**ABSTRACT**

Tertiary lymphoid Structures (TLS) mediate local antitumor immunity. Since cancer immunotherapy, TLS interests grown considerably. We examined TLS and tumor stromal blood vessels for BC molecular subtypes associated with recurrence, lymphovascular, (LV), and perineural invasion (PnI). Methods:undred BC specimens were microscopically assessed for TLS high endothelial venules and BC tumor stromal immature and mature blood vessels. Statistical study linked microscopic data to recurrence, LV, and PnI. Results: TLS subgroups have higher LV, PnI, and correlation (p<0.001), except for Lu-minal A type. LV and PnI rise in HER2+/TLS- subgroups (p<0.001). TLS/LV+ had the highest recurrence risk. The TLS subgroups are related to tumor grade and LV/PnI (p<0.001), unlike other TLS subgroups. PnI but not LV are related to TLS+/TLS- subgroup recurrence (p<0.001). Conclusion: TLS affect BC molecular subtype recurrence, LV, and PnI differently. Its antitumor immunity mediator seems closely associated to tumor aggressiveness and stromal vessel blood flow.

**RESULTS**

All patients aged between 43 and 70 years old presented with TLS while the patients over 70 years old were not able to detect external TLS. Despite the fact that there were not any direct correlations between TLS, IMBV_CD45+CD3- and BMI, we considered it important to mention that overweight (BMI>24) with Luminal A/B had the highest IMBV_CD45+CD3- vessel density, and this was statistically significant (p<0.026). The finding suggests that suboptimal tissue may promote the formation of new stromal vessels due to Luminal A/B progression independently of obesity. When we assessed the TLS, Luminal A/B cases subgroup, we found that BMI was significantly correlated to IMBV_CD45+CD3- tumor stroma vessel density (p<0.019). For the TLS+ A/B subgroup, the Luminal B/B subgroup was one of the two BC molecular subgroups together with the TNBC-BG subgroup with a high percentage of TLS positive cases. Global analysis of both TLS and TLS–/TLS+ BC cases revealed significant correlations between TLS, IMBV_CD45+CD3- (p<0.17), and IMBV_CD45+CD3- stromal blood vessels (p<0.16). In the TLS positive Luminal A/B BC subgroups, patients of younger age developed a higher number of IMBV_CD45+CD3- stromal blood vessels (p<0.011) compared to elderly patients. No significant influence of TLS presence was found on lymphovascular invasion, perineural invasion, or recurrence related to both types of tumor stromal blood vessels. The TLS negative group was characterized by different data compared to TLS positive group. Loss of TLS1A3 induced a significant peripheral invasion for elderly patients (p=0.024) being strongly correlated to metastasis status (p=0.019). Perineural invasion was significantly associated with lymphovascular invasion in TLS negative patients (p=0.19), but not with recurrence rate (p=0.85). Recurrence rate was highly influenced by BMI (p<0.001) and tumor grade (p<0.001). But not for lymphovascular or perineural invasion.

**DISCUSSIONS**

The recurrent and metastatic breast cancer phenotype is a part of a larger retrospective study including 250 breast cancer female patients enrolled (PASCA). Recurrences occurred between 2003 and 2013 from young age between 20 and 65 years old and after to all local and distant recurrence cases on histopathology. 6 of TLS subgroups were considered. In our study, recurrence. Immunohistochemical double immunoreaction was applied for a differential assessment of BC triple negative and invasive tumour blood vessels. All used clones were from monoclonal antibodies (clone, 3E10, 3A4, 4A4, 3A9, 2E1, LE10, LM1, III, A2, III) and used on 20–35 μm thick sections immunostained at a dilution of 1:100. The stained slides were analyzed at the Bioimage Therapeutic Recurrence Panel (BTP) of the TIMI-30. TLS were considered to be positive when at least 5% of the cancer cells were positive in a given section. The staining intensity was semiquantitatively scored with the following grades: 0 = no staining; 1 = weak staining; 2 = moderate staining; 3 = strong staining. The protocol for the research used was approved by the Ethics Committee of the University Timisoara, Romania. According to the protocol for the research, all patients were included in the study. For statistical analysis, the following programs were used: SPSS v. 21.0 and R v. 3.1. The statistical significance was considered for p < 0.05.

**CONCLUSIONS**

The limited literature described above related to TLSs' different impacts on BC molecular subtypes and the TLS interplay with stromal vascular components determined the research direction of this study. We aim to study the interplay between TLS and tumor stromal blood vessels (immature=CD45+CD3- vs. mature=CD45+CD3+), to find if this interrelation is BC subtypes specific and may influence lymphovascular and perineural invasion and recurrence.