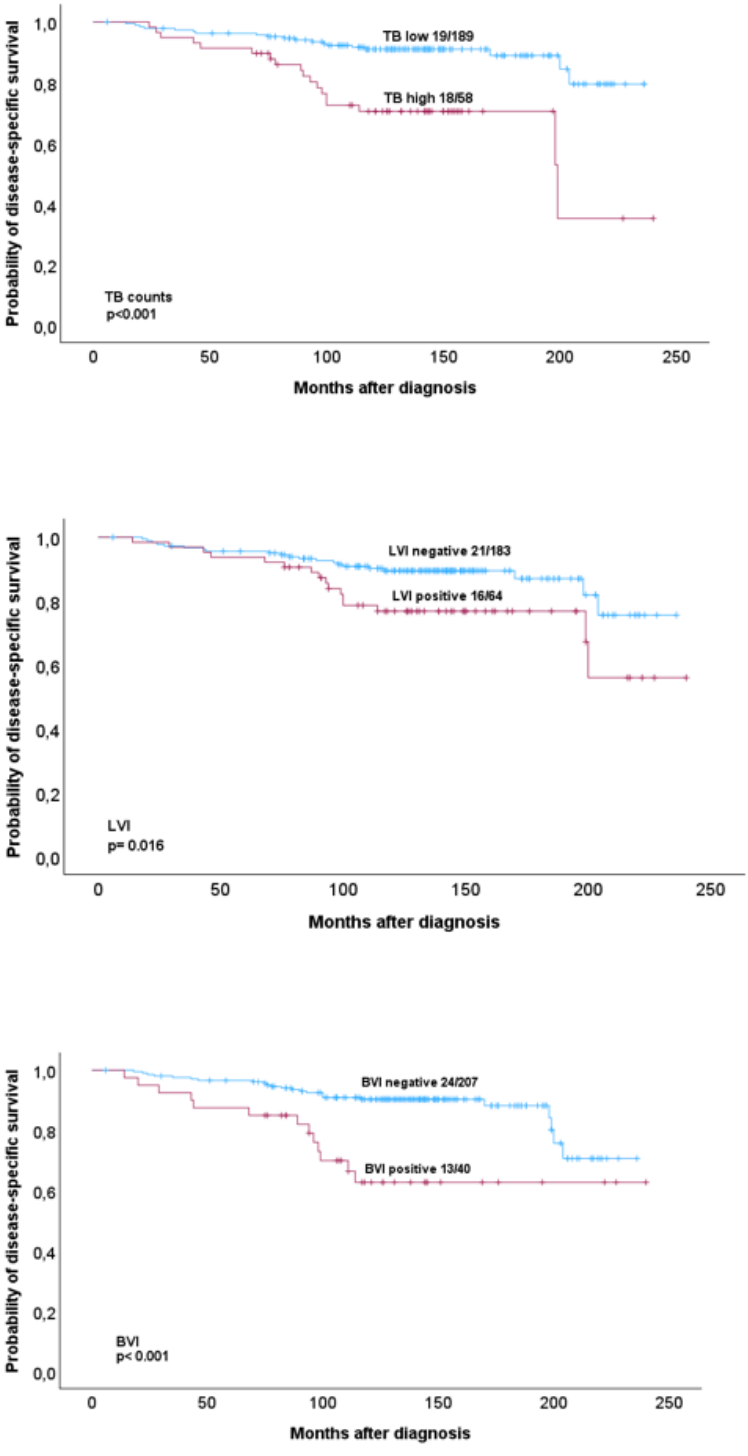


Figure 2

Estimated disease-specific survival according to presence of hotspot tumor budding (HTB), blood vessel invasion (BVI) and lymph vessel invasion (LVI). Survival curves are estimated by the Kaplan-Meier method using the log-rank test for differences between subgroups. For each category, number of events/total number of cases are given.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx
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