Editorial

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Prognosis assessment by pathologist: Is the detection of intratumoural tertiary lymphoid structures a reliable tool?☆

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Carcinogenesis is a complex multi-step process which reflects genetic alterations that drive the progressive transformation of normal human cells into highly malignant derivatives.[1] During this process, the immune system plays an important role in counterattacking the formation and progression of incipient neoplasia, late-stage tumours, and micro-metastases. This phenomenon, known as immunosurveillance, is triggered by the generation of tumour-associated antigens (TAAs) by the cancer cells through their malignant transformation. The expression of these neoantigens elicits specific immune responses favouring the recruitment and activation of both T lymphocytes and B lymphocytes, which are primed against TAAs in draining lymph nodes or tertiary lymphoid structures (TLSs) within the tumour. Regrettably, the cancer cells are able to develop some capabilities to avoid recognition and eradication by the immune system.[1]

The development of immunotherapy to effectively treat, and even cure, several previously untreatable cancers has been a major advance in clinical oncology and has generated huge interest among the different specialists devoted to the fight against cancer.[2] There is a growing awareness about the clinical relevance of understanding the complex interaction between the host immune system and their cancer through the different stages of the disease.[3] Several authors have focused their interest in evaluating the prognostic role of intratumoural TLSs.[4] The presence of these TLSs varies among different tumours, being present in the majority of patients with colorectal, ovarian and non-small-cell lung cancer (NSCLC), but rarely seen in others such as renal cell carcinoma.[5] Mature TLSs are composed by a T-cell zone with mature dendritic cells and a follicular zone in which B lymphocytes actively proliferate and differentiate in germinal centres. They are thought to play a pivotal role in the antitumoural immune responses by allowing the generation of effector and central memory T cells and plasma cells, and the presence of high densities of TLS has been associated with a favourable prognosis in several malignancies including breast, melanoma, colorectal, NSCLC, among others.[3,5,6] Regrettably, their role in patients with hepatocellular carcinoma (HCC) is still unknown.

The current issue of *Journal of Hepatology* publishes an interesting study by Calderaro *et al.* who aimed to elucidate the role of TLSs in HCC by evaluating the prognostic significance of both intratumoural and non-tumoral TLSs in a series of 273 patients with HCC treated by surgical resection in a French centre. Their results were further validated by gene expression profiling using a public data set composed of 225 patients with HBV-related HCC who underwent surgical resection in the People's Republic of China (LCI cohort).[7] Pathological examination identified TLSs in 129 tumours (47%), and their detection was associated with tumour size (≤50 mm) or histological subtype different to macrotrabecular-massive subtype. Notably, the aetiology of the underlying liver disease was not associated with the presence of TLSs. Univariate and multivariate analyses including not only pathological findings, but also relevant clinical parameters closely related to the risk of recurrence, such as levels of alpha-fetoprotein (AFP) and degree of liver function impairment, showed that intratumoural TLSs significantly correlated with a lower risk of early relapse. Furthermore, Calderaro *et al.* showed that the risk of recurrence was also significantly associated with the degree of TLS maturation (lower recurrence in those with primary or secondary follicles compared to those with only lymphoid aggregates), suggesting the hypothesis that the degree of TLS maturation also might have a prognostic impact.

This study adds a new piece to the immuno-oncology puzzle, linking anti-tumour immune response by conventional pathological assessment of resected specimens with prognosis. However, the readers should keep in mind several issues when examining this study. Firstly, the presence of TLSs was determined morphologically on haematein-eosin-saffron stained slides and the authors considered HCC as TLS positive if at least one ill-defined cluster of lymphocytes was identified in the revised slides. According to this definition, TLSs were identified in 129 tumours (47%). However, on closer inspection, in 72 out 129 tumours (56%) the TLS positivity was determined only because of the presence of clusters of lymphocytes, while secondary follicles (defined as those TLSs with well-defined

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germinal centres) were only detected in 14 patients (11% of the TLS positive tumours and 5.1% of the whole cohort). Secondary follicles are the most mature form of TLS and are clearly associated with better prognosis.[8] The authors showed that the degree of TLS maturation was correlated with the risk of early recurrence, with this risk being significantly lower in those patients with primary and secondary follicles compared to those with TLS based on the presence of aggregates. Regrettably, the authors have not shown if the mere presence of aggregates (present in 72 out of 273 cases) is also associated with lower recurrence. Furthermore, the authors have not assessed if the number and density of intratumoural TLSs in the surgical specimen are also related with lower recurrence, as shown in other cancers.[5] Besides, they have not considered if the localization of the TLS (peri *vs.* intratumor) might have additional prognostic value. Another limitation that deserves mention is the reproducibility of these findings. The authors do not describe how many slides have been reviewed from each patient, and if they have selected the slides systematically. If this is not the case, the finding of TLS and the degree of maturation might be closely related to the number of slides reviewed from each individual case. Unfortunately, the authors have not stated if those tumours categorized as TLS positive presented TLS in all revised slides; if that is not the case, the TLS detection might be seriously affected by sampling error, which may significantly limit the reliability of these results and the future applicability of this finding in clinical practice. In addition, the identification of TLSs and the assessment of the degree of maturation was done only by one pathologist, so we do not have information regarding the inter-observer agreement among different pathologists, and we do not have an external validation since the LCI cohort was not evaluated by morphological assessment of haematoxylin-eosin stained slides. Nevertheless, we should not lose sight of the accomplishment of the authors. The authors integrate the most relevant clinical (AFP levels, presence of cirrhosis and/or portal hypertension, aetiology, *etc.*) and pathological (tumour size, number of nodules, presence of vascular invasion, histological subtype, *etc.*) variables into the analysis, confirming the statistical independent prognostic value of the detection of intratumoural TLS. In addition, the results exposed in this paper are in line with the identification of an immune-class of HCC based on gene expression profiling in a large cohort of patients with HCC by Sia *et al.*[9] This immune rich class, represented by approximately one-quarter of the cases analysed, was associated with a significantly improved survival, and histologically was characterized by the presence of TLSs. Also, in the study by Calderaro *et al.*, tumours with TLSs were more frequently PD-1 high (45/78 *vs.* 19/82, p <0.001), an expected finding since PD-1 is a marker of follicular helper T cells, that are required for germinal centre formation and function.

The clinical applicability of these findings by Caldarero *et al.* remains to be elucidated. The lack of a standardized methodology for assessing the presence, density and degree of maturity of intratumoural TLSs in resected specimens, and the need of external validation force us to be cautious. Notably, if the novel immune checkpoint inhibitors which are currently under evaluation in phase III trials are able to demonstrate survival benefit in advanced HCC, we can speculate that they will be evaluated in earlier stages as adjuvant therapy after curative resection/ablation for preventing/delaying tumour recurrence, and the optimized selection of patients according to their immune status based on an strong and reproducible immune score obtained by pathological assessment may be helpful.[10] In that setting, data as exposed by Calderaro *et al.* will be very welcome. Forthcoming clinical trials will address if this morphological profile is able to define a subgroup of patients highly responsive to checkpoint inhibition or other immuno-therapeutic strategies.

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Conflict of interest

Alba Diaz does not have conflict of interest. Alejandro Forner has received lecture fees from Bayer HealthCare, MSD, Gilead and BTG, and Consultancy fees from Bayer HealthCare and Guerbet. Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

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Author names in bold designate shared co-first authorship

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