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Original contribution

Rituximab treatment circumvents the prognostic impact of tumor-infiltrating T-cells in follicular lymphoma patients☆,☆☆

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Summary Previous immunohistochemical (IHC) studies showed controversial data about the prognostic value of tumor-infiltrating lymphocytes (TILs) in follicular lymphoma (FL). To clarify this issue, a large series of FL samples from rituximab-treated patients enrolled in the randomized PRIMA trial was examined. IHC was quantified using automated image analysis in 417, 287, 418, 406, 379, and 369 patients for CD3, CD4, CD8, PD1, ICOS, and FOXP3, respectively. RNAseq analysis was used to quantify TIL-related mRNA transcripts from 148 patients. When each IHC marker was used as a continuous variable in the whole cohort, high CD3 counts were associated with better progression-free survival (PFS) ($P = .025$). When an optimal IHC cut point was applied to the whole patient population, high CD3 counts and high PD1 counts were associated with better PFS ($P = .011$ and $P = .044$, respectively), whereas none of the other TIL markers had any significant correlation with outcome. When a stringent analysis was performed by dividing the whole cohort into a training set and a validation set, none of the TIL markers showed a prognostic significance in both groups. RNAseq analysis showed a significant correlation between high levels of *CD3* and *CD8* transcripts and better PFS ($P = .001$ and $P = .037$, respectively). No prognostic correlation was found as to the level of other immune gene transcripts. These results suggest that the IHC prognostic value of TILs is circumvented by rituximab treatment, although there is a trend for high numbers of CD3+ TILs to correlate with better PFS.

1. Introduction

Follicular lymphoma (FL) is usually considered as an indolent B-cell non-Hodgkin lymphoma [1]. However, some patients present rapid disease progression, resistance to treatment, and/or transformation into aggressive lymphoma [1]. Usual clinicobiological prognostic parameters, including the follicular lymphoma international prognostic index (FLIPI), are not always efficient to predict outcome [2]. An improvement has been recently developed by integrating gene mutations in a clinicogenetic model termed *m7-FLIPI* [3]. However, the 7 genes considered in the m7-FLIPI are related to tumor cells and do not take into account the influence of the tumor microenvironment.

In this extent, it remains debated as to whether tumor-infiltrating T lymphocytes (TILs) could contribute to stratify FL patients, as suggested by gene expression profiling studies [4,5]. To translate gene expression profiling data in routine practice, numerous immunohistochemistry (IHC) studies have focused on T-cell subsets of putative interest, especially PD1+ T follicular helper (TFH) cells and FOXP3+ T regulatory cells (Tregs), which were often found to correlate with outcome [6-16]. However, discrepant data have been presented for each marker ([16] for review). For example, high amounts of intratumoral FOXP3+ T cells were often associated with a favorable outcome [6,9,15], but this impact was not confirmed in other studies [10,11,13]. Furthermore, it has been suggested that FL patients with high levels of peripheral blood Tregs had unfavorable outcome [17].

Possible explanations for these heterogeneous results include the limited number of patients in the different cohorts, the heterogeneity of treatments, and the complexity of IHC scoring that is poorly reproducible between pathologists. In fact, manual IHC scoring has been reported to be inadequate for usual lymphoma cell markers in diffuse large B-cell lymphoma [18]. Poor reproducibility was reported in the manual scoring of TIL markers in FL tissues as compared with automated microscopy [19]. Automated IHC scoring by computerized image analysis enables accurate and highly reproducible counting across tissue sections [20,21]. It thus appears as a promising alternative method to obtain more objective and standardized data, which has been used in only a few FL studies so far [22-24].

Our group has collected FL samples from a large cohort of patients enrolled in the PRIMA trial [25], which has already allowed us to reappraise the influence of macrophages in the FL microenvironment [24]. We used computer-assisted image analysis (CIA) to circumvent the poor reproducibility of manual scoring. CIA also provides extensive evaluation of relevant cutoff points, taking advantage of the continuous scale quantification in contrast with the categorical measurement of manual scoring. Using this strategy, the present study aims to reappraise the prognostic value of classical TILs markers in the PRIMA cohort. In addition to IHC, RNAseq analysis [26] was performed as a complementary approach to study the prognostic effect of these TIL markers at the mRNA level.

2. Materials and methods

2.1. Design and characteristics of the PRIMA trial

The randomized, open-label PRIMA study was undertaken in 223 centers in 25 countries [25]. Patients with previously untreated FL (n = 1135 with confirmed FL after central review) and needing systemic therapy were registered in the study and received 1 of 3 nonrandomized immunochemotherapy induction regimens used in routine practice: R-CHOP (rituximab-cyclophosphamide doxorubicin, vincristin, prednisolone), R-CVP (rituximab-cyclophosphamide, vincristin, prednisolone), or R-FCM (rituximab, fludarabine, cyclophosphamide and mitoxantrone). The 1019 patients who achieved a complete or partial response were then randomly assigned to receive 2