C-MYC is related to GATA3 expression and associated with poor prognosis in nodal peripheral T-cell lymphomas

Peripheral T-cell lymphomas (PTCLs) are a heterogeneous group of non-Hodgkin lymphomas (NHLs), characterized by aggressive clinical behavior. Nodal PTCL (n-PTCL) includes four disorders: angioimmunoblastic T-cell lymphoma (AITL), peripheral T-cell lymphoma not otherwise specified (PTCL-NOS) and anaplastic large cell lymphoma (ALCL) (ALK-positive and ALK-negative forms). While AITL and ALCL both have their own particular morphological and phenotypic features, n-PTCL-NOS comprises a variety of peripheral T-cell lymphomas. Recently, gene expression studies have identified three subgroups of PTCL-NOS that have prognostic implications. One is characterized by the overexpression of TBX21 and a better outcome, and the other two, which have a worse prognosis, have either cytotoxic markers or GATA3 overexpression.[1]

Iqbal *et al.*[1] found the GATA3-positive subgroup of tumors to be enriched in *MYC* and proliferation gene signatures, amongst others. *MYC* is one of the most frequently dysregulated oncogenes in cancer and is critical to the development of mature PTCLs.[2]

In an attempt to validate these findings, we investigated the potential relationship between the presence of C-MYC, GATA3 and a high level of Ki-67 expression in paraffin-embedded tumoral tissue in a series of 128 n-PTCL patients (including 74 AITL and 54 PTCL-NOS) using tissue microarrays (TMAs) (*Online Supplementary Information* and *Online Supplementary Table S1*). Cut-off values for these three markers were based on previous reports[1,3-5] (*Online Supplementary Information*).

We found positivity for C-MYC in 13.3% (17/128), and a high level of Ki-67 expression in 24.2% (31/128) of the cases analyzed. In this study, GATA3 expression was observed in 21.9% (28/128) of the n-PTCL cases; 20.3% (15/74) of the AITLs and 24.1% (13/54) of the PTCL-NOS patients were positive (*Online Supplementary Table S2*). Previous studies had reported GATA3 expression in 33%[1] and 45%[4] of the PTCL-NOS patients. Differences in the percentages of positivity may have been due to the different sources of the antibody used (ABCAM *vs.* Santa Cruz Biotechnology), or to differences in case selection criteria.

Significant positive associations were found between the expression of C-MYC and the presence of both Ki-67 ($P<0.001$) and GATA3 ($P=0.010$) (Table 1), but no significant correlation was found between the expression of Ki-67 and GATA3 ($P=0.754$) (Table 1). Furthermore, C-MYC and Ki-67 expression conferred poor overall survival (OS) on these patients, as indicated by the univariate analyses ($P=0.003$ and $P=0.006$, respectively) (Figure 1A). C-MYC was an independent prognostic factor in the multivariate analysis ($P=0.004$).

In the present series, the International Prognostic Index (IPI) and the Prognostic Index for T-cell lymphoma (PIT) were both related to prognosis (*data not shown*). Cases with a high IPI, PIT or Eastern Cooperative Oncology Group (ECOG) performance status score tended to have a higher percentage of C-MYC staining ($P=0.053$, $P=0.001$, and $P=0.008$, respectively) (*Online Supplementary Table S3*). On the other hand, no significant correlation of Ki-67 and GATA3 expression was found with any clinical characteristic analyzed (*Online Supplementary Table S4 and S5*). As expected, in the multivariate analysis of the clinical (IPI and PIT) and biological variables (C-MYC and Ki-67), the Cox regression model identified only PIT as an independent prognostic factor of disease-specific OS ($P<0.001$).

In the present series, C-MYC was expressed in the AITL and PTCL-NOS tumor subgroups. The correlation between C-MYC and Ki-67 was maintained in AITL patients ($P=0.007$); on the other hand, a significant positive association was found between the expression of C-MYC and the presence of both Ki-67 ($P=0.001$) and GATA3 ($P=0.004$) in the PTCL-NOS subgroup. In addition, the presence of C-MYC and Ki-67 conferred poor prognosis exclusively on the AITL patient subgroup ($P=0.008$ and $P=0.021$, respectively) (Figure 1B), while the presence of GATA3 conferred poor prognosis on the PTCL-NOS patients ($P=0.046$) (Figure 1C). In the multivariate analysis, the Cox regression again identified IPI as an independent prognostic factor of disease-specific OS in both histological tumor subtypes.

Considering patients by separate PIT risk subgroups indicated that only C-MYC maintained its prognostic implication in the low-risk PIT subgroup of AITL patients (Figure 2). Nevertheless, these results should be interpreted with caution, given the very small number of cases in this subgroup.

MYC is known to be activated by the loss of *SNF5* (SMARCB1),[2] overexpression of microRNA187,[5] and by its own gene amplification or translocation, thereby favoring the rapid appearance of T-cell neoplasms in humans.[6] Since C-MYC is involved in cell proliferation,[7] the significantly positive relationship between C-MYC expression and proliferation index is not surprising. In the present series, the presence of C-MYC and Ki-67 were both independent prognostic markers in the univariate analysis. Moreover, the significance of the C-MYC marker was also significant in the AITL tumor subgroup. Their significance was not maintained when classic clinical variables were included in the multivariate analysis, although a poor prognosis subgroup of patients within the low-risk PIT subgroup of AITL patients was identified. Previous gene expression studies had identified *MYC* as being overexpressed in AITL, adult T-cell

Table 1.

	n-PTCL			AITL			PTCL-NOS		
	C-MYC			C-MYC			C-MYC		
C-MYC	–	Ki-67	–	–	Ki-67	–	–	Ki-67	–
Ki-67	<0.001	–	GATA3	0.007	–	GATA3	0.001	–	GATA3
GATA3	0.010	0.754	–	0.251	0.307	–	0.004	0.111	–

leukemia/lymphoma, and ALCL cases.[1,8] Moreover, C-MYC overexpression is known to be related to a high proliferation index and poor clinical outcome in ALCL.[9] Cuadros *et al.*[10] identified a poor prognosis signature related to proliferation using gene expression studies. Went *et al.*[11] associated a high level of Ki-67 expression with PTCL-NOS patient outcome when associated with clinical parameters in the design of a new prognostic index called mPIT (modified Prognostic Index for T-cell lymphoma). Weisenburger *et al.*[12] found that Ki-67 expression conferred poor prognosis on PTCL-NOS patients, while our group[3] and Federico *et al.*[13] identified this proliferation rate as a prognostic factor in AITL patients. Recently, gene expression studies[1,4] have shown that a specific sub-

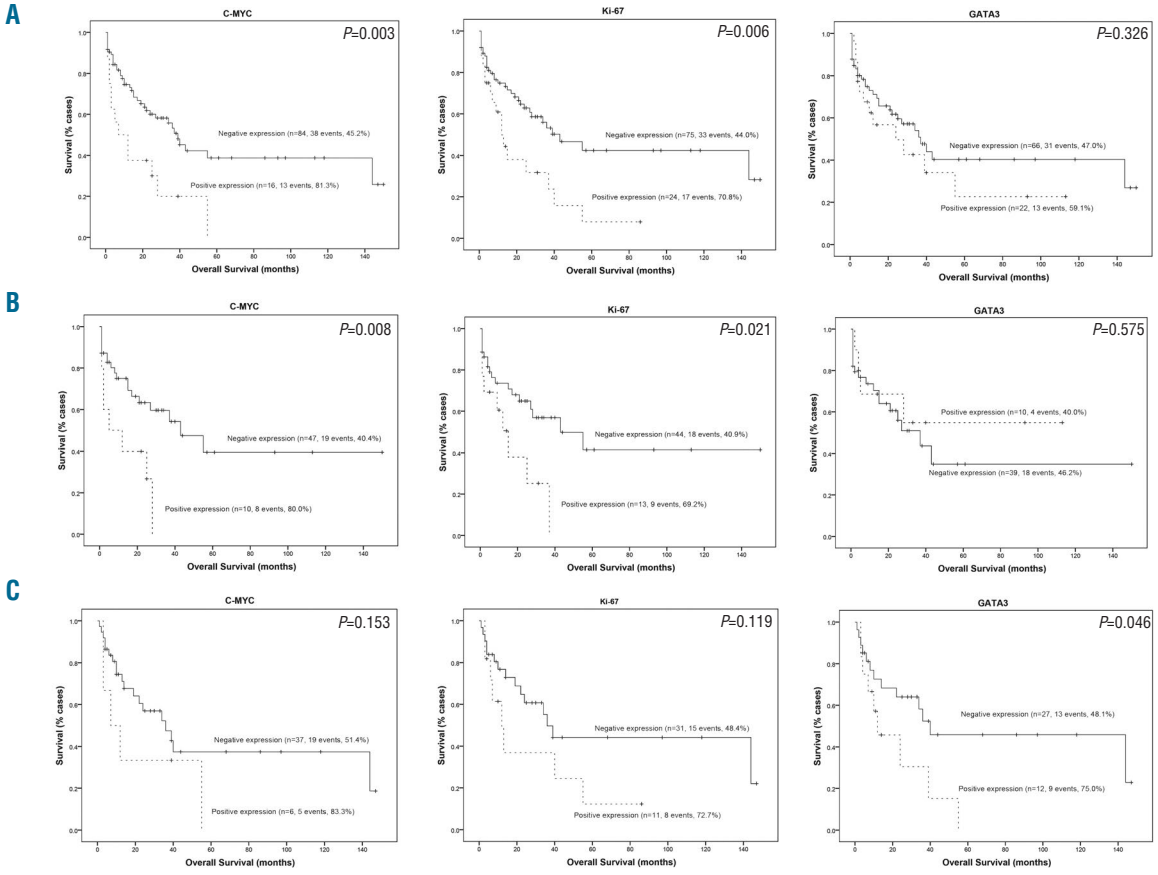

Figure 1. Kaplan–Meier survival curves of biological marker expression in the complete series (A) of n-PTCL patients and in the AITL (B) and PTCL-NOS (C) histological subgroups.

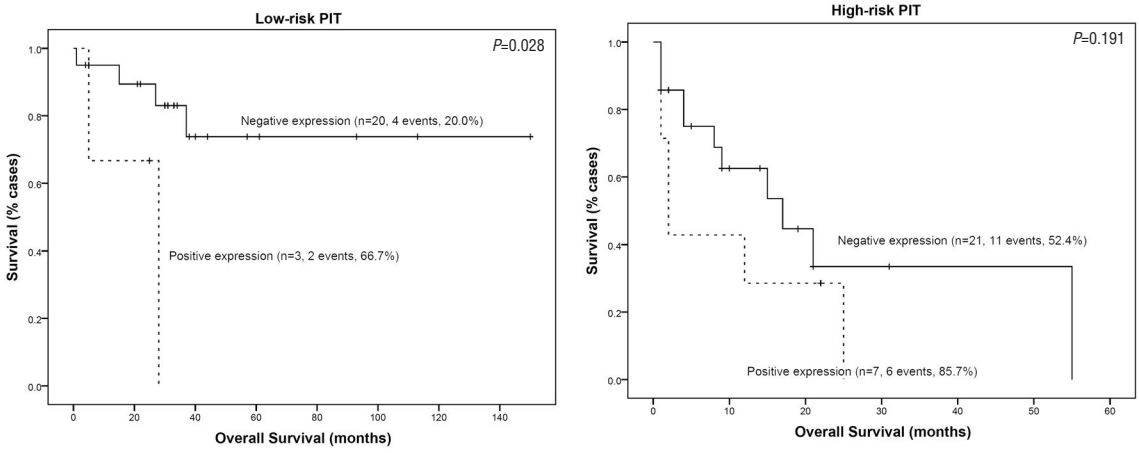

Figure 2. Kaplan–Meier survival curves of C-MYC expression in the AITL subgroup of patients in the low-risk and high-risk PIT subgroups.

group of PTCL-NOS is characterized by a high level of expression of GATA3 and poor prognosis. GATA3 and C-MYC regulate each other's expression in a manner that is not well understood, although they are critical to promoting both proliferation and differentiation of T cells.[14,15] In this study, we used gene expression arrays to validate the correlation between C-MYC and GATA3 expression proposed by Iqbal *et al*.[1] The correlation was exclusively maintained in the PTCL-NOS subgroup when considering patients with respect to morphological and phenotypic characteristics. GATA3 expression was of prognostic value in the PTCL-NOS subgroup of tumors, but not in the AITL patients.

Together, these findings validate the relevance of *MYC*[2,5,6] expression in PTCLs, and suggest a specific pathogenic role of this protein in different subgroups of n-PTCLs that warrants further investigation. Additionally, C-MYC expression identifies a group of high-risk AITL patients who would otherwise be considered to be of low-intermediate risk on the basis of their PIT score. However, these results require confirmation in an independent series of n-PTCL patients.

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