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Regular paper

The clinicopathological characteristics of C-MYC protein in angioimmunoblastic T-cell lymphoma

Yan Peng[1,2,3], Hong Yan[2,3], Jing Wang[2,3], Min Zhang[2,3] and Jie He[1,2,3]✉

[1]Anhui Provincial Hospital, Cheeloo College of Medicine, Shan Dong University, Jinan, 250012, China; [2]Department of Pathology, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, 230036, China; [3]Intelligent Pathology Institute, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, 230036, China

Aim: To investigate the relationship between expression of C-MYC protein and clinicopathologic characteristics in angioimmunoblastic T-cell lymphoma (AITL), then discuss the prognosis. Methods: Patient samples were collected and made into tissue microarray for the same experimental condition. Pathomorphological features and immunohistochemistry of specific markers were used to determine the diagnosis of AITL. Epstein-Barr virus (EBV) infection was detected by EBV-encoded small RNA by in situ molecular hybridizations. B cells atypical hyperplasia was explored by the immunohistochemistry and gene rearrangement, C-MYC protein was expressed by the immunohistochemical staining and *C-MYC* gene abnormalities were detected by fluorescence in situ hybridization (FISH). 44 AITL patients were divided into two groups and the relation among C-MYC protein, EBV infection and B cells hyperplasia was observed. We followed up the overall survival rate (OS) and progression-free survival (PFS), and analyzed C-MYC protein, and the relationship between the prognosis of survival. Results: Among 44 AITL patients, 29 cases (65.9%) were C-MYC protein-positive, and 32 (32/44, 72.3%) were EBV-positive. The expression rate of C-MYC protein in the high-risk group ranked by international prognostic index (IPI) and stage III–IV were higher than that in the low-risk group and stage I–II ($P<0.05$). The expression rate of C-MYC protein was higher in B cells excessive proliferation group, including simple hyperplasia, atypical hyperplasia, monoclonal B cells hyperplasia, and the diffuse large B lymphoma than in the low-proliferation group ($P<0.05$). The expression rate of C-MYC protein in EBV-positive patients was indifferent compared with the negative group ($P>0.05$). *C-MYC* gene rearrangement was not found in any samples, while the multi-copies of the *C-MYC* gene were found in only one case. As followed, 5-year OS was significantly low, and there was no significant difference in OS between the C-MYC protein-positive and negative groups. PFS in the positive group was significantly lower than that of the negative group. The difference in response to treatment between the two groups was statistically significant ($P<0.05$). Conclusion: We discussed the clinicopathologic characteristics and significance in AITLs, which predominantly express MYC-protein in <50% of cells, lacking *C-MYC* gene rearrangement. C-MYC protein-positive or negative expression was closely related to B cells excessive proliferation. C-MYC protein may be used as an indicator to judge the malignancy and to predict the prognosis of AITL.

Keywords: angioimmunoblastic T-cell lymphoma, C-MYC, survival prognosis

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✉e-mail: hejie23@ustc.edu.cn

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Abbreviations: AITL, angioimmunoblastic T-cell lymphoma; DAB, diaminobenzidine; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; EBER, Epstein-Barr Virus-Encoded RNA; FISH, fluorescence in situ hybridization; HE, hematoxylin-eosin; IHC, Immunohistochemistry; HRS, Hodgkin/Reed-Sternberg; IPI, international prognostic index; LDH, lactic dehydrogenase; OS, overall survival; PFS, progression-free survival; PTCL, peripheral T-cell lymphoma; TCR, T-cell receptor; TFH, follicular T helper

INTRODUCTION

Although the disease of angioimmunoblastic T-cell lymphoma (AITL) is rare, it is one of the most common types of T-cell lymphoma that originates from CD4 positive follicular helper T lymphocytes (TFH)(Basha *et al.*, 2019; Matsumoto *et al.*, 2017; Dupuis *et al.*, 2006) that has unique clinical and pathological characteristics (Hong *et al.*, 2018). More molecular insights into the multistage development of AITL were extended (Wang *et al.*, 2017). AITL is a sub-type of peripheral T-cell lymphoma (PTCL) with unique clinical symptoms, and it accounted for 2.1% of all NHL cases (Sharma *et al.*, 2014), and 15% to 20% of PTCL (Yabe *et al.*, 2019). AITL afflicts advanced-age individuals who, on average, are 60 years of age at the time of diagnosis without a notable gender predisposition (Huang *et al.*, 2020; Mourad *et al.*, 2008). AITL is associated with B-cell lymphoproliferative disorders and a constitutively activated immune system. The prognosis of patients with AITL is poor with a 5-year overall survival (OS) rate of 33% (Federico *et al.*, 2013). OS was defined as the time to death from any cause. The PFS was defined as the time to recurrence at any body parts, or all-cause death, which-ever came first. Thus, new therapeutic targets in AITL are urgently needed.

C-MYC, a proto-oncogene located on chromosome 8q24 (Palkó *et al.*, 2014), is transcribed in the human Burkitt lymphoma immunoglobulin heavy chain gene (Nwanze *et al.*, 2017), which encodes protein functions as an important transcription factor controlling a range of cellular functions, including cell cycle, cell growth, survival, cell metabolism, cell biosynthesis, adhesion and apoptosis (Wang *et al.*, 2019; Sun *et al.*, 2019). Existing research on *C-MYC* has mainly focused on Burkitt lymphoma and diffuse large B-cell lymphoma, with little focus on AITL. This study aimed to determine the role of the *C-MYC* gene in AITL, specifically investigating the clinicopathological characteristics of AITL patients, the relationship of C-MYC protein, various immunohisto-

chemical indicators, Epstein-Barr virus (EBV) infection, and the correlation of gene rearrangement.

MATERIALS AND METHODS

Patients and clinical database

Paraffin specimens of 44 AITL patients diagnosed by the Anhui provincial hospital group from 2016 to 2020 were selected and were stained with hematoxylin-eosin (HE). Two senior pathologists re-read the sections, confirmed the pathological diagnosis, and collected patients' clinical data, for example: clinical-stage, international prognostic index (IPI) score, skin rashes, B symptoms, and the level of lactate dehydrogenase (LDH). We enrolled two independent groups of 44 patients with C-MYC positive and negative groups in this study. A total of 44 patients were followed up, evaluated the association of the features with PFS and OS. All samples were anonymously coded by following the local ethical guidelines (as stipulated by the Declaration of Helsinki), and this study was approved by the Review Boards of The First Affiliated Hospital of USTC.

Tissue immunohistochemical staining

Paraffin-embedded and formalin-fixed samples were cut into 4 μm sections, and consecutive sections. Immunohistochemistry (IHC) was performed using EnVision method as previously described, the operation was performed on a Ventana automatic immunohistochemical staining instrument. The rabbit monoclonal C-MYC antibody (ab32072) was obtained from Abcam, CD20 (ZM-0039), CD3 (ZM-0417), CD2 (ZM-0278), CD4 (ZM-0418), CD10 (ZM-0283), Bcl-6 (ZM-0011), PD1 (ZM-0381), CXCL-13 (ZM-0601), CD21 (ZM-0040), Ki67 (ZM-0167) antibodies and the EnVision kit and DAB kit were acquired from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd. Known reactive lymph nodes were used as the positive control, and PBS was used as the negative control. C-MYC-positive staining sites were located in the nucleus and cytoplasm, C-MYC protein and CD20 protein index ≤5% was considered as negative staining.

In situ molecular hybridization of Epstein-Barr Virus-Encoded RNA (EBER)

The EBV Probe in Situ Hybridization Kit (Beijing Zhongshan Jinqiao Biotechnology, China, Co.LTD) was used to detect EBERs according to the following steps: (1) deparaffinization and dehydration of the paraffin sections using xylene and a series of graded ethanol; (2) pretreatment with protease K solution (1:100) for 5 minutes; (3) hybridization with digoxigenin-conjugated EBV (EBERs) probe at 37°C for 4 hours; (4) signal detection using peroxidase-conjugated anti-digo-xigenin antibody and 3,3'-diaminobenzidine (DAB); (5) counterstaining the sections with ematoxylin solution. The positive signals were brownish-yellow and localized within the nuclei.

Fluorescence *in situ* hybridization detection

The *MYC*/*IGH* fusion kit (Anbiping Pharmaceutical Technology Co., LTD, Guangzhou) was used according to the manufacturer's instructions, followed by washing and chromogenic reaction with DAPI. The *IGH* gene was labeled with green luciferin, and the *C-MYC* gene was labeled with red luciferin in the IGH/C-MYC fusion translocation probe. When an orange signal indicating fusion of the *IGH*/*C-MYC* gene appeared, one red signal and one green signal were detected in the interphase nucleus. If more than 10% of tumor cells revealed orange signals, it was considered as positive for 100 tumor cells were counted with clear signals. If 3 or more red or green signals appeared in one nucleus, it was considered as C-MYC copy number-gain. The patients with reactive hyperplasia of lymph nodes were used as a negative control, and the buffer was used as a blank control.

Gene rearrangement

The clonal rearrangement of the T-cell receptor (TCR) gene in AITL was detected by PCR, DNA was extracted using a special extraction kit for paraffin tissue DNA from the QIAGEN Company. BIOMED-2 primer design and gene rearrangement were used to detect *TCR* gene rearrangement. Positive criteria for *TCR* gene clonal rearrangement: electrophoretic bandwidth <1 mm, sharp edge, an electrophoretic product within the predetermined position range is positive for gene clonal rearrangement; bands that were not in a predetermined position were considered as false positive.

Statistical analysis

SPSS 16.0 statistical software was mainly used for the data analysis. Log-rank analysis was used to test whether there was a difference between two groups. Patient survival data were analyzed with the Kaplan-Meier method. Results that showed $P<0.05$ were considered statistically significant. Overall survival (OS) was calculated starting from the diagnosis date ending on the date of death or the date of the last follow-up (June 2021). Progression-free survival (PFS) was calculated starting from the diagnosis date and ending on the first date of disease progression, relapse, or death from any cause or the last date of a follow-up.

RESULTS

Baseline patient characteristics

A total of 44 patients, 32 male (72.7%) and 12 female (27.3%) with the median age of 64 years (43 to 86 years), were included in the analysis. According to Ann arbor's clinical-stage, there were 20 cases with stage I–II and 24 cases with stage III–IV. The IPI, a prognostic index, namely for aggressive B-cell NHL, demonstrated a percentage of 34.1 (15 cases) in 0–2 points (low-risk group to medium-low-risk group) and a percentage of 65.9 (29 cases) in 3–5 points (medium-high-risk group to high-risk group). Twenty-four cases (54.5%) had LDH content <248 U/L, and 20 cases (45.5%) had LDH content ≥248 U/L. Fourteen cases (31.8%) were without B symptoms, 30 cases (68.2%) had B symptoms. Twenty-seven cases were without rash (61.4%), and seventeen cases had a rash (38.6%). There were 29 cases (65.9%) with C-MYC protein-positive. There were statistically significant differences in the positive rate of C-MYC protein expression between IPI score and clinical stage ($P<0.05$). There was no statistical significance in the positive rates of C-MYC protein expression and gender, age, B symptoms, LDH level, rash, and EBV infection (Table 1).

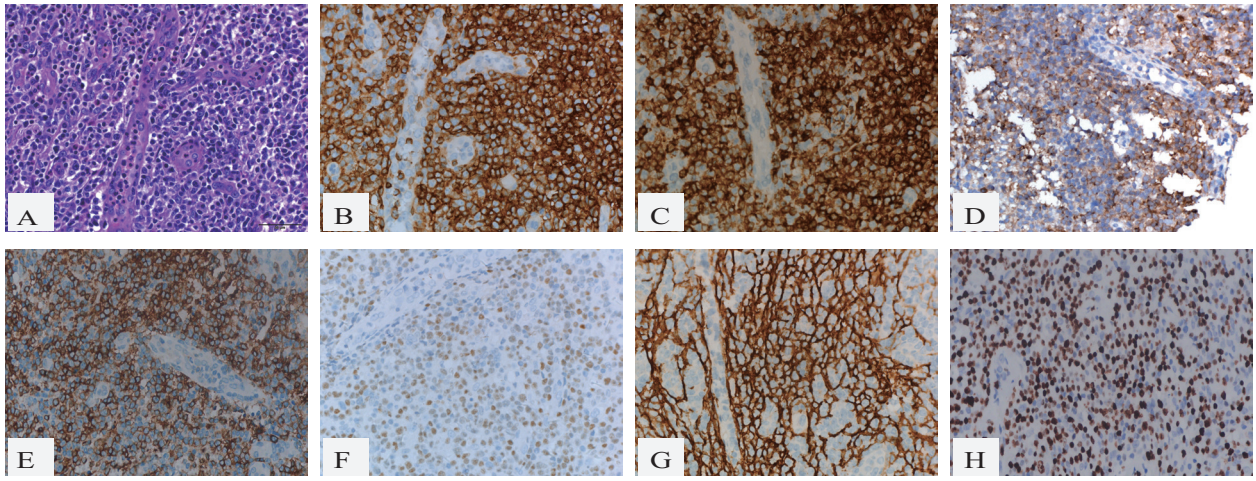

Figure 1. Angioimmunoblastic T-cell lymphoma showing typical histological and immunophenotypic features.
AITL is characterized by polymorphous background infiltrate composed of medium-sized atypical lymphocytes, with markedly high endothelial branched small veins (**A**). Atypical T-lymphocytes are CD4-positive (**B**), CD10-positive (**C**), CXCL13-positive (**D**), PD1-positive (**E**), BCL6-positive (**F**). The expanded CD21-positive follicular dendritic cell meshwork (**G**) and high expression Ki67 (**H**). (A: HE staining, ×400; B–H: immunostaining, ×400).

The pathological morphology and immunophenotypic characteristics of AITL

The typical histological morphology of AITL is characterized by infiltration of heterotypic T lymphocytes in the lymph nodes, with markedly high endothelial branched small veins and marked proliferation of follicular dendritic cells (Fig. 1A). Thus, we examined the expression of AITL-related specific markers in patient tissues. Results of immunochemistry showed 40 patients with CD4 positive cases (Fig. 1B), 38 patients with CD3 positive cases, 36 patients with CD2 positive cases, 34 patients with CD5 positive cases, and only 9 patients with CD7 positive cases (20.1%). Subsequent detection of germinal center-specific markers showed that 94.7% cases expressing PD1 (Fig. 1C), 70% cases expressing CXCL-13 (Fig. 1D), 45% cases ex-

Table 1. The clinicopathologic characteristics of patients included in this study

	C-Myc protein		χ^2-value	*P*-value
	–	+		
Age			0.006	0.606
≤ 60	5	10		
> 60	10	19		
Gender			0.004	0.621
Female	4	8		
Male	11	21		
Clinical stages			4.036	0.043
I–II	10	10		
III–IV	5	19		
IPI (Points)			3.214	0.017
0–2	9	6		
3–5	6	23		
LDH (U/L)			0.557	0.331
<248	7	17		
≥248	8	12		
B symptoms			0.024	0.568
Without	5	9		
With	10	20		
Rash			0.017	0.573
Without	9	18		
With EBV infection EBER-negative EBER-positive	6 2 13	11 10 19	2.179	0.127

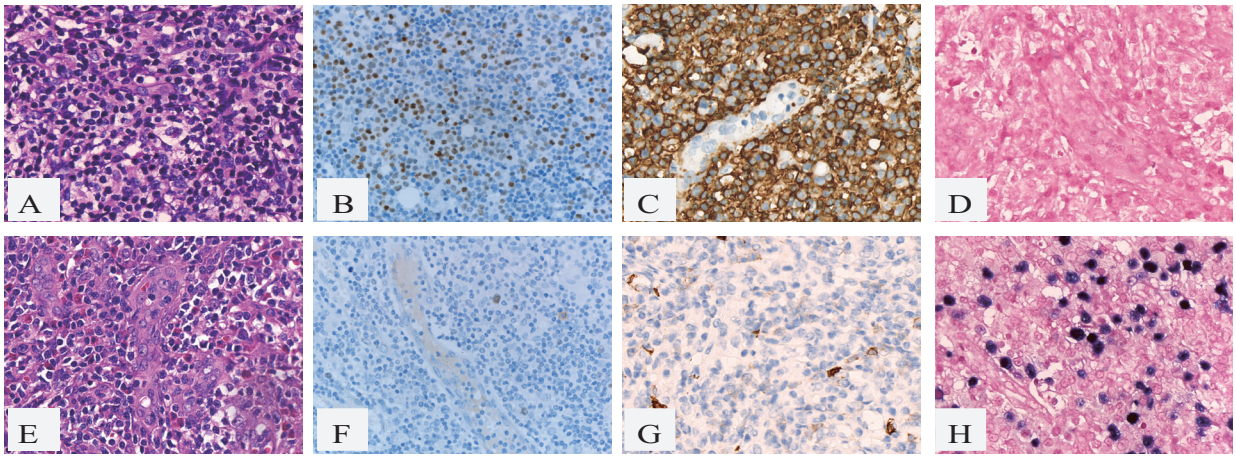

Figure 2. All the cases of AITL were divided into two groups:(A–D, E–H)
(A) Represent HE stain of AITL sample of B lymphocytes hyperplasia, B lymphocytes decreased or low hyperplasia (E). Immunostaining in a C-MYC positive (B) and C-MYC negative group (F). The group with positive expression of C-MYC protein showing strong staining for CD20 (C), negative EBER (D). The group with negative expression of C-MYC protein is weakly expressing CD20 (G), and highly expressing EBER (H)respectively. (A and E: HE staining×400; B–C, F–G: immunostaining, ×400; D and H: *in situ* molecular hybridization of Epstein-Barr Virus-Encoded RNA, ×400).

Table 2. The relationship between the expression of C-MYC and B cells proliferation

B lymphocytes hyperplasia	C-MYC protein		χ^2-value	*P*-value
	–	+		
CD20+ B cells			6.046	0.017
B cell low expression	8	5		
B cells high expression	7	24		

pressing CD10 (Fig. 1E), and 60% cases express Bcl-6 (Fig. 1F). B lymphocytes were obviously bigger than the normal B immunoblast cells, nucleis became obviously bigger and stained deeply, such as the Hodgkin/Reed-Sternberg (HRS)-like cells and mummified cells. The follicular dendritic cells were expressing CD21 were expressed in all cases (Fig. 1G). AITL showed a high proliferation index for Ki67 (Fig. 1H).

Relationship between EBV infection and C-MYC protein

Thirty-two of the 44 patients were positive for EBER (positive rate: 72.7%, shown in Fig. 2D, H) and the expression of C-MYC protein in patients with positive and negative EBV infection was not statistically significant ($P<0.05$) (Table 1). The negative groups have infected at percentage of 86.6 (13/15), C-MYC positive group was 76% (19/29). The more EBER+ cases were susceptible to the negative groups.

Relationship between the proliferation of B cells and C-MYC protein

The B cells proliferation was observed in 31 cases of 44 AITL patients, and there was a significant difference between ($P<0.05$) with and without the proliferation of B cells (Table 2). In addition, 8 cases of AITL showed monoclonal B cells clonal proliferation, three cases of complicated or secondary DLBCL showed not only κ and λ monoclonal restricted expressions but also increased C-MYC protein expression. Another 5 cases with the clonal proliferation of B cells showed a positive expression of C-MYC protein. Among 16/23 cases showed positive expression of C-MYC protein. CD20 immunohistochemical staining highlights the proliferation of B cells (shown in Fig. 2C, F).

Table 3. The relationship between C-MYC protein and *TCR* gene rearrangement and *C-MYC* gene copies

	C-MYC protein		χ^2-value	*P*-value
	–	+		
TCR gene rearrangement			0.201	0.464
–	4	6		
+	11	23		
C-MYC gene copies			0.162	0.579
–	14	26		
+	1	3		

Relationship between *TCR* gene rearrangement and C-MYC protein

AITL gene rearrangement results showed that *TCR* rearrangement occurred in 34 (77.3%) out of 44 AITL patients, and there was no significant difference between C-MYC protein expression and TCR rearrangement (Table 3).

Expression of *C-MYC* gene in AITL

No translocation of the *C-MYC* gene was found in 44 AITL cases, but 4 of them had multiple copies of the gene (3 or 4 copies, shown in Fig. 3). The rate of *C-MYC* gene abnormalities in C-MYC protein-positive

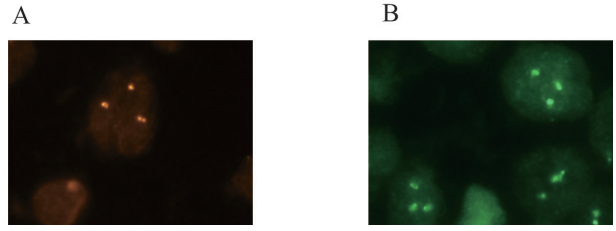

Figure 3. FISH detection for multiple copies of the *C-MYC* gene and Ig in AITL.
(**A**) The case has multiple copies of the *C-MYC* gene (4 copy numbers). (**B**) The case has multiple copies of immunoglobulin heavy chains (3 copy numbers).

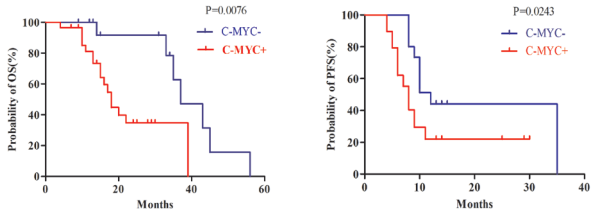

Figure 4. Comparison of survival rate.
The OS and PFS between the C-MYC positive and C-MYC negative groups (**A**). OS=Overall survival. PFS=progression-free survival (PFS).

cases and C-MYC protein negative cases was 6.82% (3/44) and 2.27% (1/44), respectively, with no statistically significant difference (Table 4). Correspondingly, 3 copies of B-cell immunoglobulin heavy chains also showed multi-copies among the patients at 60 years or older, and at the clinical stage of III–IV.

The disease remission rate of patients with C-MYC protein in AITL

44 AITL specimens, 29 were positive for C-MYC protein, and OS of patients in the C-MYC protein-positive group showed a statistically significant difference (P=0.0076). PFS of patients in the C-MYC protein-positive group was significantly lower than that in the C-MYC protein-negative group (P=0.0243) (shown in Fig. 4).

DISCUSSION

Peripheral T-cell lymphoma (PTCL) is a rare, heterogeneous group of mature T-cell neoplasms that comprise 10–15% of non-Hodgkin lymphoma cases in the United States, with the second most common subtype being the angioimmunoblastic type, accouting for 18.5% of the cases worldwide. In other words, AITLs have a unique epidemiology and pathobiology and are usually clinically aggressive with poor outcomes.

AITL is based on the biology of normal CD4+ T-cell subsets, which is emerging from the estimation of these subtypes. The 2017 World Health Organization update introduced a new category of nodal peripheral T-cell lymphoma with T-follicular helper phenotype (PTCL-TFH) defined by the expression of at least 2 or 3 TFH markers (Basha *et al.*, 2019; Zhou *et al.*, 2007). All of our cases were confirmed to be TFH phenotype by immunohistochemical staining. The rudimentary immunophenotype of a follicular T helper (T_{FH}), the cell of origin for AITL, is CD3, CD4, and CD10 and Bcl-6 positive (Marafioti *et al.*, 2010; Yuan *et al.*, 2005). The T-cell receptor is α–β with often aberrant loss of CD5 and/or CD7. Furthermore, T_{FH} expression of PD-1 (Dorfman *et al.*, 2006), CXCL-13 (Grogg *et al.*, 2006), ICOS and CD200 (Dorfman & Shahsafaei, 2011) continue to support an expression pattern that distinguishes AITL from a spectrum of benign lymphoproliferative disorders to other PTCL subtypes that have a TFH cell of origin. Our results showed that CD4, CD3, CD2, and CD5 were positive in almost all cases, but CD7 was positive in only 6 cases. At the same time, PD1 and CXCL-13 were expressed in most cases, the near-uniform expression of cytoplasmic CXCL13 has improved the diagnostic confidence. Bcl-6 was expressed in more than half of the cases, while CD10 was expressed in less than half of the cases. This suggests that the nature of each case needs to be specially treated.

This study aimed to examine the relationship between protein expression and gene abnormality of C-MYC protein in AITL and to examine clinical parameters, pathological characteristics, immune phenotype. We analyzed 44 cases of AITL, performed C-MYC protein and AITL-specific markers immunohistochemical studies, detected the EBV infection by in situ molecular hybridizations, B-cells hyperplasia by immunohistochemical staining and gene rearrangement, and evaluated for *C-MYC* gene abnormalities by FISH.

The discovery that T cells and B cells are both required for antibody production in an immune response produced the concept of T-cell help to B cells. It was followed by the finding that the culture of naive CD4+ T cells, which drives B-cell proliferation. When B-cell proliferation was out of control, it led to the B cells atypical hyperplasia, monoclonal hyperplasia might even subsequently progress to diffuse to the large B cell lymphoma. In the present study, cases were divided into 2 groups, according to the expression of C-MYC protein. Our findings showed that the B-cell excessive proliferation often appeared in the C-MYC protein-positive group, which had stimulated the expression of C-MYC protein.

The reactivation of EBV in AITL is thought to be an event secondary to an associated immune dysfunction. EBV+ B cells could have been detected very early in the disease course of AITL, which implies that it may also play a role in the development of AITL (Zhou *et al.*, 2007). B cells excessive proliferation first showed that the number of B cells was significantly increased, the histomorphology of atypical hyperplasia was that the cell volume became obviously bigger, nuclei became bigger and stained deeply, nucleoli seemed obvious, such as in B immunoblast cells, HRS cell and mummified cells. The B cellexcessive proliferation was detected by CD20 immunohistochemical staining, called CD20+ B cells. A few cases of extranodal EBV-associated B-cell lymphomas arising in patients with angioimmunoblastic T-cell lymphoma (AITL) have been reported (Poon *et al.*, 2019). Many reviews have insisted that EBV infection is a driving factor that stimulates B cell proliferation and monoclonal proliferation, developing into diffused large B cell lymphoma, EBV-positive (EBV+) B-cell lymphomas, after AITL treatment (Hashimoto *et al.*, 2021; Hoffmann *et al.*, 2016).

It has been reported that composite DLBCL and PTCLs, with the frequency of C-MYC protein overexpression, were significantly higher than that of PTCL-independent DLBCL. Two patients were simultaneously diagnosed with AITL and DLBCL, the other patient was diagnosed with AITL one year ago, biopsy lymph nodes were re-examined and progressed into DLBCL. Another 28 patients, who were occasionally found with B-cell hyperplasia proliferation, including monoclonal and restricted expression proliferation, were not susceptible to EBV infection. These cases with the high expression of C-MYC protein, EBV infection was irregular in the percentage of EBER+ cells. The relationship between EBV and MYC signaling pathways was even more complex, C-MYC positive and negative groups seemed to have little correlation with EBV infection. EBV virus was both detected in the positive and negative group of C-MYC protein, which had no obvious difference in the percentage of EBER+ cells and proportion of infections (Fig. 2). Some studies suggest that MYC protein expression is up-regulated in the presence of EBV (Hoffmann

et al., 2016). However, in our cases, the overlap between MYC expression and EBV could not support such findings. Still, other studies suggested that C-MYC may liberate cells from the EBV-driven process. EBV infection promoted the B cells atypical hyperplasia, whether or not the other unknown factors also led to the B cells atypical hyperplasia. The mechanisms of action among them are still unclear, additional studies are needed to further elucidate the relationship between EBV and MYC in these rare composite lymphomas.

MYC protein expression levels were not necessarily correlated with *C-MYC* gene status, which was similar to our conclusion that C-MYC protein expression is independent of gene state. Previous reports showed that C-MYC protein expression was 50% and 70% in some lymphomas, but *C-MYC* gene rearrangement was still lacking. Regarding the expression of MYC in T cell lymphomas, Chisholm and others (Chisholm *et al*., 2015) performed immunohistochemistry for MYC on cases of peripheral T cell lymphoma and ALK+ anaplastic large cell lymphoma and found that these neoplasms predominantly express MYC in <50% of cells. Our findings, together with the previous reviews, have suggested that MYC is expressed in T cell lymphomas in less than 50% of cells in AITL. Our results revealed that the *C-MYC* gene rearrangements do not necessarily correlate with C-MYC protein expression. Maybe there was a unique mechanism for C-MYC protein expression in AITL.

Additionally, we followed up with 44 patients and recorded their survival and prognosis. AITL showed variable biological and clinical presentations. The survival rate of patients with AITL did not correlate with T-cell clonality, the presence of EBV-infected cells, EBV-DNA copy number. The EBER status did not affect OS or PFS, the recent review also suggested that among younger patients, an EBER+ status might be associated with an indolent clinical course and a better prognosis compared to an EBER status (Eladl *et al*., 2020). The patients with high expression of EBER were more likely to appear in the C-MYC negative expression group, which had a relatively good prognosis.

In this study, only 3 AITL patients with diffuse large B cell lymphoma have worse prognosis than the simple AITL cases, and their survival time was obviously shortened. We still observed a significant reduction in survival time, a faster progression of the disease, which was consistent with the previous study. The review that patients with composite lymphoma with diffuse large B cell lymphoma and AITL showed worse OS. AITL frequent mutations in TET2, DNMT3A, IDH2, and RHOA (Yao *et al*., 2020; Sakata-Yanagimoto *et al*., 2014; Odejide *et al*., 2014), the prognostic impact of these mutations is not widely investigated (Hsu *et al*., 2020). TET2 and DNMT3A mutations were identified in CD20+ cells, whereas several mutations, including NOTCH1 mutations, were detected only in CD20+ cells (Ohshima *et al*., 2018). The CD20+cell staining can reflect the B cells atypical/monoclonal hyperplasia. Most of the CD20+ B-cells were corresponding to the C-MYC positive group, with rapid disease progression and poor prognosis.

There were some limitations to this study. First, the data were obtained in one endemic area in China, while the distribution of clinical characteristics might be different in some areas. Second, our study involved a limited number of cases which is a reflection of the fact that this lymphoma is infrequent in pathology practice. The cases were divided into two groups, where each group became smaller. Moreover, our study mainly focused on histological morphology and immunohistochemical level, the molecular mechanism is not involved. We knew that further investigations were needed to verify and supplement these results.

In conclusion, AITL with B cells atypical hyperplasia are significantly related with the expression of C-MYC protein, and C-MYC positive patients had significantly lower PFS than C-MYC negative ones, which further indicated that C-MYC might become a new marker of AITL consistent with the above markers.

Declarations

Acknowledgements: We thank the patients who participated in this study.

Statement of Ethics: This study was approved by the Ethics Committee and Institutional Review Board of The First Affiliated Hospital of USTC, and written informed consent was obtained from each patient included in the study.

Conflict of Interest Statement: The authors declare no conflicts of interest.

Author Contributions: Each author contributed equally to this study.

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