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ORIGINAL ARTICLE

Title**: *GATA 3* Gene Overexpression Is A Biomarker of Adverse Prognosis for Peripheral T-Cell Lymphoma, Not Otherwise Specified and for ALK-Negative Anaplastic Large Cell Lymphoma: An Exploratory Analysis of 80 Latin American Cases of nodal PTCLs**

Running title: Prognostic and Diagnostic Aspects of the *GATA 3* Gene in nPTCLs.

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Abstract:

Background: Nodal peripheral T-cell lymphomas (nPTCLs) encompass a heterogeneous group of mature and aggressive lymphoid malignancies, including peripheral T-cell lymphoma, not otherwise specified (PTCL/NOS), angioimmunoblastic T-cell lymphoma (AITL) and anaplastic large cell lymphoma (ALCL) ALK-positive and ALK-negative. Their differential diagnosis and prognosis are an issue in the clinical practice. Accurate biomarkers to refine the different subtypes of nPTCLs and to stratify their prognosis are essential to improve their treatment approach. The aim of this study was to test the prognostic impact of *GATA3* gene expression, and its capability to discriminate the different subtypes of nPTCLs.

Patients and Methods: From 2000 to 2017, 80 patients with nPTCLs were eligible for *GATA3* gene expression analysis that was assessed retrospectively by quantitative real time PCR (qRT-PCR) of neoplastic biopsies in Formalin-Fixed Paraffin-Embedded samples (FFPE).

Results: Median age was 49 years old (IqR 34-59), 43/80 (53.7%) were male. Median follow-up was 1.72 years. Of them, 36.3% were classified as PTCL/NOS, 31.2% ALK-negative ALCL, 21.2% ALK-positive ALCL and 11.3% AITL. The majority of cases had advanced stage (III/IV). Two-year estimated overall survival (OS) and progression-free survival (PFS) were 52.2% and 39.5%, respectively. The median *GATA3* gene expression level was 0.49% (range 0 – 7.07) in all cohort, it was 0.11% for ALK-positive ALCL, 0.46% for ALK-negative ALCL, 0.86% for PTCL/NOS and 0.67% for AITL. The difference of *GATA3* gene expression among distinct variants of nPTCLs was statistically significant ($p < 0.001$). *GATA3* gene expression levels ≥ 0.71% discriminate PTCL/NOS from ALK-negative ALCL and AITL with sensitivity of 62% and specificity of 80.3%. *GATA3* gene expression levels ≥ median was associated with poor 2-year OS for PTCL/NOS (46.7% x 21.4%, $p=0.04$) and for ALK-negative ALCL (85.7% x 54.5%, $p=0.04$).

Conclusion: Despite the relative small and heterogeneous group of patients with nPTCLs, *GATA3* gene overexpression may be an important biomarker associated with poor prognosis in PTCL/NOS and ALK-negative ALCL. Moreover it may also discriminate different subtypes of nPTCLs. Further studies with larger series of patients should confirm our findings.

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Key words: nodal peripheral T-cell lymphomas; peripheral T-cell lymphoma, not otherwise specified (PTCL/NOS); ALK-negative anaplastic large-cell lymphoma (ALCL/ALK-); Angioimmunoblastic T-cell lymphoma (AITL); diagnosis; prognosis; GATA3 gene expression

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Introduction

Peripheral T-cell lymphomas (PTCLs) constitute a heterogeneous group of rare malignancies of mature T lymphocytes [1]. It corresponds to 10-15% of all non-Hodgkin's lymphomas (NHLs) and have a characteristic geographic distribution, predominantly in Southeast Asia and Latin America, where its incidence accounts for 20-25% of all NHLs [1]. PTCLs comprise approximately 22 distinct diseases, classified according to their clinical aspects in predominantly nodal (nPTCL), predominantly extralymphonodal, primary cutaneous and disseminated or leukemic [2].

The nPTCL group represents 60-70% of all PTCLs, including peripheral T-cell lymphoma, not otherwise specified (PTCL/NOS), angioimmunoblastic T-cell lymphoma (AITL) and anaplastic large-cell lymphoma (ALCL), which is divided in ALK (*anaplastic lymphoma kinase)* positive and negative [1].

Diagnosis of nPTCL can be challenging, thus requiring integrated histopathological, immunophenotypical and molecular analysis. Molecular methods are expensive, difficult to apply and not always generally available. However, the differential diagnosis among the different types of nPTCLs established on morphology and phenotype is difficult even for the most experienced hematopathologists, because these entities have overlapping morphological and immunophenotypic aspects [3].

nPTCLs frequently present a poor prognosis, since most patients display unfavorable clinical and laboratory features at diagnosis, as high expression of P-glycoprotein (Pgp) and *TP53* gene mutation, as well as worse response to anthracycline-based therapy [4, 5]. Therefore, new powerful prognostic biomarkers are needed to improve therapy, and to allow a more personalized approach to these patients.

The *GATA3* gene, located on the short arm of chromosome 10 (10p14) encodes the transcription factor GATA3 expressed on immune cells, such as mature T lymphocytes [6]. This protein works as a transcription factor acting for maintenance and proliferation of T lymphocytes, regulating self-renewal of hematopoietic stem cells, and playing an essential role in Th2 lymphocyte differentiation [7, 8].

GATA3 has been consolidated as biomarker for diagnosis of several neoplasms, including breast cancer, urothelial tumors, germ cell neoplasms and mesotheliomas [9, 10, 11]. Moreover, *GATA3* gene expression has been associated in the prognosis of PTCL/NOS. Iqbal et al., studied

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106 patients with PTCL/NOS and found that overexpression of *GATA3* gene was associated with poor OS when compared to cases with lower expression levels (0.9 versus 2.08 years, p = 0.01) [3].

We previously demonstrated that overexpression of *CCNA2* gene and CHECK1 protein were associated with poor prognosis in nPTCLs [12]. Here, we aimed to test and confirm the hypothesis that *GATA3 gene* expression would be associated with overall survival and progression-free survival and could be used as a marker to differentiate histopathological subtypes of nPTCLs.

Patients and Methods

Patients

After local Ethical Committee approval, under the number 02975012.0.0000.0068, 117 patients from 18 to 86 years old with PTCL/NOS, AITL, ALK-positive ALCL and ALK-negative ALCL treated from January 2000 to December 2017 at a single reference center for cancer in Brazil were retrospectively identified. Thirty seven patients (31.6%) of these 117 were excluded due to lack of adequate sample for RNA extraction. Patients with symptomatic heart failure and/or ejection fraction < 30%, primary cutaneous ALCL and with unavailable tumor samples were further excluded from the analysis.

Eighty patients met the eligibility criteria. Baseline features, including age, gender, histopathological subtype, Ann Arbor stage, number of extranodal sites involved with lymphoma, B symptoms, bone marrow (BM) infiltration, performance status (PS) by the Eastern Cooperative Oncology Group (ECOG), bulky mass ≥ 10 cm, LDH dosage, International Prognostic Score (IPI) and Prognostic Index for T-cell Lymphoma (PIT) score were taken from medical records by a specific researcher.

The approach of patients followed the current institutional protocol at that time. Before treatment, patients should be tested for HIV, B and C hepatitis and HTLV-1, comprehensive biochemical and echocardiography. Disease stage was characterized using computerized tomography (CT) scan of the neck, chest, abdomen and pelvis and unilateral bone marrow (BM) biopsy stained with Hematoxylin & Eosin (H&E) and immunohistochemistry (IHC). In selected patients, central nervous system prophylaxis was done with four cycles of intrathecal injection

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of methotrexate (12 mg) and dexamethasone (2 mg) during the first four cycles of chemotherapy. Two additional cycles of high dose methotrexate (3000 mg sqm) following the last cycle of therapy was also delivered for patients with sinuses, kidney, adrenal, breast or gonads lymphoma, paravertebral mass and involvement of ≥ 2 extranodal sites with intermediate-high and high risk IPI/PIT. The main endpoints evaluated were overall response rate (ORR), overall survival (OS) and progression-free survival (PFS).

Histology and Immunohistochemistry

Two experts in hematopathology reviewed all cases following the WHO criterion [1]. Hence, cuts of 5µm thick obtained from FFPE samples and stained with H&E were analyzed. A IHC panel screening included the monoclonal antibodies Ki67 (Dako, K55, 1/1600), CD20 (Dako, L26, 1/1000), CD3 (Dako, F7.2.38, 1/500) and CD30/Ki-1 (Cell Marque, Ber-H2, 1/1000). The subsequent diagnostic approach was guided according to the screening results.

At screening analysis, atypical small/medium size cells with polymorphic atypical lymphoid infiltrate, marked vascular proliferation and irregular follicular dendritic cells (FDC) arrangement was suggestive of AITL. The confirmatory IHC panel included T-helper Follicular (THF) markers as CD10 (Novocastra, S6C6, 1/2000), ICOS (Abcam, SP98, 1/100), CXCL-13 (Abcam, Ab112521, 1/300), PD-1 (Abcam, NAT105, 1/1000); vascular markers [CD31 (Dako, JC/70A, 1/100), CD34 (Dako, QBEand-10, 1/2000)], FDC antigens [CD21 (Novocastra, 2G9, 1/800), CD23 (Biocare, 1B12, 1/1000)] and EBER by *in situ* hybridization for EBV.

In cases of ALCL hypothesis by demonstration of atypical lymphoid infiltrate with large and pleomorphic “hallmark cells” expressing CD30 higher than 75%, additional IHC with ALK1 (Spring, SP8, 1/400) was carried out to discriminate between ALK-positive ALCL and ALK-negative variants. The IHC panel of ALK-negative ALCL cases was expanded with CD45 (Dako, 2B11+PD7/26, 1/2000), CD15 (Dako, Carb-3, 1/50) and PAX-5 (Dako, Dak-Pax5, 1/200) to exclude Hodgkin's lymphoma. The presence of atypical lymphoid proliferation with small/medium size cells with no criterion for any other nPTCL was indicative of PTCL/NOS and additional IHC with CD2 (Monossan, AB75m, 1/200), CD4 (Spring, SP35, 1/400), CD7 (Novocastra, CD7-272, 1/3000) and CD8 (Dako, C8/144B, 1/800) was carried out.

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Treatment, response criteria and follow-up

ALK-positive ALCL patients received 6 to 8 cycles of CHOP-21 (cyclophosphamide 750 mg/sqm i.v on D1, doxorubicin 50 mg/sqm i.v on D1, vincristine 1.4 mg/sqm [maximum 2.0 mg] i.v on D1 and prednisone 100 mg/day p.o D1 to D5). ALK-negative ALCL, PTCL/NOS and AITL patients were treated with 6 to 8 cycles of CHOEP-21 (CHOP plus etoposide 100 mg/sqm i.v D1 to D3). Except ALK-positive ALCL, patients under 65 years with no significant comorbidities received autologous hematopoietic stem cell transplantation (ASCT) in first complete remission (CR) or in partial response (PR) as consolidative approach. ASCT in ALK-positive ALCL was done only in the setting of second CR/PR. Interim response after the fourth cycle and final response was assessed as recommended by Cheson et al., 2014 based on clinical aspects, LDH dosage, CT scan and bone marrow biopsy if BM was involved at diagnosis [13]. The follow up was carried out every two months in the first year, every three months in the second year, every four months in the third year, every six months in the fourth year, and annually after the fifth year.

Molecular Biology

Total RNA was isolated from FFPE tumor samples (5µm thick) using Qiagen FFPE Rneasy Kit (Valencia, CA, USA) after deparaffinization with xylol, following the manufacturer's recommendation. RNA quality and integrity were assessed on spectrophotometer (NanoDrop 1000 Spectrophotometer V3.7, Thermo Fisher Scientific, Wilmington, DE, USA) and cDNA was synthesized from 1.0 µg of total RNA using GoScript Reverse Transcriptase Kit (Promega, Madison, Wisconsin, USA) according to the manufacturer's protocol. Subsequently, cDNA integrity was checked with *GUSB* gene (Hs99999908_m1) (TaqMan Gene Expression Assays, ThermoFischer) as control. Finally, *GATA3* gene (Hs00231122_m1) expression analysis was carried out by qRT-PCR.

The PCR standard curves were assembled with known concentration of total RNA obtained from a pool of lymphocytes of non-neoplastic lymph nodes. Briefly, qRT-PCR was performed in 7500 FAST Real Time PCR System (Applied Biosystems, Foster City, CA, USA) using Applied Biosystems Taqman Gene Expression Assays (Applied Biosystems) and TaqMan Universal PCR Master Mix (Applied Biosystems) from five serial dilutions of cDNA at 10 x scale. *GUSB* gene samples with Ct value > 34 were not accepted as previously recommended, and gene expression results were shown as relative values [14].

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Statistical Analysis

Variables were described in absolute and relative frequencies and assessed for association with *GATA3* gene expression levels. The association of *GATA3* gene expression with histopathological variants of nPTCLs was assessed by Kruskal-Wallis and post-hoc Dunn's test. The *GATA3* expression cut-off point able to discriminate the distinct subgroups of nPTCLs was determined using the receiver operator characteristic curve (ROC) method. To evaluate the prognostic of *GATA3* gene expression in the different variants of nPTCLs, numerical continuous variables obtained were transformed in categorical variables and categorized in values below, equal or higher than the median of *GATA3* gene expression. OS and PFS curves were estimated by Kaplan-Meier method. OS was considered from the date of diagnosis until death, and PFS from the date of diagnosis until progression of disease, death or last follow-up. The data were censored at last follow-up and log-rank and Peto-peto tests were used to compare the survival curves of nPTCLs subgroups. The tests were performed using STATA 12.0 software with a *p* value ≤ 0.05 considered statistically significant. Due to the relative small number of patients a multivariate analysis was not performed.

Results

Clinical and laboratory features

Table 1 displays the clinical characteristics of 80 patients included in the study. Their median age was 49 years (interquartile range [IQR] 34 - 59 years) and 53.7% (43/80) were male. Of them, 36.3% (29/80) were classified as PTCL/NOS, 31.2% (25/80) ALK-negative ALCL, 21.2% (17/80) ALK-positive ALCL and 11.3% (9/80) AITL. The majority of cases (77/80, 96.2%) had advanced stage (III/IV), 30.0% (24/80) had ECOG ≥ 2, 61.2% (49/80) and 58.7% (47/80) presented intermediate-high and high-risk score at IPI and PIT, respectively. B symptoms were found in 82.5% (66/80) of patients, whereas 58.7% (47/80) presented LDH ≥ ULN and 37.5% (30/80) had ≥ 2 extranodal sites involved with lymphoma. Bulky mass ≥ 10 cm in the longest axis was observed in 37.5% (30/80), and BM infiltration in 13.7% (11/80) of cases.

The data presented in **Table 2** summarize the main results obtained for the ALCL group, in which 64.7% (11/17) of ALK-positive and 44.0% (11/25) of ALK-negative ALCL were younger than 40 years old. A high proportion of ALK-positive patients presented intermediate-high and

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high-risk IPI 52.9% (9/17) and PIT 52.9% (9/17) was observed. In ALK-negative ALCL, 56.0% (14/25) and 48.0% (12/25) had intermediate-high and high-risk IPI and PIT, respectively. ALK-positive ALCL presented high rate of progression and relapse (8/17, 47%) in comparison to ALK-negative ALCL (3/25, 12%). Only 23.5% (4/17) of ALK-positive ALCL patients underwent ASCT, being all of them in the setting of relapsed and refractory disease, while 52.0% (13/25) of ALK-negative ALCL received ASCT in first CR/PR.

CHOP regimen was used in first-line for 36.2% (29/80) of patients, CHOEP for 38.7% (31/80), 15.0% (12/80) of patients received other protocols and 10.0% (8/80) did not undergo any therapy in general because of early death. Overall, patients received a median of 6 cycles (range 1 - 8) of chemotherapy. Twenty-five per cent (20/80) of patients were consolidated with radiotherapy and 35.0% (28/80) were submitted to ASCT.

Outcomes

Complete remission (CR) was achieved after treatment in 37/80 patients (47.5%) and partial remission (PR) in 9/80 (11.2%), with overall remission rate (ORR) of 57.5% (95% CI: 45.9-68.5). The remainders 8 patients (10%) presented Progressive Disease (PD) or were primary refractory , and 31.3% (25/80) died during the first line of treatment. Of them, 22/25 (88%) died of relapse or progressive disease and 3/25 (12%) with infections complications (**Table 1**).

The median follow-up for the all cohort of nPTCLs was 1.72 years (0.02-23.99) with a median OS of 3.16 years (95% CI: 1.01-8.25) and 2-year OS of 52.2% (95% CI: 40.7-62.5). The median PFS was 0.91 years (95% CI: 0.58-2.30) with 2-year PFS of 39.5% (95% CI: 28.7-50.1) (**Figure 1**). Patients with ALK-positive ALCL had a median OS of 6.59 years (95% CI: 0.33 - not reached) and 2-year OS of 58.8% (95% CI: 32.5-77.8). In the ALK-negative ALCL group the median OS was not reached, and 2-year OS was 72.0% (95% CI: 50.1 - 85.5). For PTCL/NOS the median OS was 1.28 years (95% CI: 0.73 - 2.59 years) and 2-year OS was 33.4% (95%CI: 17.0 - 50.8). In AITL subtype, the median OS was 1.15 years (95% CI: 0.17 - 8.51) and 2-year OS was 44.4% (95% CI: 13.6 - 71.9) (**Figure 2A**).

The median PFS and 2-year PFS were respectively 0.58 years (95% CI: 0.22 - 6.59), and 47.1% (95% CI: 23.0 - 68.0) for ALK-positive ALCL. The median PFS was not reached, and 2-year PFS was 68.0% (95% CI: 46.1 - 82.5) for ALK-negative ALCL. In the subgroup of PTCL/NOS, the median PFS was 0.68 years (95% CI: 0.43 - 0.89) and 2-year PFS was 8.0% (95% CI: 1.53 - 22.6).

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AITL patients presented a median PFS of 1.09 years (95% CI: 0.17 - 5.40), and 2-year PFS of 44.4% (95% CI 13.5 - 71.9) (**Figure 2B**).

***GATA3* gene expression impact on overall survival and progression-free survival**

The median *GATA3* gene expression level in the full cohort of nPTCLs was 0.49% (range 0 - 7.07), and 0.11% (range 0 - 2.09) for ALK-positive ALCL, 0.46% (range 0.04 - 1.96) for ALK-negative ALCL, 0.86% (range 0.18 - 7.07) for PTCL/NOS and 0.67% (range 0.24 - 1.55) for AITL. The difference of *GATA3* gene expression among the distinct variants of nPTCLs was statistically significant ($p < 0.001$).

The median OS for the 80 nPTCL patients was 4.28 years (95% CI: 0.77 - not reached) for *GATA3* gene expression < 0.49% and 2.59 years (95% CI: 0.63 - 8.25) for *GATA3* ≥ 0.49% p = 0.56. The 2-year OS was 51.2% (95% CI: 34.6 - 65.5) for *GATA3* < 0.49% and 53.0% (95% CI: 36.5 - 67.0) for *GATA3* ≥ 0.49%, p = 0.61. The median PFS was 0.91 years (95% CI: 0.33 - 3.06 years) for *GATA 3* < 0.49% and 0.89 years (95% CI: 0.50 - 3.56) for *GATA 3* ≥ 0.49% p = 0.87. The 2-year PFS was 43.4% (95% CI: 27.7 - 58.2) for *GATA 3* < 0.49% and 35.4% (95% CI: 21.0 - 50.2) for *GATA 3* ≥ 0.49%, p = 0.93.

In the ALK-positive ALCL subgroup *GATA3* gene expression did not have prognostic impact on OS and PFS. In this group (n = 17), the median OS was not reached for *GATA 3* < 0.11%, and was 3.16 years (95% CI: 0.22 - not reached) for *GATA3* ≥ 0.11%, p = 0.74. The 2-year OS was 60.0% (95% CI: 25.3 - 82.7) for *GATA3* < 0.11% and 57.1% (95% CI: 17.2 - 83.7) for *GATA 3* ≥ 0.11%, p = 0.85. The median PFS was 1.79 years (95% CI: 0.22 - not reached) for *GATA3* < 0.11% and 0.58 years (95% CI: 0.06 - not reached) for *GATA 3* ≥ 0.11%, p = 0.23. The 2-year PFS was 50.0% (95% CI: 25.3 - 75.3) for *GATA3* < 0.11% and 42.9% (95% CI: 9.7 - 73.4) for *GATA 3* ≥ 0.11%, p = 0.44.

In the PTCL/NOS (n=29) group, *GATA3* gene overexpression was statistically significantly associated with poor OS, but not with PFS. The median OS was 1.29 years (95% CI: 0.73 - 6.96) for *GATA3* < 0.86% and 0.83 years (95% CI: 0.04 - 1.98) for *GATA3* ≥ 0.86%, p=0.021. The 2-year OS was 46.7% (95% CI: 21.2 - 68.7) for *GATA3* < 0.86% and 21.4% (95%CI: 5.2 - 44.8) for *GATA 3* ≥ 0.86%, p = 0.049. The median PFS was 0.76 years (95% CI: 0.04 - 1.52 years) for *GATA3* < 0.86% and 0.65 years (95% CI: 0.43 - 0.89) for *GATA3* ≥ 0.86 %, p = 0.67. The 2-year-PFS was 10% (95% CI: 0.7 - 33.5) for *GATA 3* < 0.86% and 2% (95%CI: 0.4 - 27.5) for *GATA 3* > 0.86%, p = 0.91 (**Figure 3**).

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In patients with ALK-negative ALCL (n=25), *GATA3* overexpression was statistically significantly associated to worse OS and PFS. The median OS was not reached for *GATA 3* < 0.46% and was 8.25 years (95% CI: 0.10 - not reached) for *GATA3* > 0.46%, p=0.07. The 2-year OS was 85.7% (95% CI: 53.9 - 96.2) for *GATA 3* < 0.46% and 54.5% (95% CI: 22.9 - 78.0) for *GATA 3* ≥ 0.46%, p = 0.041. The median PFS was not reached for *GATA 3* < 0.46% and 4.55 years (95%CI: 0.10 - not reached) for *GATA3* ≥ 0.46%, p= 0.05. The 2-year PFS was 78.6% (95% CI: 47.2 - 92.5) for *GATA 3* < 0.46% and 54.5% (95%CI: 22.9 - 78.0) for *GATA 3* ≥ 0.46%, p = 0.08 (**Figure 4**).

In the AITL subgroup, the small number of patients (n = 9) did not allow appropriate tests to assess the prognostic impact of *GATA3* gene expression.

GATA3 gene expression may discriminate different nPTCLs subtypes

We identified a statistically significant difference in the distribution of *GATA3* gene expression among different nPTCLs subtypes (p < 0.001). *GATA3* gene expression was significantly higher in PTCL/NOS in comparison to ALK-positive ALCL, ALK-negative ALCL and AITL (**Table 3** and **Figure 5**). The area under the curve was 0.752 (0.644-0.861), with a sensitivity of 62.0% and specificity of 80.3% for a cut-off of 0.71% of *GATA3* gene expression, and a predictive positive value (PPV) of 64.3% and predictive negative value (PNV) of 78.8% to establish the differential diagnosis between PTCL/NOS and other nPTCLs non-ALK+ (ALK negative ALCL and AITL) [n = 63]. *GATA3* gene expression < 0.71% favored ALK-negative ALCL or AITL diagnosis, while *GATA3* gene expression ≥ 0.71% favored PTCL/NOS diagnosis (p=0.03 PTCL/NOS x ALK negative ALCL, p=0.04 PTCL/NOS x AITL).

Discussion

In this study, we demonstrated for the first time that overexpression of *GATA3* gene represents a biomarker of poor prognosis in ALK-negative ALCL. We have also confirmed its unfavorable role for PTCL/NOS, in agreement with previous studies [3, 15].

Prez-Andreu et al. were the first authors to describe the adverse prognosis of the *GATA3* gene in hematological neoplasms [16]. They evaluated 511 pediatric patients with acute lymphoblastic leukemia (ALL) using gene expression profile microarray, and were successful to show that *GATA3* polymorphism rs3824662 was associated with somatic abnormalities resembling a gene signature pattern typical of Ph positive ALL, such as *CRLF2* rearrangement,

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JAK gene mutation and *IKZF1* deletion. Single nucleotide polymorphism in *GATA 3* was also associated with early response at therapy and increased risk of disease recurrence [16].

Afterwards, Iqbal et al. analyzed 121 patients diagnosed with PTCL/NOS and identified two major molecular subgroups of patients. In one of them, there was a prevalence of *GATA3* gene expression (33%, 37/121), and in the other subgroup there was a predominance of the transcriptional factor T-box (*TBX21)* gene expression (49%, 59/121). However, 9.9% (12/121) of patients did not fit in any group. The group with *GATA3* gene overexpression showed poor overall survival with a median OS of 0.9 years in comparison to 2.08 years observed in the *TBX21* group, p = 0.01. This was the first study to demonstrate an unfavorable prognosis associated with overexpression of *GATA3* gene in a specific subtype of nPTCL [3]. However, in this casuistic, patients with ALCL were not studied.

Zhang et al. also analyzed the transcriptions factors *GATA3* and *T-bet* in 109 patients diagnosed with PTCL. In addition, they studied the non-neoplastic immune component of the microenvironment by IHC staining using the macrophage antigen CD68, aiming to quantify the tumor-associated macrophage (TAM) content. In univariate analysis, high *GATA3 gene* expression correlated with poor survival and abundant tumor macrophage infiltration. They concluded that *GATA3* gene overexpression is predictive of poor prognosis in PTCL and suggested that non-neoplastic T-cells could induce type-M2 macrophage differentiation dependent of *GATA3* [15].

Recently, Heavican et al., demonstrated that PTCL-GATA3 (PTCL overexpressing GATA3) exhibited more aberrant genome than PTCL-TBX21 with frequent partial/complete chr7 gain, partial/complete chr8q gain, and chr17q gain. *MYC* (8q24.21) was amplified in half of cases with concomitant higher mRNA expression, along with an enrichment of *MYC* target genes. Chr17q gain encompassed *STAT3* gene, and 9 of 11 cases with *STAT3* gain had a *MYC* gain. *TP53* mutations were also associated with PTCL-GATA3 [17].

Surprisingly, we also found that *GATA3* gene overexpression was significantly associated with poor prognosis in ALK-negative ALCL. In contrary to ALK-positive ALCL subtype, ALK-negative ALCL is a genetic heterogeneous disease, in which 30% of them express the *DUSP22* gene that is associated with better prognosis, and 8% exhibits *P63* gene mutation related to poor outcomes [18]. However, *GATA3* gene has not been described before as a marker of prognosis in this entity. Although this data has yet to be confirmed, our results might be underestimated, as few in common cases of T-cell lymphoma studies were evaluated here. Moreover, we speculate if the presence of *GATA3* gene and *DUSP22* gene could be excluding genetic

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abnormalities in ALK-negative ALCL, since they confer different prognosis in this population. Furthermore, we inquire if ALK-negative ALCL cases presenting high expression of *GATA3*, as described above for PTCL/NOS, also have higher genetic abnormalities associated with poor prognosis as *MYC* gene hyperexpression and *TP53* mutations. However, in our study, we did not analyze *DUSP22* gene expression, neither performed analysis of *TP53* gene or *MYC* gene, not being able to verify its hypothesis.

GATA3 gene is expressed in several immune cells during the development of T-lymphocytes, and in mature T-cells and natural killer (NK) cells. It encodes a transcription factor essential for regulation of T-lymphocyte survival and induction of Th2 differentiation. It also induces expression of IL-5, IL-4 and IL-13, and controls IL-10 expression and suppresses Th1 phenotype development through chromatin conformation modification [6, 7, 8]. Previous studies have described *GATA3* gene and its respective protein as biomarkers to differentiate the diagnosis of several neoplasms, including breast cancer, urothelial tumors, germ cell cancer, tumors of neuroendocrine lineage, mesothelioma and paraganglioma [19, 20, 21]. Based on the premise of *GATA3* gene being expressed in precursor and mature T cell neoplasm [3, 15, 22], our group sought to study its potential role as a marker to differentiate distinct nPTCL histopathological variants.

We were able to demonstrate that *GATA3* gene expression exhibited a heterogeneous distribution in the different histological variants of nPTCLs ($p < 0.001$). Moreover, we successfully found a cut-off of *GATA3* gene expression (0.71%) capable of differentiating the subtype PTCL/NOS from both AITL ($p = 0.04$) and ALK-negative ALCL variants ($p = 0.03$). The group of PTCL/NOS patients presented statistically significant higher levels of *GATA3* gene expression in comparison to other subtypes ($p < 0.001$). *GATA3* gene expression ≥ 0.71% favored the PTCL/NOS diagnosis, while expression lower than 0.71% favored the diagnosis of ALK-negative ALCL or AITL.

These molecular findings are particularly important as the variants of nPTCLs present enormous overlapping clinical, laboratorial and histological aspects, making their differentiation truly challenging in the clinical practice. Even for the most expert hematopathologists, the differentiation between PTCL/NOS and ALK-negative ALCL or AITL is quite problematic [1]. This analysis can be more troublesome particularly if tumor sample is scarce, preventing analysis of the complete architecture of the lymph node that is commonly found in samples obtained by core-biopsy [1, 23, 24].

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An additional issue to distinguish PTCL/NOS and AITL from ALK-negative ALCL is that the Ki-1 antigen (CD30) that is universally present in ALK- negative ALCL, can be expressed in 30% to 40% of PTCL/NOS and in 15% to 20% of AITL [1]. Even using the morphological criterion of more than 75% of hallmark cells expressing CD30, and a pattern of sinusoidal infiltration that characterizes anaplastic lymphomas, this differential diagnosis is still problematic. In fact, only the ALK-positive ALCL subtype is apparently less complicated to be individualized, since the presence of ALK helps in its differentiation from other nPTCL variants [1]. Besides that, the differentiation of PTCL/NOS CD30+ from ALK-negative ALCL is essential. According to the International T-cell group, the 5-year OS for ALK-negative ALCL is 50%, and 20-30% for PTCL/NOS [25]. In addition, ALK-negative ALCL benefits from ASCT consolidation in first line of therapy and with the addition of brentuximab-vedotin (anti CD30) in its chemotherapy regimen [26,27].

In our cohort, similar to the one reported by the European and North American international multicentric studies of PTCLs [25,28], there was a predominance of the male gender. However, even though there was a predominance of the PTCL/NOS variant, we observed more cases of ALCL than AITL, in opposition to the International T-cell Lymphoma Project (ITCLP) report [25]. In addition, our patients presented higher frequency of dismal clinical aspects, such as more B symptoms, advanced stage of disease, high risk of IPI and poor ECOG. We presume that this was partly due to diagnosis delay and the difficult access of the patients in our health services, that is a characteristic of developing countries. However, we also could not rule out the contribution of unfavorable biological characteristics intrinsic to these neoplasms to these phenotypic findings.

Conclusion

In conclusion, this study confirmed *GATA3* gene overexpression as a potential biomarker of poor prognosis associated with unfavorable OS for PTCL/NOS patients. In addition, we demonstrated for the first time that *GATA3* overexpression confers poor prognosis for ALK-negative ALCL patients, and can differentiate PTCL/NOS variant from other non-ALK-positive nPTCLs, as ALK-negative ALCL and AITL. Our results should be confirmed in future studies with different cohort of patients. Experimental studies investigating the pathway of *GATA3* gene in the pathogenesis of nPTCLs will also contribute to a better understanding of these diseases and to the further development of new targeted therapies.

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Abbreviations

PTCLs: peripheral T-cell lymphomas; NHL: non-Hodgkin lymphomas; nPTCLs: nodal peripheral T-cell lymphomas; PTCL/NOS: peripheral T-cell lymphoma, not otherwise specified; AITL: angioimmunoblastic T-cell lymphoma; ALK: anaplastic lymphoma kinase; ALCL: anaplastic large cell lymphoma; Pgp: P-glycoprotein; OS: overall survival; PFS: progression-free survival; BM: bone marrow; PS: performance status; ECOG: Eastern Cooperative Oncology Group; LDH: lactate dehydrogenase; IPI: International Prognostic Index; PIT: Prognostic Index for T-cell Lymphoma; HIV: human immunodeficiency virus; HTLV: human T-cell leukemia/lymphoma virus; CT: computerized tomography; H&E: Hematoxylin & Eosin; IHC: immunohistochemistry; ORR: overall response rate; CR: complete response; PR: partial response; PD: progressive disease; WHO: World Health Organization; FFPE: formalin-fixed paraffin-embedded; FDC: follicular dendritic cells; THf: T-helper follicular; EBV: Epstein-Barr virus; ASCT: autologous stem cell transplantation; CHOP: cyclophosphamide, doxorubicin, vincristine and prednisone; CHOEP: CHOP plus etoposide; qRT-PCR: quantitative real time – polymerase chain reaction; Ct: cycle threshold; ROC: receiver operator characteristic curve; IqR: interquartile range; ULN: upper limit of normality; PPV: predictive positive value; PNV: predictive negative value; ALL: acute lymphoblastic leukemia; Ph: Philadelphia chromosome; TAM: tumor-associated macrophage content; NK: natural killer; ITCLP: International T-cell Lymphoma Project.

Ethics approval and consent to participate: This study was approved by the local Ethic Committee (Sao Paulo University Ethic Committee) in April 2016, under the number 02975012.0.0000.0068. All participants signed an Informed Consent Form, agreeing to participate in this study.

Consent for publication: Not Applicable

Competing interests: The authors declare that they have no competing interests

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 doi:10.20944/preprints202004.0509.v1

Bibliographic references

1 – Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles G, Zelenetz AD, Jaffe ES. The 2016 revision of World Health Organization classification of lymphoid neoplasms. *Blood*. 2016; 127 (20): 2375-2390.

2 – Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood*. 2011; 117(19): 5019-5032.

3 – Iqbal J, Wright G, Wang C, Rosenwald A, Gascoyne RD, Weisenburger DD, Greiner TC, Smith L, Guo S, Wilcox RA, Teh BT, Lim ST, Tan SY, Rimsza LM, Jaffe ES, Campo E, Martinez A, Delabie J, Braziel RM, Cook JR, Tubbs RR, Ott G, Geissinger E, Gaulard P, Piccaluga PP, Pileri SA, Au WY, Nakamura S, Seto M, Berger F, de Leval L, Connors JM, Armitage J, Vose J, Chan WC, Staudt M. Gene expression signatures delineate biological and prognostic subgroups in peripheral T-cell lymphoma. *Blood*. 2014; 123(19):2915-2923.

4 – MA H, Abdul-Hay M. T-cell lymphomas, a challenging disease: types, treatments and future. *Int J Clin Oncol*. 2017; 22 (1): 18-51.

5 – Kansara R, Savage KJ. The problem with cyclophosphamide, doxorrubicin, vincristine and prednisone for the treatment of peripheral T-cell lymphoma. *Leuk Lymphoma*. 2014; 55(4): 727-729.

6 – Wang Y, Misumi I, Gu AD, Curtis TA, Su L, Whitmire JK, Wan YY. GATA-3 controls the maintenance and proliferation of T cells downstream of TCR and cytokine signaling. *Nat Immunol. 2013*; 14(7): 714-722.

7 – Cheng Y, Zhang J, Hou W, Wang D, Li F, Zhang Y, Yuan F. Immunoregulatory effects of sinomenine on the T-bet/GATA-3 ratio and Th1/Th2 cytokine balance in the treatment of mesangial proliferative nephritis. *Int Immunopharmacol*. 2009; 9(7-8): 894-899.

8 – Frelin C, Herrington R, Janmohamed S, Barbara M, Tran G, Paige CJ, Benveniste P, Zuñiga-Pflucker JC, Souabni A, Busslinger M, Iscove NN. GATA-3 regulates the self-renewal of long-term hematopoietic stem cell. *Nat Immunol. 2013*; 14(10): 1037-1044.

9 – Davis DG, Siddiqui MT, Oprea-Illies G, Stevens K, Osunkoya, Cohen C, Li XB. GATA-3 and FOXA1 expression is useful to differentiate breast carcinoma from other carcinomas. *Hum Patho*l. 2016; 47(1): 26-31.

10 – Ortiz-Rey JA, Chantada de la Fuente D, Peteiro-Cancelo MA, Gómez de Maria C, San Miguel Fraile MP. Usefulness of GATA-3 as a marker of seminal epithelium in prostate biopsies. *Actas Urol Esp*. 2017; 41(9): 577-583.

11 – Ronaghy A, Xiao GQ, Santagada E, Hasanovic A, Unger P. Expression of GATA-3 in testicular and gynecologic mesothelial neoplastic and non-neoplastic tissues. *Int J Gynecol Pathol*. 2018; 37(3): 284-289.

12 – de Pádua Covas Lage LA, Levy D, Xavier FD, Reis DC, de Oliveira Costa R, Gonçalves MC, Rocha V, Zerbini MCN, Pereira J. Proliferative, pro-inflammatory and angiogenesis regulator gene expression profile defines prognosis in different histopathological subtypes of nodal peripheral T-cell lymphoma. *Oncotarget*. 2019; 10(50): 5136-5151.

13 – Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, Lister TA. Recommendations for initial evaluation, staging and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol*. 2014; 32(27): 3059-3068.

14 – Huggett JF, Foy CA, Benes V, Emslie K, Garson JA, Haynes R, Hellemans J, Kubista M, Mueller RD, Nolan T, Pfafll MW, Shipley GL, Vamdesonpele J, Wittwer CT, Bustin SA. The digital MIQE guidelines: Minimun information for publication of quantitative digital PCR experiments. *Clin Chem*. 2013; 59(6): 892-902.

15 – Zhang W, Wang Z, Luo Y, Zhong D, Luo Y, Zhou D. GATA3 expression correlates with poor prognosis and tumor-associated macrophage infiltration in peripheral T-cell lymphoma. *Oncotarget*. 2016; 7(40): 65284-65294.

16 - Prez-Andreu V, Roberts KG, Harvey RC, Yang W, Cheng C, Pei D, Xu H, Gastier-Foster J, E S, Lim JY, Chen IM, Fan Y, Devidas M, Borowitz MJ, Smith C, Neale G, Burchard EG, Torgerson DG, Klussman FA, Villagran CR, Winick NJ, Camitta BM, Raetz E, Wood B, Yue F, Carroll WL, Larsen E, Bowman WP, Loh ML, Dean M, Bhojwani D, Pui CH, Evans WE, Relling MV, Hunger SP, Willman CL, Mullighan CG, Yang JJ. Inherited GATA3 variants are associated with Ph-like childhood acute lymphoblastic leukemia and risk of relapse. *Nat Genet*. 2013; 45(12): 1494-1498.

17 – Heavican TB, Bouska A, Yu J, Lone W, Amador C, Gong Q, Zhang W, Li Y, Dave BJ, Nairismagi NL, Greiner TC, Vose J, Weisenburger DD, Lachel C, Wang C, Fu K, Stevens JM, Lim ST, Ong CK, Gascoyne RD, Missiaglia E, Lemonier F, Haioun C, Hartmann S, Pedersen MB, Lagisnestra MA, Wilcox RA, The BT, Yoshida N, Ohshima K, Seto M, Rosenwald A, Ott G, Campo E, Rimza LM, Jaffe ES, Braziel RM, d'Amore F, Inghirami G, Bertoni F, de Leval L, Gaulard P, Staudt LM, McKeithan

TW, Pileri S, Chan WC, Iqbal J. Genetic drivers of oncogenic pathways in molecular subgroups of peripheral T-cell lymphomas. *Blood*. 2019; 133(15): 1664-1676.

18 – Parrilla-Castellar ER, Jaffe ES, Said JW, Swerdlow SH, Ketterling RP, Knudson RA, Sidhu JS, His ED, Karikehalli S, Jiang L, Vasmatzis G, Gibson SE, Ondrejka S, Nicolae A, Grogg KL, Allmer C, Ristow KM, Wilson WH, Macon WR, Law ME, Cerhan JR, Habbermann TM, Ansell SM, Dogan A, Maurer MJ, Feldman AL. ALK-negative anaplastic large cell lymphoma is a genetically heterogeneous disease with widely disparate clinical outcomes. *Blood*. 2014; 124(9): 1473-1480.

19 – Tozbikian GH, Zynger DL. A combination of GATA 3 and SOX 10 is useful for diagnosis of metastatic triple-negative breast cancer. *Hum Pathol*.2019; 85:221-227.

20 – Agarwal H, Babu S, Rana C, Kumar M, Singhai A, Shankwar SN, Singh V, Sinha RJ. Diagnostic utility of GATA3 immunohistochemical expression in urothelial carcinoma. *Indian J Pathol Microbiol.* 2019; 62(2): 244-250.

21 – Mete O, Kefeli M, Caliskan S, Asa SL. GATA3 immunoreactivity expands the transcription fator profile of neuroendocrine tumors. *Mod Pathol*. 2019; 32(4): 484-489.

22 – Dorfman DM, Morgan EA, Pelton A, Unitt C. T-cell transcription factor GATA3 is an immunophenotypic marker of acute leukemias with T-cell differentiation. *Hum Pathol*. 2017; 65: 166-174.

23 – Lage LA, Cabral TC, Costa RO, Gonçalves MC, Levy D, Zerbini MC, Pereira J. Primary nodal peripheral T-cell lymphomas: diagnosis and therapeutic considerations. Rev Bras Hematol Hemoter. 2015; 37(4): 277-284.

24 – Sachsida-Colombo E, Barbosa Mariano LC, Bastos FQ, Rassi AB, Lage LA, Barreto A, Siqueira S, Pereira J. A difficult case of angioimmunoblastic T-cell lymphoma to diagnose. Rev Bras Hematol Hemoter. 2016; 38(1): 82-85.

25 - Vose J, Armitage J, Weisenburger D. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. *J Clin Oncol*. 2008; 26(25): 4124-4130.

26 – d'Amore F, Relander T, Lauritszen GF, Jantunen E, Hagberg H, Anderson H, Holte H, Österborg A, Merup M, Brown P, Kuittinen O, Erlanson M, Ostenstad B, Fargeli UM, Gadeber OV, Sundstrom C, Delabie J, Ralfkiaer E, Vornanem M, Toldbod HE. Up-front autologous stem cell transplantation in peripheral T-cell lymphoma: NLG-T-01. *J Clin Oncol*. 2012; 30(25): 3093-3099.

 doi:10.20944/preprints202004.0509.v1

27 – Pro B, Advani R, Brice P, Bartlett NL, Rosenblatt JD, Illidge T, Matous J, Ramchandren R, Fanale M, Connors JM, Fenton K, Huebner D, Pinelli JM, Kennedy DA, Shustov A. Five-year results of brentuximab vedotin in patients with relapsed or refractory systemic anaplastic large cell lymphoma. *Blood*. 2017; 130(25): 2709-2717.

28 - Petrich AM, Helenowski IB, Bryan LJ, Rozell SA, Galamaga R, Nabhan C. Factors predicting survival in peripheral T-cell lymphoma in the USA: a population- based analysis of 8802 patients in the modern era. Br J Haemathol. 2015; 168(5): 708-718.

Table 1: Clinical characterization of all cohort of nPTCL (n=80)

Male, n(%)	43 (53.7%)
Age, median (IqR)	49 (34 – 59)
Histopathological subtype, n(%) ALK-positive ALCL ALK-negative ALCL PTCL, NOS AITL	 17 (21.2%) 25 (31.2%) 29 (36.3%) 09 (11.3%)
IPI int-high e high-risk n(%)	49 (61.2%)
PIT int-high e high-risk n(%)	47 (58.7%)
Extranodal areas ≥ 2 n(%)	30 (37.5%)
LDH ≥ UVN, n(%)	47 (58.7%)
Bone marrow involvement n(%)	11 (13.7%)
Bulky disease n(%)	30 (37.5%)
ECOG ≥ 2 n(%)	24 (30.0%)
B-symptoms n(%)	66 (82.5%)
CS Ann Arbor III e IV n(%)	77 (96.2%)
Primary treatment, n(%) CHOP	 29 (36.2%)

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CHOEP	31 (38.7%)
Others	12 (15.0%)
Not done	08 (10.0%)
Number of cycles, median (range)	06 (01 – 08)
Radiotherapy, n(%)	20 (25.0)
ASCT, n(%)	28 (35.0)
Interim response, n(%)	
Complete	27 (33.7%)
Partial	25 (31.2%)
Progressive/Refractory	09 (11.2%)
Not evaluated	19 (23.7%)
Final response, n(%)	
Complete	37 (47.5)
Partial	09 (11.2)
Progressive/Refractory	08 (10.0)
Mortality during first line treatment	25 (31.3)

IqR: interquartile range; ALCL/ALK+: anaplastic large cell lymphoma ALK positive; ALCL/ALK-: anaplastic large cell lymphoma ALK negative; PTCL/NOS: peripheral T-cell lymphoma, not otherwise specified; AITL: angioimmunoblastic T-cell lymphoma, IPI: International Prognostic Index; PIT: Prognostic Index for Peripheral T-cell Lymphomas; LDH: lactate dehydrogenase; ECOG: Eastern Cooperative Oncology Group; CS: clinical stage; CHOP: cyclophosphamide, doxorubicin, vincristine and prednisone; CHOEP: cyclophosphamide, doxorubicin, etoposide, vincristine and prednisone; ASCT: autologous stem cell transplantation.

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Table 2: Characteristics related to prognosis in the cohort of ALCL patients

	ALCL/ALK+ (n=17)	ACLCL/ALK- (n=25)
Age < 40 years, n(%)	11 (64.7)	11 (44.0)
IPI int-high e high-risk, n(%)	09 (52.9)	14 (56.0)
PIT int-high e high-risk, n(%)	09 (52.9)	12 (48.0)
ASCT n(%)	04 (23.5)	13 (52.0)
R/R disease n(%)	08 (47.0)	03 (12.0)

ALCL/ALK+: anaplastic large cell lymphoma ALK positive; ALCL/ALK-: anaplastic large cell lymphoma ALK negative; IPI: International Prognostic Index; PIT: Prognostic Index for T-cell Lymphomas; ASCT: autologous stem cell transplantation; R/R: Refractory/Relapsed disease.

Table 3: Relative expression of *GATA3* gene and discriminatory potential between different histopathological subgroups of nPTCL.

Comparision among groups	Z	P-value
AITL vs ALK-negative ALCL	1.12	0.52
AITL vs ALK-positive ALCL	2.77	0.27
ALK-negative vs ALK-positive ALCL	2.24	0.07
AITL vs PTCL, NOS	-0.71	**0.04**
ALK-negative vs PTCL, NOS	-2.60	**0.03**
ALK-positive vs PTCL, NOS	-4.64	**< 0.001**

ALCL/ALK+: anaplastic large cell lymphoma ALK positive; ALCL/ALK-: anaplastic large cell lymphoma ALK negative; PTCL/NOS: peripheral T-cell lymphoma, not otherwise specified; AITL: angioimmunoblastic T-cell lymphoma.

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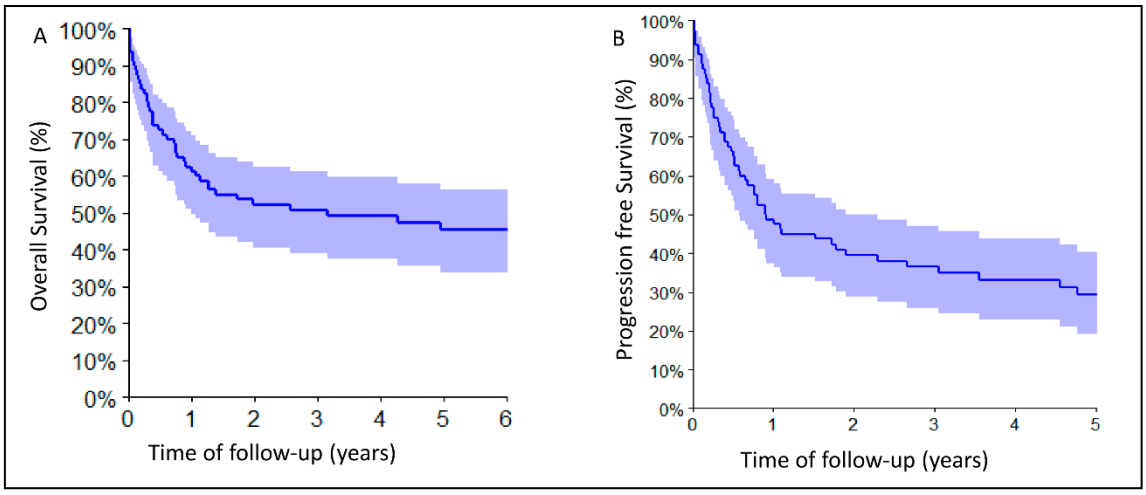

Figure 1 – Overall survival (A) and Progression-free survival (B) in total cohort of nPTCLs (n=80)

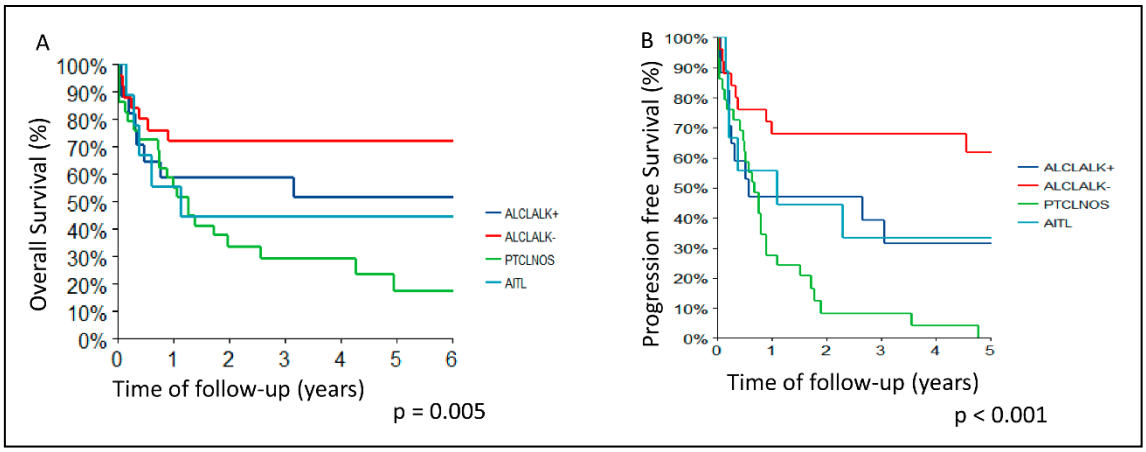

Figure 2 – Overall survival (A) and Progression-free survival (B) by nPTCL subtypes (n=80)

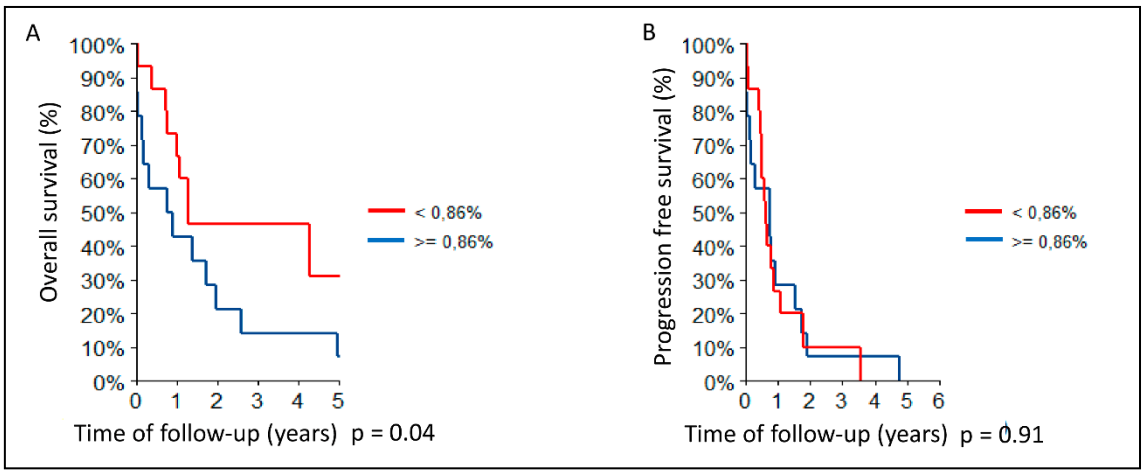

Figure 3 – Overall survival (A) and Progression-free survival (B) in PTCL/NOS (n=29) according to GATA3 gene expression (< or ≥ median)

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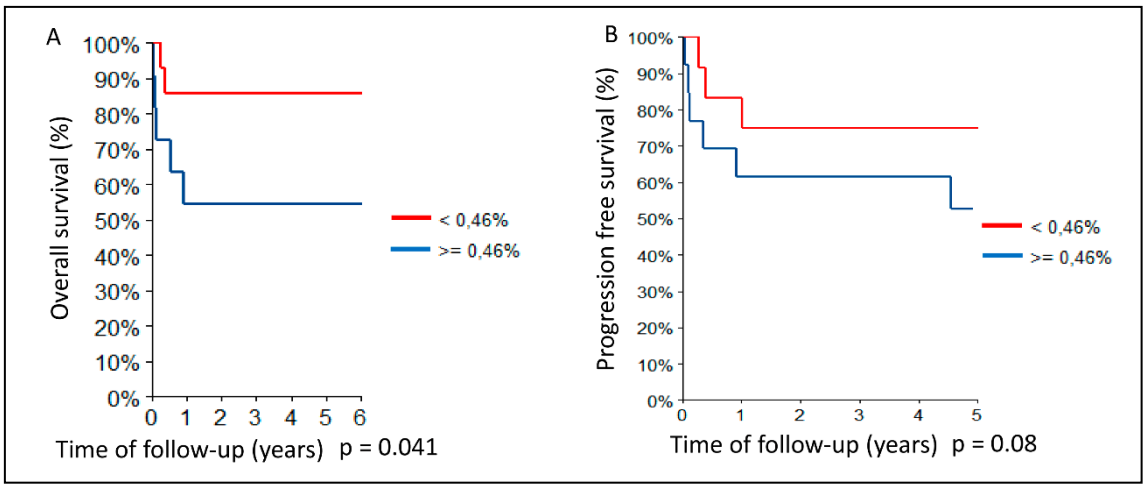

Figure 4 – Overall survival (A) and Progression-free survival (B) in ALK-negative ALCL (n=25) according to GATA3 gene expression (< or ≥ median)

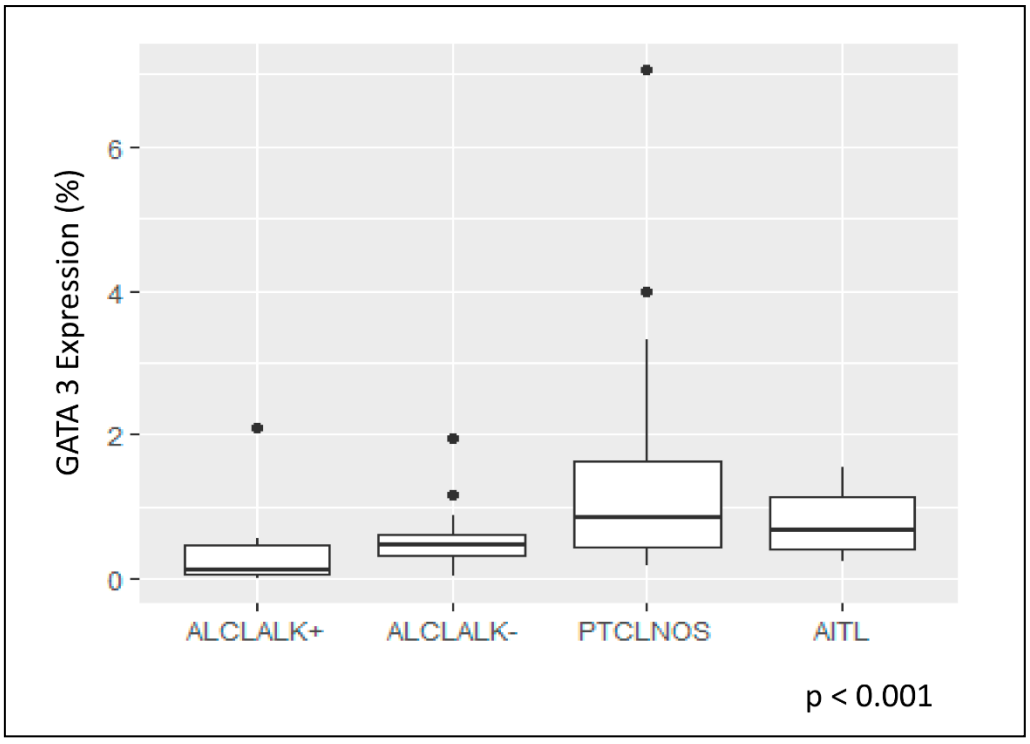

Figure 5 – Relative levels of GATA3 gene expression in the different subgroups of nPTCLs (n=80)