RESEARCH ARTICLE
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GATA3 Immunohistochemical Staining in Classical Hodgkin Lymphoma and its Diagnostic Utility in Differential Diagnosis

Klasik Hodgkin Lenfomada GATA3'ün İmmünhistokimyasal Boyanması ve Ayırıcı Tanıda Kullanılabilirliği

Altay A.Y. et al.: GATA3 Expression in Classical Hodgkin Lymphoma

Ali Yılmaz Altay[1], Begüm Yeni Erdem[2], Gülçin Yegen[1]
[1]İstanbul University, İstanbul Faculty of Medicine, Department of Pathology, İstanbul, Türkiye
[2]University of Health Sciences Türkiye, Kartal Dr. Lütfi Kırdar City Hospital, Clinic of Pathology, İstanbul, Türkiye

Ali Yılmaz Altay, M.D., İstanbul University, İstanbul Faculty of Medicine, Department of Pathology, İstanbul, Türkiye
+90 212 414 24 00
alialtay07@gmail.com

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ABSTRACT

Objective: Classical Hodgkin lymphoma (CHL) is a common lymphoid neoplasm with a wide range of differential diagnosis. Although it has a specific immunophenotype, aberrant expression of antigens can cause problems at its diagnosis. In this study we evaluated the usefulness of GATA3 in differential diagnosis of classical Hodgkin lymphoma.

Material and Method: One hundred CHL cases and a control group of 106 lymphoma cases, which include anaplastic large cell lymphoma (ALK (+) and (-)), EBV (+) large B cell lymphoma, T-cell/histiocyte rich B cell lymphoma, primary mediastinal large B cell lymphoma, nodular lymphocyte predominant Hodgkin lymphoma and mediastinal grey zone lymphoma, were included in the study. GATA3 immunohistochemistry were applied to all cases and its nuclear expression was accepted as positive. Expression status of GATA3 was compared in between the CHL and the control group, as well as among each lymphoma subtype. In addition, whether the biopsy type effects its diagnostic performance is assessed. In CHLs relationship with EBV status and GATA3 expression is evaluated.

Results: GATA3 expression was significantly higher in CHL cases compared to the control group ($p<0,001$). When compared with the individual subgroups GATA3 is still found to be useful in differential diagnosis except for ALK (-) ALCL ($p=0,678$) and mediastinal gray zone lymphomas ($p=0,327$). GATA3 expression is significantly higher in EBV (-) CHLs ($p=0,02$). In core needle biopsies its diagnostic performance is limited ($p=0,178$).

Conclusions: GATA3 is a useful marker for differentiating CHL from B-cell non-Hodgkin lymphomas but its efficiency is limited in ALK (-) ALCL and mediastinal grey zone lymphomas. Due to the heterogeneous reaction diagnostic value is limited in core needle biopsies.

Keywords: classical Hodgkin lymphoma, GATA3, anaplastic large cell lymphoma, incisional/excisional biopsy

ÖZET

Amaç: Klasik Hodgkin lenfoma (KHL), geniş bir ayırıcı tanı yelpazesine sahip, sık görülen bir lenfoid neoplazidir. Özgün bir immünfenotipe sahip olmasına rağmen beklenenin dışında olabilen immünfenotiplerde tanısal zorluk olabilir. Bu çalışmada GATA3'ün klasik Hodgkin lenfoma ayırıcı tanısındaki kullanışlılığını değerlendirdik.

Gereç ve Yöntem: Yüz KHL olgusu ve anaplastik büyük hücreli lenfoma (ALK (+) ve ALK (-)), EBV (+) büyük B hücreli lenfoma, T hücreleri ve histiyositlerden zengin büyük B hücreli lenfoma, primer mediastinal büyük B hücreli lenfoma, nodüler lenfosit predominant Hodgkin lenfoma ve mediastinal gri zon lenfomadan oluşan 106 olguluk kontrol grubu çalışmaya alındı. Tüm olgulara GATA3 immünohistokimyası uygulandı ve çekirdek boyanması pozitif olarak kabul edildi. GATA3 ifadesi KHL grubu ile bir bütün olarak kontrol grubu ve onu oluşturan lenfoma alt grupları ile karşılaştırıldı. Ayrıca biyopsi tipinin GATA3'ün tanısal performansına etkisi ve KHL grubu içerisinde GATA3 ifadesinin EBV ile olan ilişkisi değerlendirildi.
Sonuçlar: KHL vakaları ile kontrol grubu kıyaslandığında GATA3 ifadesi KHL vakalarında anlamlı şekilde yüksek bulunmuştur ($p<0,001$). Alt gruplarla karşılaştırıldığında GATA3'ün, ALK (-) anaplastik büyük hücreli lenfoma ($p=0,678$) ve mediastinal gri zon lenfomaları ($p=0,327$) dışında ayırıcı tanıda yararlı olduğu görülmektedir. EBV (-) KHL'lerde GATA3 ekspresyonu anlamlı derecede yüksektir ($p=0,02$). İğne biyopsilerinde ise GATA3'ün tanısal performansı sınırlıdır ($p=0,178$).
Tartışma: GATA3, KHL'yi B hücreli non-Hodgkin lenfomalardan ayırmak için yararlı bir belirteçtir ancak ALK (-) anaplastik büyük hücreli lenfoma ve mediastinal gri zon lenfomalarında katkısı sınırlıdır. Ayrıca kısmi pozitiflik gösterebilmesi nedeniyle iğne biyopsisi gibi sınırlı örneklemelerde tanısal katkısı sınırlı olabilmektedir.

INTRODUCTION

Classical Hodgkin lymphoma (CHL) is a relatively common lymphoid neoplasm with distinct morphology and immunohistochemical profile[1]. Although, it is a a B cell neoplasm most of the B cell antigen expression were lost due to defective B cell programming. Furthermore, aberrant T cell antigen expression can be detected [1]. Due to the morphological and immunohistochemical properties, there is a wide range of differential diagnosis including T and B cell non-Hodgkin lymphomas[1–3]. Diagnosis might be challenging, especially in limited biopsies, because of the overlapping features among the entities.

GATA3 is a transcription factor that differentiates T helper cells to Th2 subtype[4] and is reported to be aberrantly expressed in CHL[4,5]. There are a few studies suggesting it as a useful marker in the differential diagnosis of CHL[6–8] but as they didn't cover the entire spectrum of entities that are included in the differential diagnosis of CHL, its diagnostic utility is still not clear. Here we aimed to assess the usefulness of GATA3 in the differential diagnosis of CHL by evaluating the expression status of GATA 3 in CHL and a wide range of entities that are in the differential diagnosis.

MATERIAL AND METHOD

Case Selection

One hundred CHL cases diagnosed between 2006 and 2023 were included in the study. Control group consisted of 106 cases which include ALK (+) (10 cases) and ALK (-) (46 cases) anaplastic large cell lymphoma (ALCL), EBV (+) diffuse large B cell lymphoma (DLBCL) (10 cases), T-cell/histiocyte rich large B cell lymphoma (THRLBCL) (15 cases), primary mediastinal large B cell lymphoma (PMLBCL) (10 cases), nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) (10 cases), and mediastinal grey zone lymphoma (MGZL) (5 cases).

Immunohistochemical Analysis

GATA3 (L50-823, Biocare mouse monoclonal) immunohistochemistry (IHC) was performed on the 3 µm thick sections of the formaline-fixed paraffin-embedded tissues representing the tumor, by using an automated immuno-stainer (Ventana Medical System-Benchmark XT/ISH Staining module). Nuclear staining accepted as positive regardless of staining extent and intensity. Cases with a staining extent of less than 50% of neoplastic cells are accepted as partial positive.

Statistical Analysis

Chi-square and Fischer exact test was performed for statistical analysis. A p-value less than 0,05 was accepted as statistically significant.

RESULTS

The demographic features of CHL and the control group were summarized in Table 1. CHL subtypes included in the study are as follows: 39 nodular sclerosis (NS), 21 mixed cellularity (MC), 20 lymphocyte rich (LR) and 20 lymphocyte poor (LP). Seventeen of the NS subtype were grade 2 and 2 were syncytial variant. In LR subtype 11 cases showed nodular and 9 cases showed diffuse growth pattern. EBV status, determined by EBV-LMP1 immunohistochemistry, was available for 88 CHL cases. EBER in situ hybridization was available for one case. Thirty-three cases (44%) (9 NS, 15 MS, 8 LR and 1 LP) were EBV positive. Eighty-three of CHL cases (83%) were diagnosed with excisional biopsy (29 NS, 18 MC, 17 LR, 19 LP) and 17 cases (17%) were diagnosed with core needle biopsy

(CNB) (10 NS, 3 MC, 3 LR, 1 LP). Sixty-nine of CHL cases (69%) were positive with GATA3 (29 NS, 13 MC, 13 LR, 14 LP) (Figure 1). Partial positivity was observed in 10 CHL cases (4 NS, 2 MC, 3 LR, 1 LP). In most cases, staining was observed in the majority of cells but not all. Staining intensity was also more prominent than the background T lymphocytes. GATA3 expression did not differ in CHL histological subtypes (p>0,05).

In the control group 61 (32 ALCL (5 ALK (+), 27 ALK (-)), 8 EBV (+) DLBCL, 10 THRLBCL, 9 NLPHL, 2 MGZL) were excisional biopsies, 45 (24 ALCL (5 ALK (+), 19 ALK (-)), 2 EBV (+) DLBCL, 5 THRLBCL, 10 PMLBCL, 1 NLPHL, 3 MGZL) were CNB. Thirty-eight of 106 cases (36%) (33 ALCL (3 ALK (+), 30 ALK (-)), 1 EBV (+) DLBCL, 1 THRLBCL, 1 PMLBCL, 2 MGZL) showed GATA3 positivity (Figure 2). Partial positivity was observed in 10 cases (7 ALCL (2 ALK (+), 5 ALK (-)), 1 EBV (+) DLBCL, 1 THRLBCL, 1 PMLBCL).

GATA3 positivity was significantly higher in CHL than in the control group (p<0,001). When the control group was divided into B cell and T cell non-Hodgkin lymphomas (NHL), the difference was statistically significant in B cell NHLs (EBV+DLBCL, THRLBCL, PMLBCL, MGZL) (p<0,001), whereas the difference was insignificant in T cell NHLs (ALCLs) (p=0,221). When the expression of GATA3 in CHL is compared with each lymphoma subtype GATA3 positivity was still significantly higher in CHL vs EBV (+) DLBCL (p<0,001), CHL vs THRBCL (p<0,001), CHL vs PMLBCL (p<0,001) and CHL vs NLPHL (p<0,001). However, the difference between CHL vs ALCL and CHL vs MGZL was not statistically significant (p=0,221 and p=0,327 respectively). When compared with ALCL subgroups individually, GATA3 positivity in CHL vs ALK (+) ALCL is statistically significant (p=0,031) but the significance disappears in CHL vs ALK (-) ALCL (p=0,678).

To find whether EBV influences GATA3 expression in CHL we compared the expression status of GATA3 in EBV-LMP1 (+) and EBV-LMP1 (-) CHL cases. GATA3 positivity was significantly higher in EBV (-) CHL cases (p=0,02).

Lastly, as GATA3 is not expressed in all neoplastic cells we analyzed whether the biopsy type affects the utility of GATA3 in differential diagnosis. Although GATA3 expression was significantly different between CHL excisional vs control excisional (p<0,001), the statistical significance was lost in the CHL CNB vs control CNB setting (p=0,178).

Details about GATA3 expression and statistical analysis are summarized in Table 2.

DISCUSSION

CHL, with its morphologic and immunohistochemical features, has a wide variety of entities in its differential diagnosis which requires different clinical management. In this study we evaluated GATA3's usefulness in CHL differential diagnosis.

Although a mature B cell neoplasm, CHL has a defective B cell program that results in loss of the expression of most of the B cell antigens and aberrant expression of other antigens as well as T cell markers[1,2]. GATA3 is one of the such transcription factors (TF) that is reported to be expressed in Reed-Sternberg cells, which is normally expressed in T lymphocytes and regulates Th2 differentiation[4]. It is speculated that NFkB and Notch-1 activation in CHL causes the GATA3 expression[5].

In our study, GATA3 is found to be a reliable marker in the differential diagnosis of CHL and B cell non-Hodgkin lymphomas such as EBV (+) DLBCL, THRLBCL, PMLBCL and NLPHL. Our results are consistent with other studies[6,8] except for PMLBCL. In their study, Kezlarian et al reported GATA3 positivity in 75% of the PMLBCL cohort. Whereas we only have one case that shows partial positivity with GATA3. Similar results to ours were reported by Kim et al[7]. Compared to the CHL cases, the positivity of GATA3 in above mentioned B-cell NHLs tends to be limited and the staining intensity of GATA3 is equal to reactive T lymphocytes that reside in the microenvironment. Whereas in CHL GATA3 staining intensity is generally higher than accompanying T-cells. In their recent study Papoudou-Bai A et al[9] discussed the staining extent of GATA3 in CHL cases and offered a cut off value of 26% for GATA3 positivity. Staining extent is important for most immunohistochemical markers and more diffuse and homogenous expression increases their diagnostic reliability. However, in neoplasms like CHL which contain dispersed and relatively small number of neoplastic cells, we believe giving a staining extent is difficult and can be unreproducible. In our cohort when GATA3 positivity is present, it is seen in most of the neoplastic cells. For the cases which show partial positivity roughly less than 50% of cell show GATA3 expression. It also should be noted that GATA3 expression is not a requirement for CHL diagnosis. Our data and literature indicate 69%, 80%[6] and 54%[9] positivity rates respectively. GATA3 positivity is a supportive feature for the diagnosis of CHL and its differential diagnosis from B-NHLs and NLPHLs but, its negativity does not exclude the diagnosis of CHL.

Our results with ALCL and MGZL tell us a different story. Although GATA3 is still useful in the differential diagnosis of CHL and ALK (+) ALCL, although ALK positivity makes GATA3 obsolete.

The important issue is the GATA3 positivity in ALK (-) ALCL. GATA3 positivity is reported for primary cutaneous ALCL[10] but at least to our knowledge its positivity in systemic ALK (-) ALCL wasn't discussed in the literature. We believe this finding is important because in some cases antigenic overlap between CHL and ALK (-) ALCL can cause a real diagnostic dilemma[1,2]. GATA3, along with TBX-21 (T-bet) is used for subclassifying peripheral T-cell lymphoma, NOS to Th1 (TBX-21 expression) and Th2 (GATA3 expression) subgroups via gene expression profiling (GEP)[11]. With their target cytokines, CCR5 and CXCR3 respectively, a four-antibody IHC panel is proposed for Th1 and Th2 distinction[11]. This subgrouping approach hasn't been reported for ALCL and thus we don't know its relationship with the current ALK (-) ALCL subtypes. There are reported similarities in oncogenic pathways in ALCL and CHL like BATF3 and STAT3[12]. STAT3 activation can be important regarding GATA3 because STAT3 activation is also seen in PTCL-GATA3 subgroup[11,13]. GATA3 expression in CHL and ALCL can be a byproduct of this similarity.

We evaluated the relationship between GATA3 expression and EBV status by EBV-LMP1 in CHL. The expression of GATA3 is significantly lower in EBV-LMP1 (+) CHL. Although our finding is based on EBV-LMP and not confirmed by EBER, it is in concordance with GATA3 negativity in EBV (+) DLBCLs. This can be attributed to EBV positive cases have fever genetic mutations, especially in NFkB and JAK/STAT pathway[14–16] in CHL.

As an alternative to excisional biopsy, CNB of lymph nodes has been a widely accepted procedure for diagnosis. As the evaluated tissue is limited in CNBs the differential diagnosis of CHL can become challenging due to the polymorphic background that may mask the neoplastic cells. Furthermore, heterogeneous immunoreactions in neoplastic cells increase the diagnostic difficulty. Reliable diagnostic markers play an important role to be more conclusive and to reduce the risk of misdiagnosis. The effect of the biopsy type on GATA3's diagnostic utility wasn't discussed in the literature. In our study we evaluated whether GATA3's contribution to diagnosis differed depending on the biopsy type. Its contribution to diagnosis was not significant in CNB, which is probably due to heterogeneous expression of GATA3 in neoplastic cells.

STUDY LIMITATIONS

Main limitations of the study are limited EBV status detection by EBER in situ hybridization and unavailability of ALK (-) ALCL subgrouping by DUSP22 and TP63 fluorescence in situ hybridization. For the first limitation, latency type of EBV (type 2a) in CHL helps us to overcome this issue. EBV-LMP1 is enough to detect EBV presence. Second situation arise due to the unexpected high expression of GATA3 in ALK (-) ALCL cases. Although the lack of subgroup information of ALK (-) ALCL did not affect interpretation of GATA3, whether GATA3 expression is higher in a specific ALK (-) ALCL subgroup is a question to be answered.

CONCLUSION

GATA3 is a useful marker in the differential diagnosis of CHL from B-cell NHLs and NLPHL. However, it does not have an additive value in differential diagnosis of CHL vs ALK (-) ALCL and MGZL. Since it is not expressed in all neoplastic cells, its contribution to diagnosis is limited in CNB.

Declarations:

Disclosure of potential conflicts of interest: None

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Ethics approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of the Istanbul University, Istanbul Medical Faculty (No: 2022/2042)

Author Contributions:

Design: Ali Yılmaz Altay, Gülçin Yegen

Data collection and processing: Ali Yılmaz Altay, Begüm Yeni Erdem

Analysis or interpretation: Ali Yılmaz Altay, Gülçin Yegen, Begüm Yeni Erdem

Literature Search: Ali Yılmaz Altay, Begüm Yeni Erdem

Writing: Ali Yılmaz Altay, Gülçin Yegen

Abbreviations:

cHL: Classical Hodgkin lymphoma

NHL: Non Hodgkin lymphoma

ALK: Anaplastic lymphoma kinase

ALCL: Anaplastic large cell lymphoma
EBV: Epstein Barr virus
DLBCL: Diffuse large B cell lymphoma
THRLBCL: T-cell/Histiocyte rich large B cell lymphoma
PMLBCL: Primary mediastinal large B cell lymphoma
NLPHL: Nodular lymphocyte predominant Hodgkin lymphoma
MGZL: Mediastinal grey zone lymphoma
NS: Nodular sclerosis
MS: Mixed cellularity
LR: Lymphocyte rich
LP: Lymphocyte poor
TF: Transcription factor
CNB: Core needle biopsy
LMP1: Latent membrane protein 1
GATA3: GATA binding protein 3
EBER: Epstein Barr virus encoded small RNAs

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Table 1: Demographic features and biopsy types of cases	
Classical Hodgkin lymphoma (n=100)	
Gender	Male: 61
	Female: 39
Age (Median)	33 (4-88)
Biopsy type	Excisional: 83
	Core needle biopsy: 17
Anaplastic large cell lymphoma ALK+ (n=10)	
Gender	Male: 6
	Female: 4
Age (Median)	20,5 (10-40)
Biopsy type	Excisional: 5
	Core needle biopsy: 5
Anaplastic large cell lymphoma ALK- (n=46)	
Gender	Male: 36
	Female: 10
Age (Median)	50 (14-85)
Biopsy type	Excisional: 27
	Core needle biopsy: 19
EBV+ Diffuse large B cell lymphoma (n=10)	
Gender	Male: 8
	Female: 2
Age (Median)	54 (11-81)
Biopsy type	Excisional: 8
	Core needle biopsy: 2
T cell and histiocyte rich large B cell lymphoma (n=15)	
Gender	Male: 12
	Female: 3
Age (Median)	45 (27-83)
Biopsy type	Excisional: 10
	Core needle biopsy: 5
Primary mediastinal large B cell lymphoma (n=10)	
Gender	Male: 3
	Female: 7
Age (Median)	39 (17-62)
Biopsy type	Excisional: 0
	Core needle biopsy: 10
Nodular lymphocyte predominant Hodgkin lymphoma (n=10)	
Gender	Male: 7
	Female: 3
Age (Median)	36,5 (10-65)
Biopsy type	Excisional: 9
	Core needle biopsy: 1
Mediastinal grey zone lymphoma (n=5)	
Gender	Male: 2
	Female: 3
Age (Median)	27 (15-72)
Biopsy type	Excisional: 2
	Core needle biopsy: 3

Table 2: Expression status of GATA3 among subgroups			
Comparison of GATA3 expression between CHL and control group			
	GATA3 +	GATA3-	p
CHL (N=100)	69	31	P<0,001
Control group(N=106)	38	68	
Comparison of GATA3 expression between CHL and control group (n)			
CHL (100) vs control group (106)			p<0,001
CHL (100) vs B-NHL group (40)			p<0,001
CHL (100) vs ALCL (56)			p=0,221
CHL (100) vs ALK (+) ALCL (10)			p=0,031
CHL (100) vs ALK (-) ALCL (46)			p=0,678
CHL (100) vs EBV (+) DLBCL (10)			p<0,001
CHL (100) vs THRLBCL (15)			p<0,001
CHL (100) vs PMLBCL (10)			p<0,001
CHL (100) vs NLPHL (10)			p<0,001
CHL (100) vs MGZL (5)			p=0,327
Comparison of GATA3 expression between CHL subtypes			
NS (39) vs non-NS (61)			p=0,397
NS grade 1 (20) vs NS grade 2+syncytial variant (19)			p=0,461
NS (39) vs MC (21)			p=0,347
NS (39) vs LR (20)			p=0,490
NS (39) vs LP (20)			p=0,765
MC (21) vs LR (20)			p=0,837
MC (21) vs LP (20)			p=0,585
LR (20) vs LP (20)			p=0,736
Comparison of GATA3 expression between EBV (+) and EBV (-) CHL cases			
EBV (+) (33) vs EBV (-) (56)			p=0,02
Comparison of biopsy types between CHL and control group			
CHL CNB (17) vs control CNB (45)			p=0,178
CHL excisional (83) vs control excisional (61)			p<0,001

CHL: Classical Hodgkin lymphoma, GATA3: GATA binding protein 3, B-NHL: B cell non Hodgkin lymphoma, ALCL: Anaplastic large cell lymphoma, ALK: Anaplastic lymphoma kinase, EBV: Epstien Barr virus, DLBCL: Diffuse large B cell lymphoma, THRLBCL: T cell and histiocyte rich large B cell lymphoma, PMLBCL: Primary mediastinal large B cell lymphoma, NLPHL: Nodular lymphocyte predominant Hodgkin lymphoma, MGZL: Mediastinal grey zone lymphoma, NS: Nodular sclerosis, MC: Mixed cellularity, LR: Lymphocyte rich, LP: Lymphocyte poor, CNB: Core needle biopsy

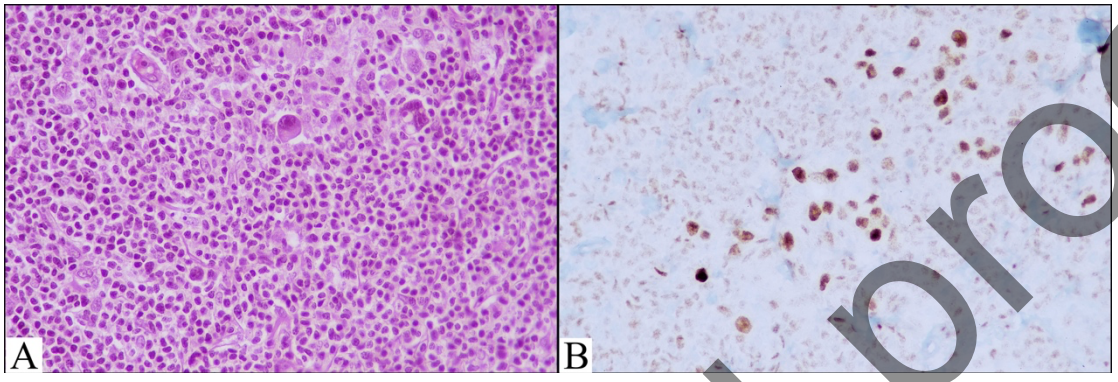

Figure 1: A: Classical Hodgkin lymphoma nodular sclerosis subtype. HE x400. B: GATA3 positivity in Hodgkin and Reed-Sternberg type neoplastic cells. GATA3 staining intensity is greater than the background T lymphocytes. IHC x400

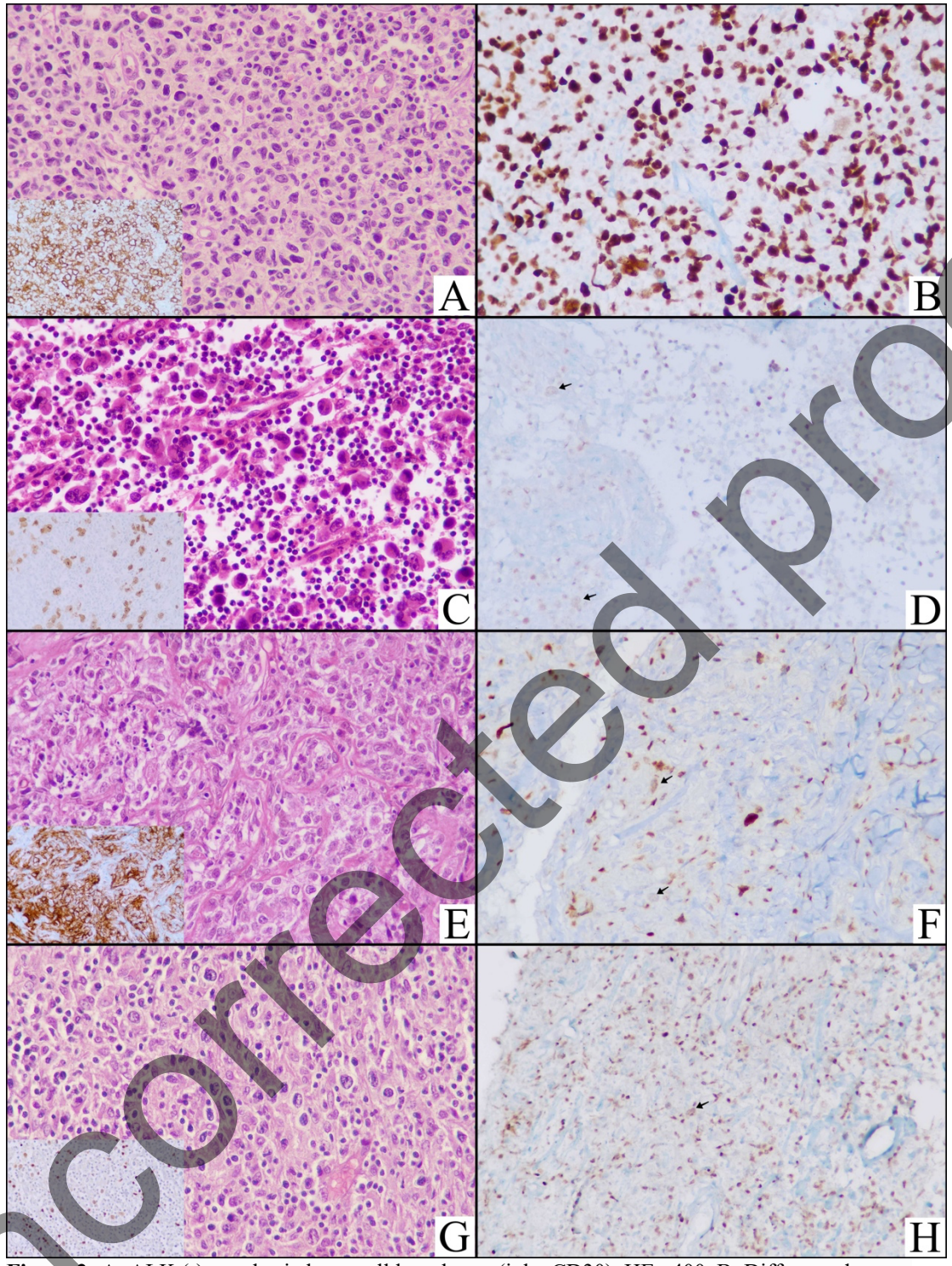

Figure 2: A: ALK (-) anaplastic large cell lymphoma (inlet CD30). HE x400. B: Diffuse and strong GATA3 positivity at neoplastic cells. IHC x400. C: ALK (+) anaplastic large cell lymphoma (inlet ALK1). HE x400. D: Weak GATA3 positivity observed in very few neoplastic cells. IHC x400. E: Primary mediastinal large B cell lymphoma (inlet CD23). HE x400. F: Weak to moderate GATA3 positivity detected in few neoplastic cells. IHC x400. G: T cell and histiocyte rich large B cell lymphoma (inlet pax5). H: Weak to moderate GATA3 positivity detected in very few neoplastic cells. IHC x400.