Expression of TFH Markers and Detection of RHOA p.G17V and IDH2 p.R172K/S Mutations in Cutaneous Localizations of Angioimmunoblastic T-Cell Lymphomas

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滤泡生发中心T细胞

- 滤泡生发中心CD4+ T辅助性细胞（T_{FH}）
- Bcl-6、CD10、CD4、PD1、CD57阳性
- CXCL-13、CXCR5、ICOS阳性
- 血管免疫母细胞性T细胞淋巴瘤
- *滤泡性T细胞淋巴瘤*
- *淋巴结伴有T_{FH}表型的外周T细胞淋巴瘤*
- *原发皮肤CD4阳性小/中T细胞增殖性疾病*

血管免疫母细胞性T细胞淋巴瘤AITL

- 是一种特殊类型的外周T细胞淋巴瘤
- 形态学上以淋巴结内成簇的透明T细胞增生（部分病例），明显的高内皮静脉和FDC细胞增生为特征（T细胞淋巴瘤几乎无FDC网）
- 是一类和EBV关系密切的疾病
- 没有典型的临床表现时应谨慎诊断

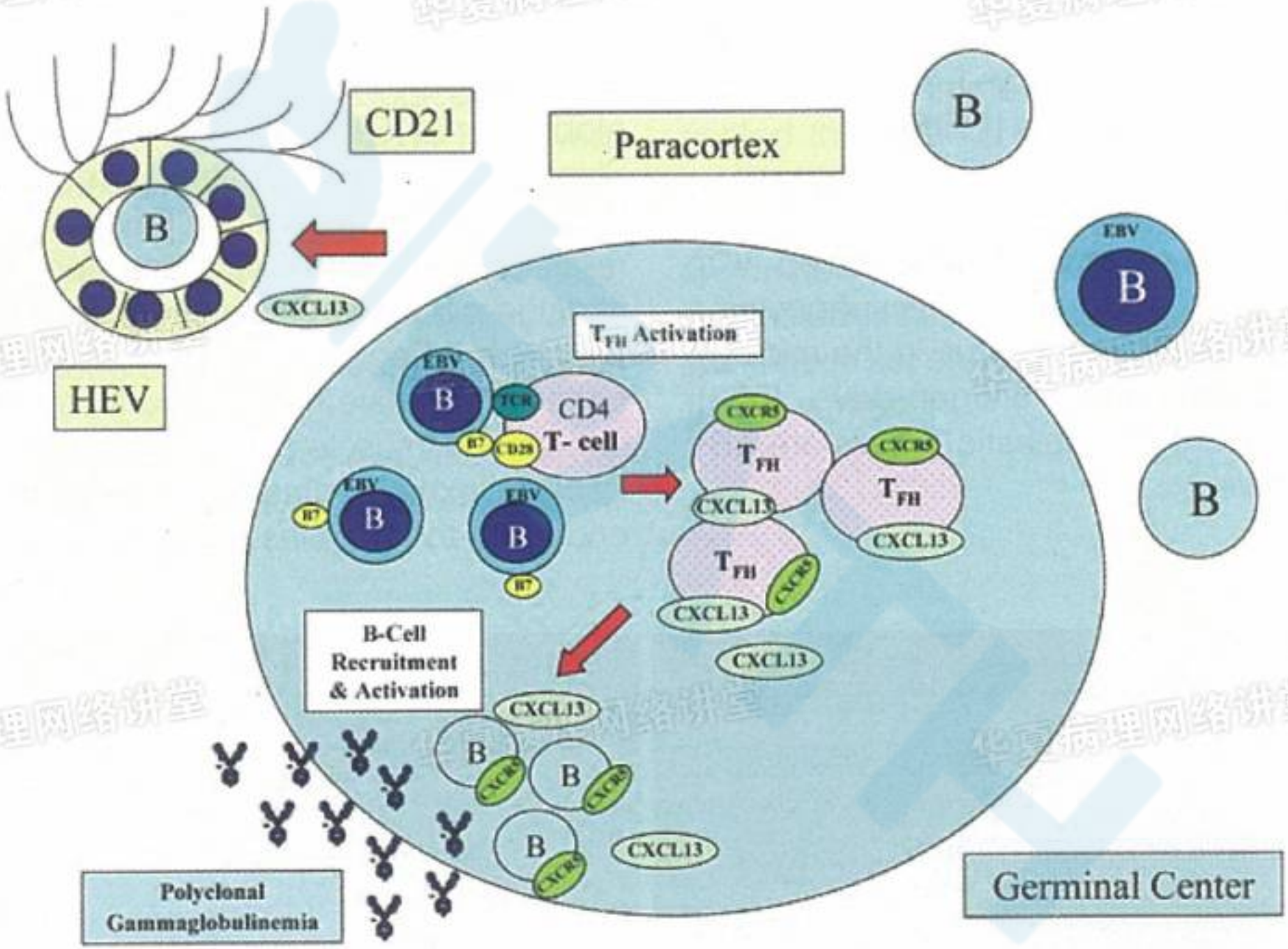
AITL临床特点

- 好发于老年人，男女比例约1:1，常出现全身多发淋巴结肿大
- “B”症状多见，皮疹，肝脾常肿大
- β2微球蛋白及LDH常升高，多克隆性高丙种球蛋白血症，溶血性贫血
- 几乎均原发于淋巴结，结外罕见，可累及结外，如皮肤
- 侵袭性临床经过，治疗后伴发感染性并发症的危险性高

AITL累及皮肤

- 大约50%的AITL会发生病变
- 常表现为广泛的斑丘疹，类似病毒性皮疹、药物疹、风疹、紫癜、红斑性鳞状斑块、痒疹、红皮病、糜烂和坏死性病变
- 皮肤病变以非特异性表浅血管周侵犯为特征，由嗜酸性粒细胞和无非典型性的淋巴细胞组成，伴有毛细血管增生。
- 可见混杂的浆细胞和组织细胞。有些病例具有克隆性T细胞受体重排。

AITL 发生机制


CD21
Paracortex
B
EBV
B
HEV
CXCL13
T_{FH} Activation
EBV
B
TCR
CD4
T- cell
B7
CD28
CXCR5
T_{FH}
CXCL13
EBV
B
B7
EBV
B
B7
B-Cell
Recruitment
& Activation
CXCL13
B
B
B
CXCL13
Polyclonal
Gammaglobulinemia
Germinal Center

AITL病理学特点

- 低倍镜下结构破坏或部分破坏，背景细胞成分混杂:反应性小淋巴细胞，嗜酸性粒细胞，浆细胞，组织细胞
- 滤泡旁高内皮静脉（HEV）增生
- 可见胞质透亮的非典型性T淋巴细胞
- 散在分布的B免疫母细胞
- 部分病例可见R-S样细胞散在分布

AITL形态学特点

组织学模式（3型）

模式一

滤泡增生，副皮质区扩张，淋巴结结构部分保存

模式二

滤泡萎缩，副皮质区显著扩张

模式三

淋巴结结构破坏，滤泡消失

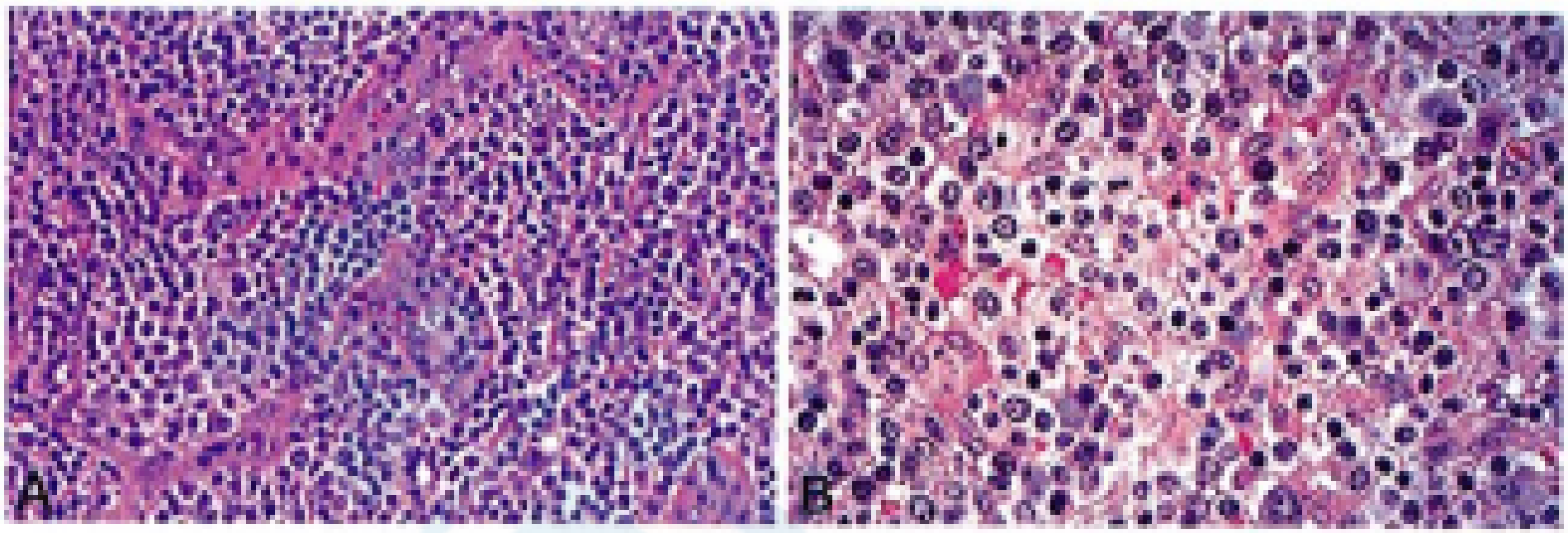


图 11.73　血管免疫母细胞性 T 细胞淋巴瘤。A 典型肿瘤细胞呈中等大小的核，胞浆丰富，淡染/透明。B 浸润灶由中－大的淋巴样细胞组成，有丰富的透明胞浆。

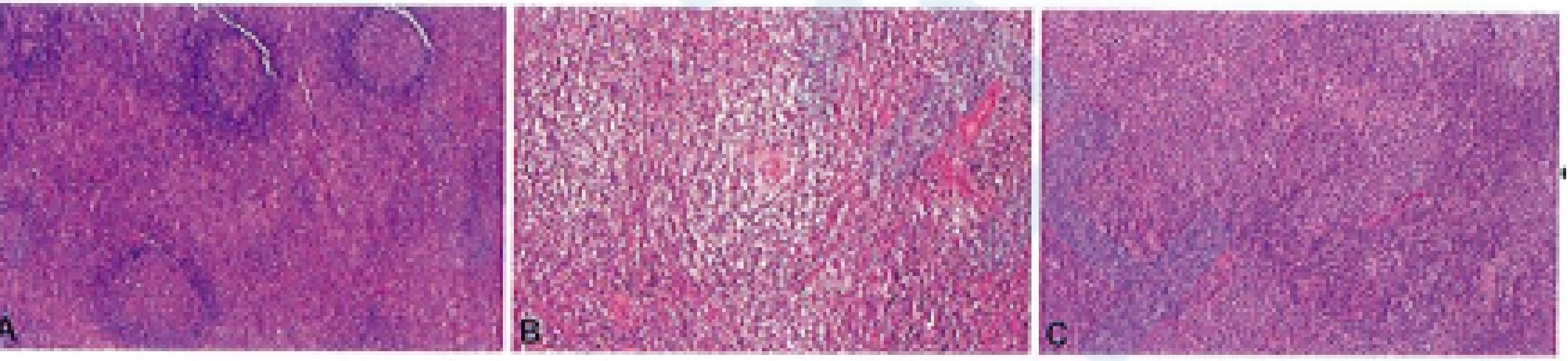
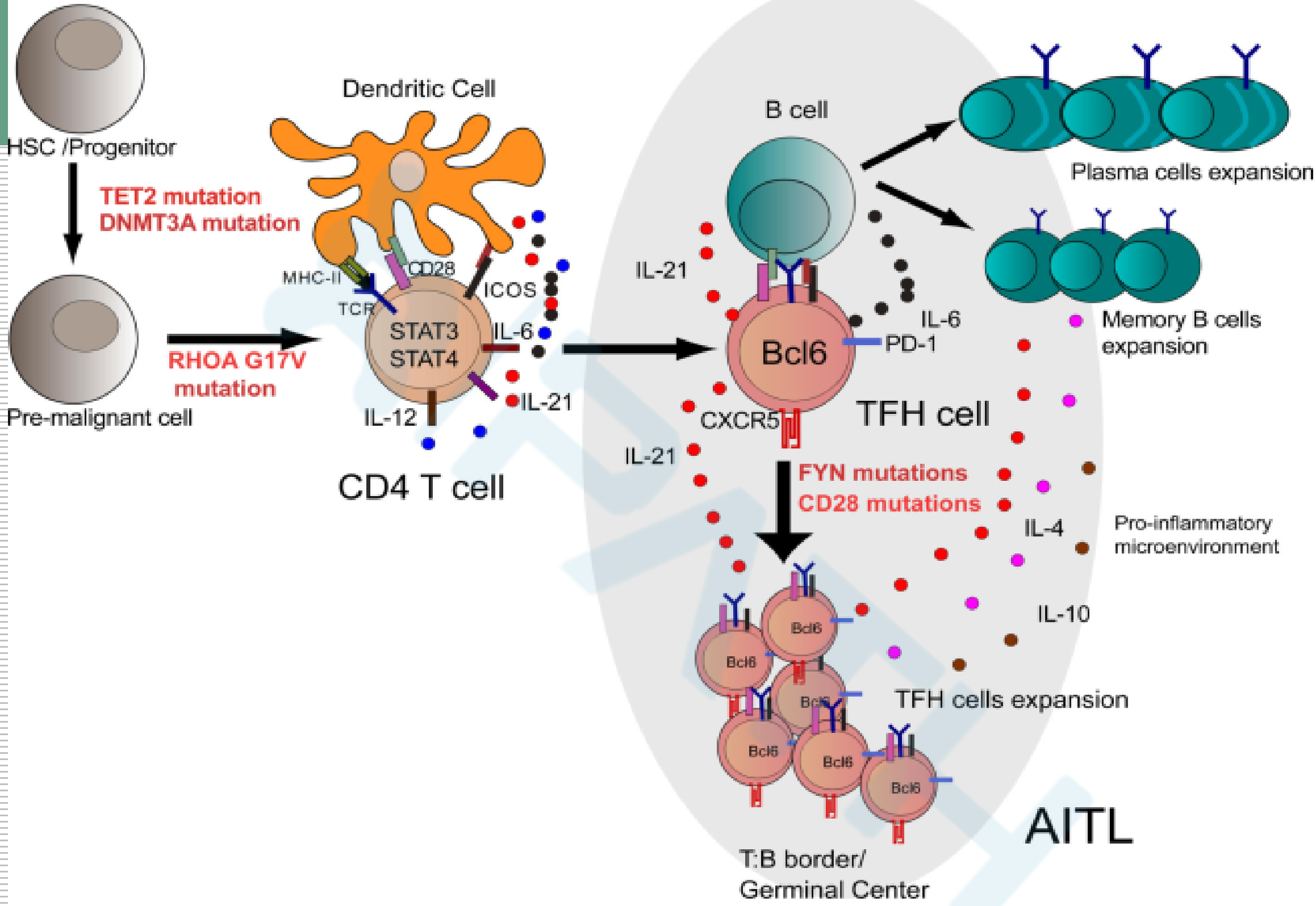


图 11.74　血管免疫母细胞性 T 细胞淋巴瘤的组织学模式。A 此例为早期病例，滤泡增生，副皮质区扩张（模式 1）。B 此例滤泡衰竭/萎缩，和 Castleman's 病相似，副皮质区显著扩张（模式 2）。C 典型形态学改变为正常结构破坏，显著血管增生和异型淋巴细胞聚集（模式 3）。

AITL免疫表型

- T_{FH}细胞阳性（CD4、CD10、PD-1、BCL-6、CXCL13、ICOS）
- 广泛的CD21+ FDC网围绕高内皮小静脉
- 散在分布的大B免疫母细胞，EBV常+
- 多克隆性/罕见的情况下单克隆性浆细胞
- ＞90%TCR克隆性重排
- 10-30%IG克隆性重排



The current model for AITL development suggest the existence of a premalignant lesion in the TET2 or DNMT3A epigenetic regulators, followed by a secondary mutation in RHOA G17V or IDH2 R172 that results in the malignant transformation of mature T-cells with a TFH phenotype

MATERIALS AND METHODS

- Patient Selection

  (1) A diagnosis of AITL established in an LN biopsy, according to the criteria of the World Health Organization classification.

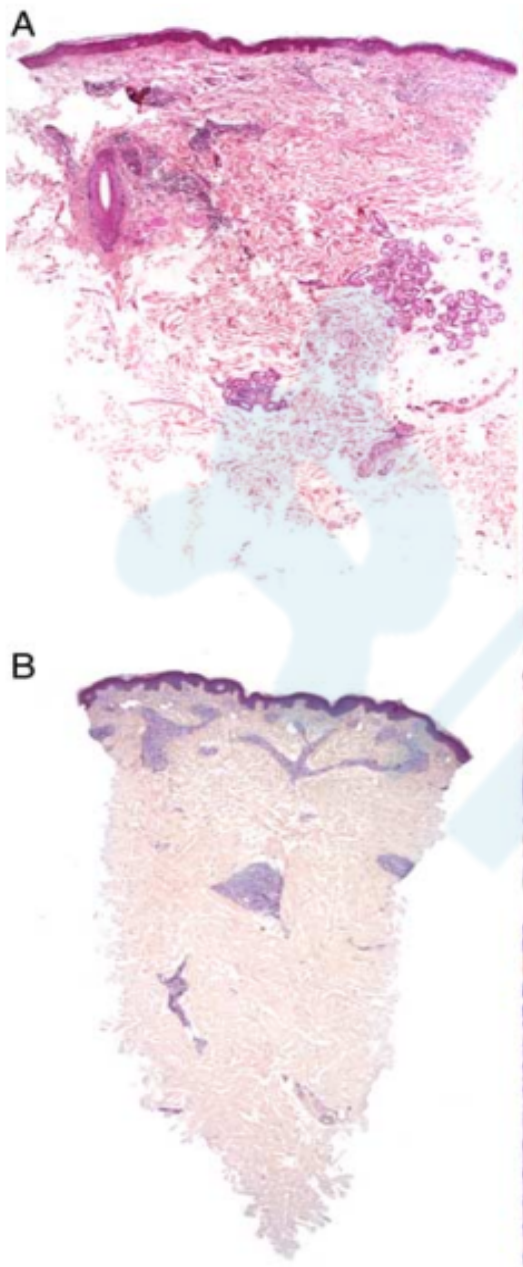
  (2) A skin biopsy performed because of skin manifestations.

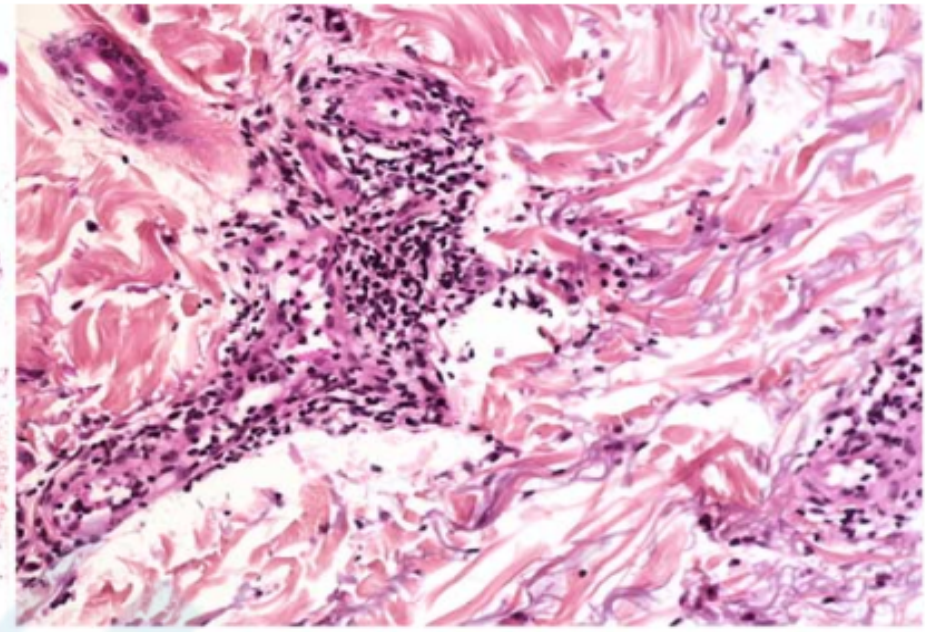
  (3) A diagnosis of cutaneous AITL lesion, after retrospective review by 2 of us. Only cases with features of neoplastic T-cell infiltration in the skin were retained, that is, cases showing a significant expression of CD10 and/or CXCL13 and/or PD1 in the lymphocytic infiltrates, and/or a T-cell antigen loss (among CD2, CD3, CD5, CD7), and/or a T-cell clone in the skin

- Among the 46 initially included patients, 5 were excluded after histopathologic reassessment because of a nonspecific infiltrate without an identifiable neoplastic component by both T-cell clonality studies and phenotypic analyses.
- These 5 cases were finally diagnosed as a druginduced or virus-induced exanthema (n = 4, including 1 with a probable drug rash with eosinophilia and systemic symptoms, DRESS) and a lymphocytic vasculitis (n = 1) and included in the control group

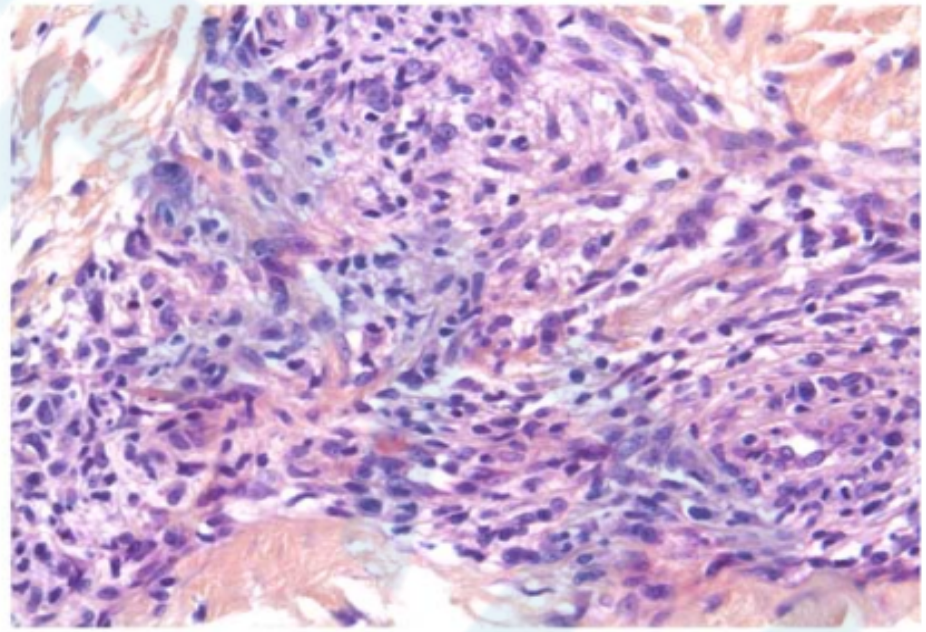
- Control group
  5 AITL patients with non-neoplastic skin infiltrates
  non-AITL patients with inflammatory dermatoses
  - including 14 patients with a cutaneous adverse reaction (DRESS or severe maculopapular eruption)
  - 3 patients with a chronic idiopathic erythroderma
  - 4 with a Sézary syndrome (including 1 with both the skin and a LN) was selected

- Pathologic Assessment of Cases
  - Pattern 1 (equivocal), low-density perivascular lymphocytic infiltrate with inconspicuous atypical cells, resembling an inflammatory dermatosis
  - Pattern 2 (suspicious), dense perivascular infiltrates with atypical cells and occasional inflammatory cells
  - Pattern 3 (AITL-like), dense dermal infiltrates with the classic features of nodal AITL, including atypical lymphocytes, vascular hyperplasia, and inflammatory cells
  - “Other” comprised cases not fulfilling the criteria for the 3 patterns mentioned above

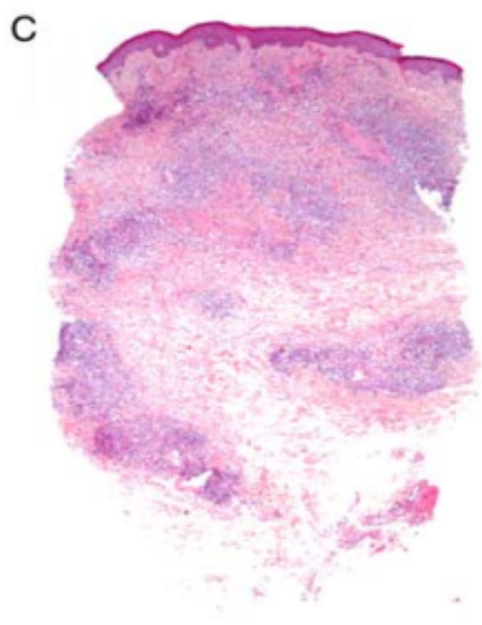


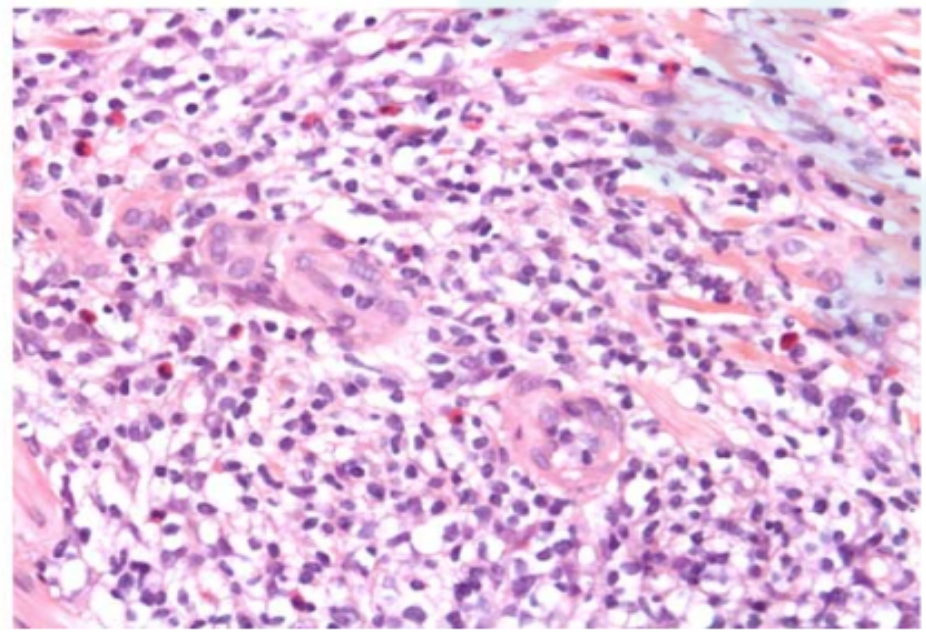


Pattern 1



Pattern 2





Pattern 3

- Immunohistochemical Studies
- In Situ Hybridization

TABLE 1. Summary of Antibodies and Methods Used for Immunohistochemistry and In Situ Hybridization

Target	Clone	Vendor	Antibody Dilution	Retrieval Conditions	Instrument
CD2	AB75	Novocastra-Menarini	1:100	pH9	Bond-MAX or Bond III
CD3	F.7.2.38	Dako	1:50	pH9	Bond-MAX or Bond III
CD4	4B12	Leica	1:100	pH9	Bond-MAX or Bond III
CD5	4C7	Novocastra-Menarini	1:100	pH9	Bond-MAX or Bond III
CD7	LP15	Leica	1:50	pH9	Bond-MAX or Bond III
CD8	C8/144B	Dako	1:200	pH9	Bond-MAX or Bond III
CD20	L26	Dako	1:500	pH6	Bond-MAX or Bond III
CD10	56C6	Leica	1:50	Ph9	Bond-MAX or Bond III
CXCL13	53610	R&D Systems	1:50	pH6	Bond-MAX or Bond III
ICOS	Rabbit polyclonal	Spring Biosciences	1:100	pH9	Bond-MAX or Bond III
PD1	NAT105	Abcam	1:100	pH6	Bond-MAX or Bond III
BCL6	LN22	Menarini	Prediluted	pH9	Bond-MAX or Bond III
IDH2 R172K	Mouse monoclonal	NewEAST	1:100	pH9	Manual staining
EBV (EBER)	Bond ISH probe EBER	Leica	Nonrelevant	Enzyme	Bond-MAX or Bond III
Kappa	Bond ISH kappa probe	Leica	Nonrelevant	Enzyme	Bond-MAX or Bond III
Lambda	Bond ISH lambda probe	Leica	Nonrelevant	Enzyme	Bond-MAX or Bond III

ISH indicates in situ hybridization.

- T-Cell Clonality Studies
- Allele-specific PCR for the Detection of the RHOA p.G17V and IDH2 p.R172K/S Mutational Status

TABLE 2. Primers Used for Allele-specific PCR

Target	Primer	Sequence	Amplification Pairs	Targets
RHOA	Forward MUT G17V	ATTGTTGGTGATGGAGCCTGTAT	Forward MUT/reverse R1	*RHOA* p.G17V
	Forward WT	ATTGTTGGTGATGGAGCCTGTGG	Forward WT/reverse R1	*RHOA* wt
	Reverse R1	CTCACCCTGCTTTCCATCC	Forward MUT/reverse R2 for qPCR	*RHOA* p.G17V
	Reverse R2	ACACCTCTGGGAACTGGTCCT		
	Internal probe	GCTTGCTCATAGTCTTCAGCA		
IDH2	Forward MUT R172K	CCAAGCCCATCACCATTGGCGA	Forward MUT R172K/reverse R1	*IDH2* p.R172K
	Forward MUT R172S	AAGCCCATCACCATTGGCAGC	Forward MUT R172S/reverse R2	*IDH2* p.R172S
	Forward WT	CCAAGCCCATCACCATTGGCAG	Forward WT/reverse R3	*IDH2* wt
	Reverse R1	CCCACTCCTTGACACCACTGCC		
	Reverse R2	GGTCTGCCACAAAGTCTGTGGCC		
	Reverse R3	ATGGCTAGGCGAGGAGCTCCAG		

qPCR indicates quantitative polymerase chain reaction.

RESULTS

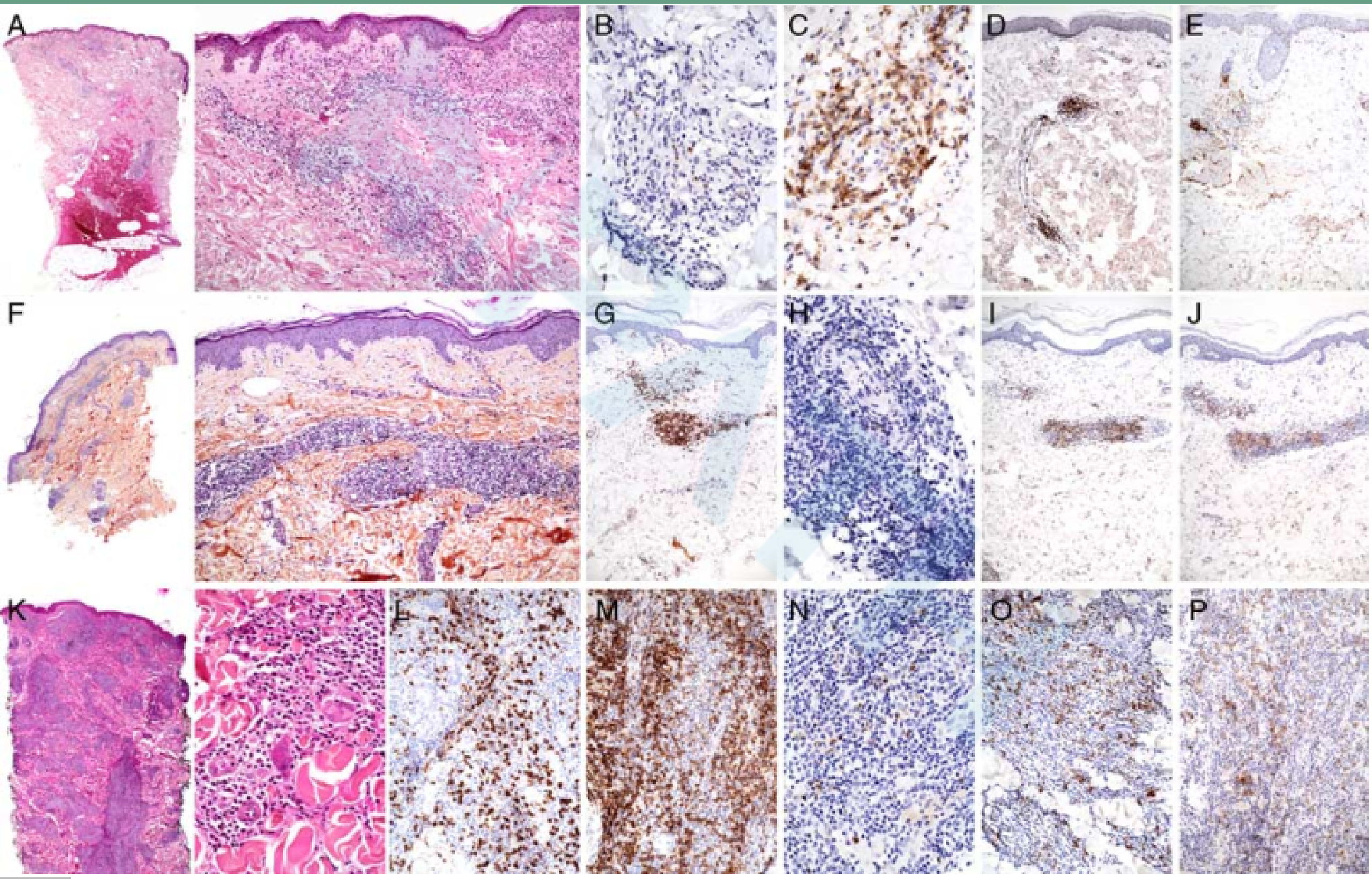


TABLE 3. Histopathologic Results

Epidermal Changes	Value (N = 41) (n/N [%])	Comment(s)
Epidermotropism	7/40 (17.5)	Pautrier's abscesses (n = 3) Folliculotropism (n = 1) Syringotropism (n = 1)
Inflammatory changes	11/41 (27)	
Eczematiform	2/41 (5)	
Interface dermatitis	5/41 (12)	
Other	4/41 (10)	
Dermal changes		
Density		
1	10/41 (24)	
2	16/41 (39)	
3	15/41 (37)	
Architecture		
Perivascular	34/41 (83)	
Periadnexal	20/41 (49)	
Diffuse	10/41 (24)	
Subepidermal band-like	3/41 (7)	
Atypical lymphocytes	26/41 (63)	
Lymphocytes with clear cytoplasms	15/26 (58)	
Other (pleomorphic/ Sézariform)	9/26 (34)	
Both aspects	2/26 (8)	
Inflammatory cells	28/41 (68)	
Eosinophils	6/41 (15)	
Neutrophils	5/41 (12)	
Plasma cells	13/41 (32)	
Histiocytes/histiocytic granulomas	16/41 (39)	
Vascular lesions		
Vasculitis	1/41 (2)	
Capillary hyperplasia	5/41 (12)	
Morphologic groups		
Pattern 1 (low-density perivascular infiltrates)	11/41 (27)	
Pattern 2 (atypical perivascular infiltrates)	13/41 (31.5)	
Pattern 3 (nodal AITL-like)	4/41 (10)	
Other patterns	13/41 (31.5)	Band-like (n = 2) Large cells (n = 1) Inflammatory lesions (n = 3) Plasmocytoid LPD (n = 2) EBV$^+$ DLBCL (n = 2) Other (n = 3)

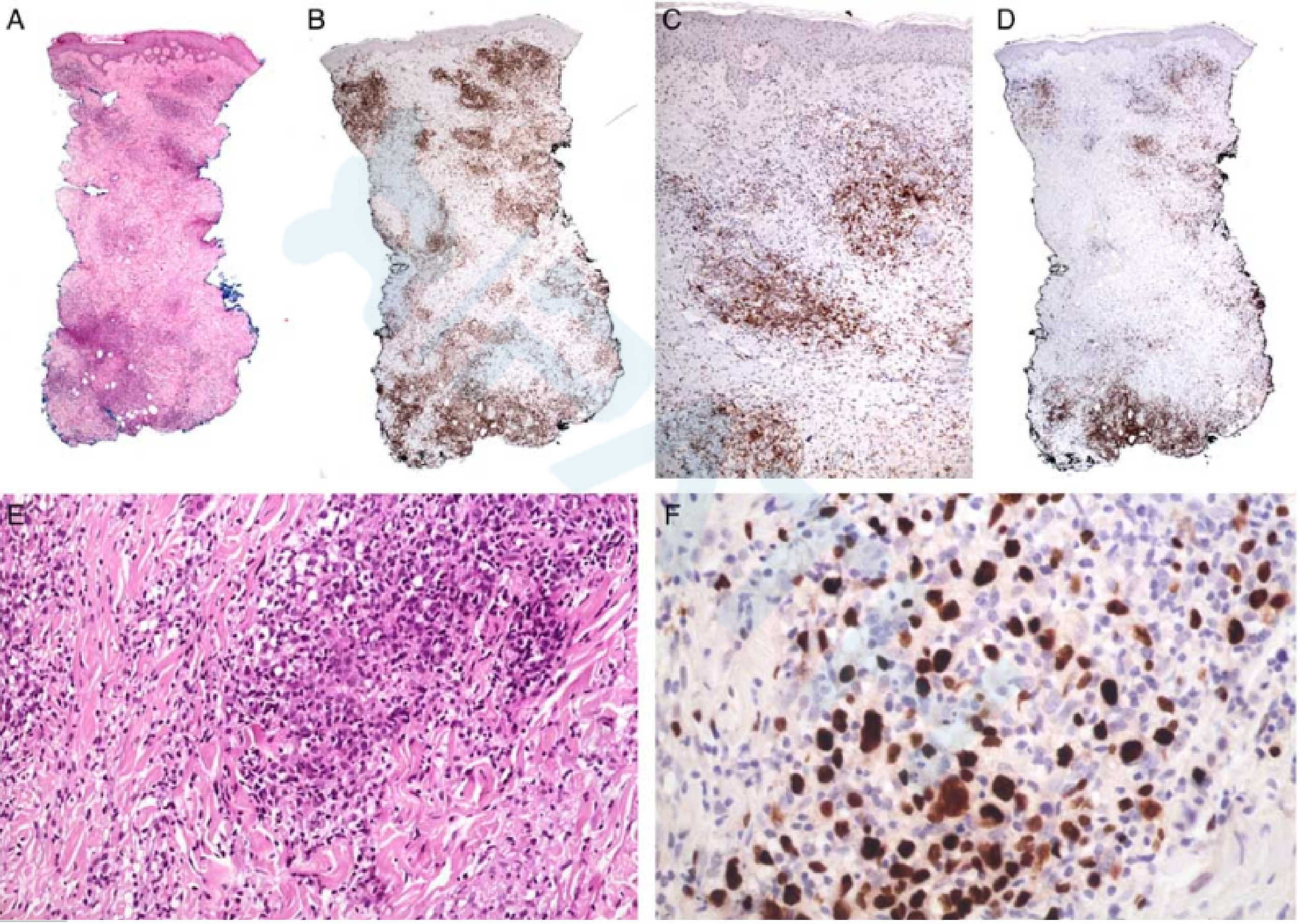
DLBCL indicates diffuse large B-cell lymphoma; LPD, lymphoproliferative disease.

TABLE 4. Phenotypic Studies

Phenotypic Marker	n/N (%) All Cases (N = 41)	Pattern 1 (N = 11)	Pattern 2 (N = 13)	Pattern 3 (N = 4)
T-cell antigen loss				
CD2	0	0	0	0
CD3	2/41 (5)	0	2/13 (15)	0
CD5	0	0	0	0
CD7	17/40 (42)	2/11 (18)	6/13 (46)	1/4 (25)
CD4/CD8 ratio				
≤1	7/37 (19)	2/10 (20)	2/11 (18)	0
>1-2	6/37 (16)	2/10 (20)	3/11 (27)	1/3 (33)
>2-<5	9/37 (24)	4/10 (40)	2/11 (18)	2/3 (67)
≥5	15/37 (41)	2/10 (20)	4/11 (37)	0
CD20$^+$	30/39 (77)	7/10 (70)	9/12 (75)	3/4 (75)
EBV$^+$ lymphocytes (EBER ISH)	10/38 (26)	1/11 (10)	1/13 (8)	3/4 (75)
CD10	20/40 (50)	3/10 (30)	6/13 (54)	4/4 (100)
1	7/20 (35)	0	3/6 (50)	1/4 (25)
2	9/20 (45)	2/3 (67)	2/6 (33)	3/4 (75)
3	4/20 (20)	1/3 (33)	1/6 (17)	0
CXCL13	32/38 (84)	7/11 (64)	11/12 (91)	4/4 (100)
1	10/32 (32)	1/7 (14)	4/11 (36)	1/4 (25)
2	11/32 (34)	4/7 (57)	3/11 (28)	1/4 (25)
3	11/32 (34)	2/7 (29)	4/11 (36)	2/4 (50)
PD1	31/33 (94)	11/11 (100)	6/6 (100)	4/4 (100)
1	2/31 (7)	1/11 (9)	0	1/4 (25)
2	11/31 (35)	5/11 (45.5)	1/6 (17)	1/4 (25)
3	18/31 (58)	5/11 (45.5)	5/6 (83)	2/4 (50)
ICOS	35/36 (97.5)	10/10 (100)	12/12 (100)	4/4 (100)
1	1/35 (3)	1/10 (10)	0	0
2	16/35 (46)	5/10 (50)	6/12 (50)	2/4 (50)
3	18/35 (51)	4/10 (40)	6/12 (50)	2/4 (50)
BCL6	26/31 (84)	9/11 (82)	6/6 (100)	3/4 (75)
1	20/26 (77)	8/9 (89)	5/6 (83)	1/3 (33)
2	6/26 (23)	1/9 (11)	1/6 (17)	2/3 (67)
3	0	0	0	0
TFH phenotype 1	32/38 (84)	9/11 (82)	8/11 (73)	4/4 (100)
TFH phenotype 2	22/32 (65)	6/11 (55)	6/11 (55)	4/4 (100)

The expression of the various phenotypic markers was divided into 3 categories representing the proportion of neoplastic positive cells within the T-cell infiltrate (1, <5%; 2, 5% to 50%; 3, >50%). The TFH phenotype was defined by the expression of at least 2 (TFH phenotype 1) or 3 (TFH phenotype 2) TFH markers among CD10, CXCL13, PD1, ICOS, and BCL6 by >5% of lymphocytes.

ISH indicates in situ hybridization.


A
B
C
D
E
F

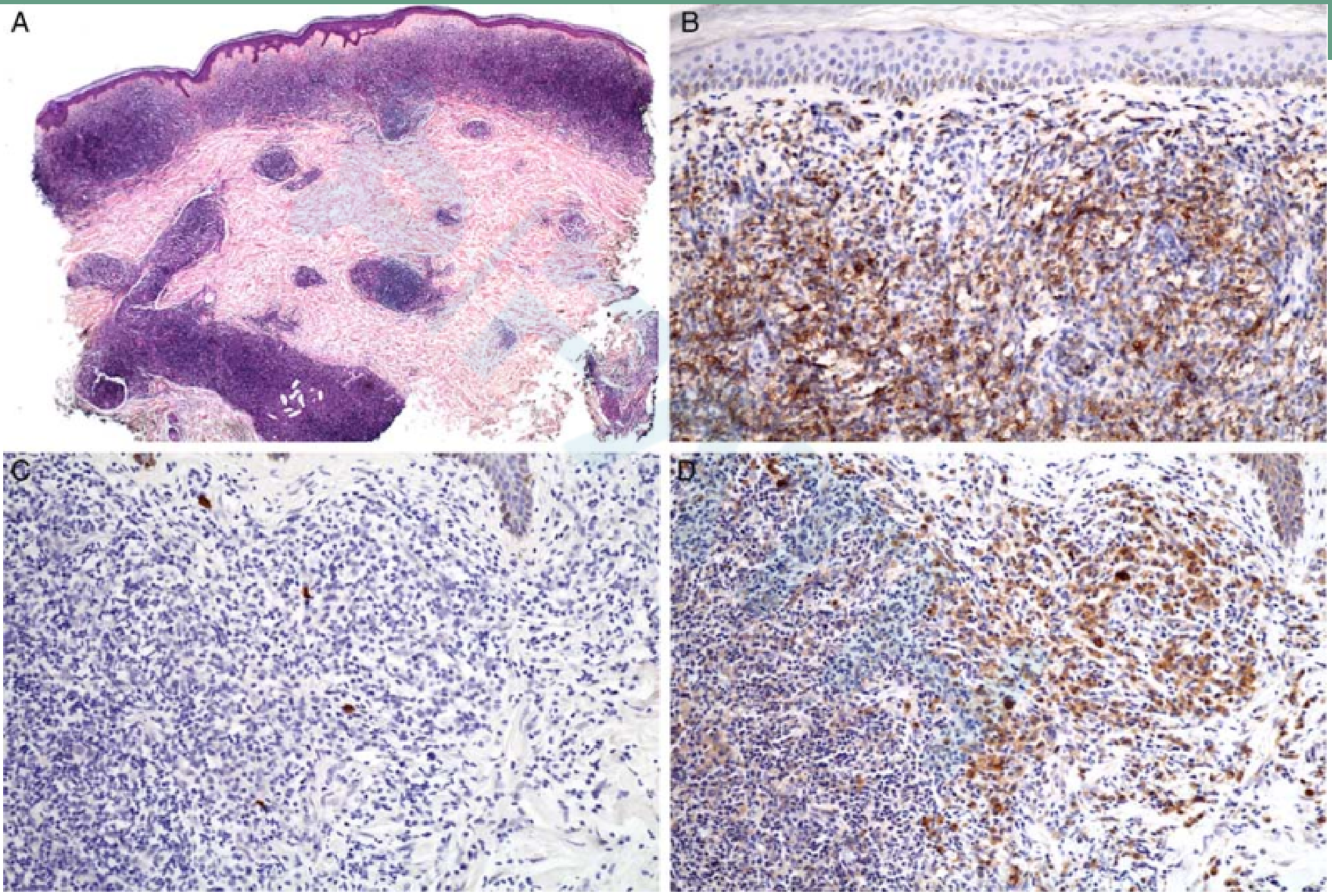
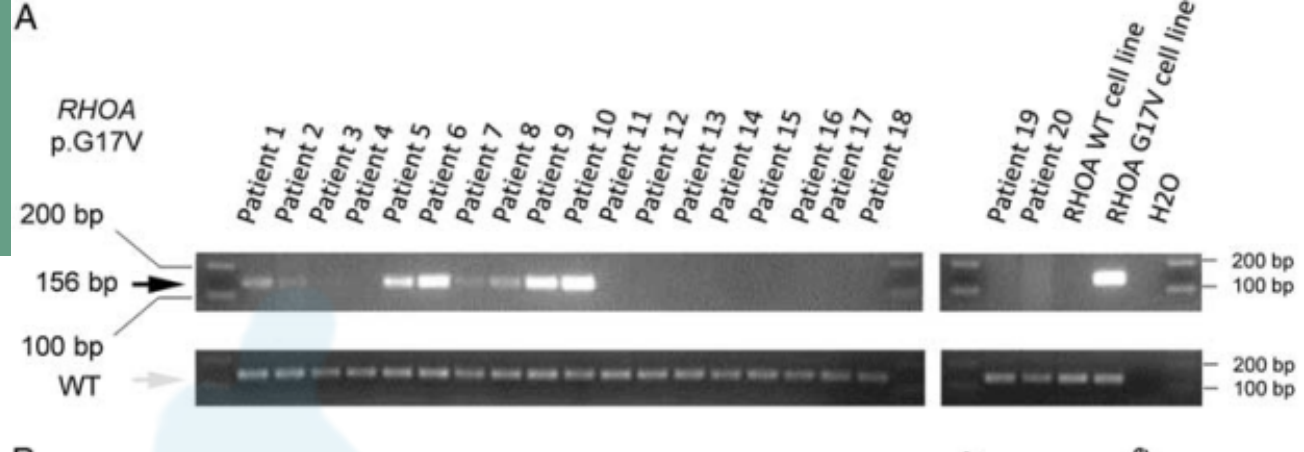
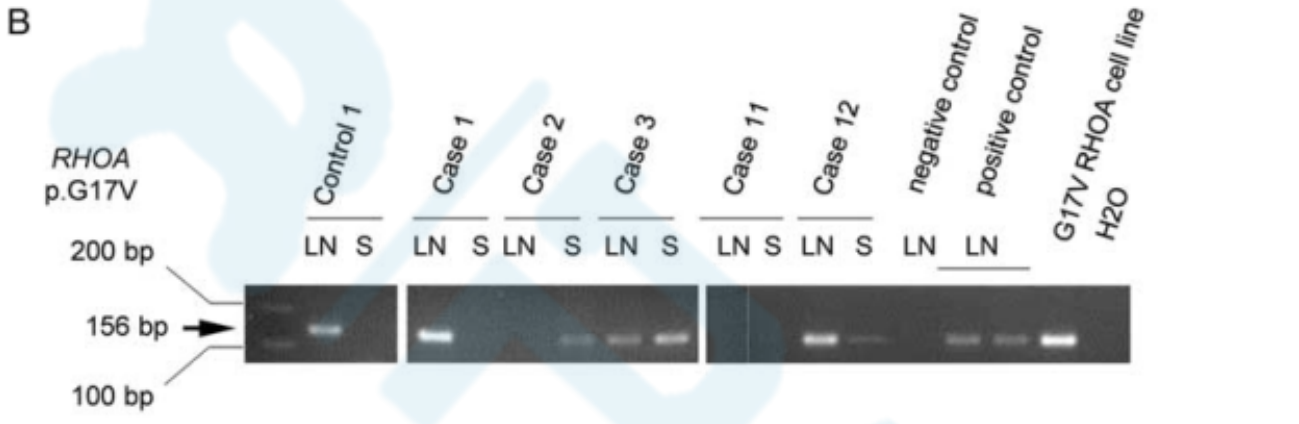

A
B
C
D

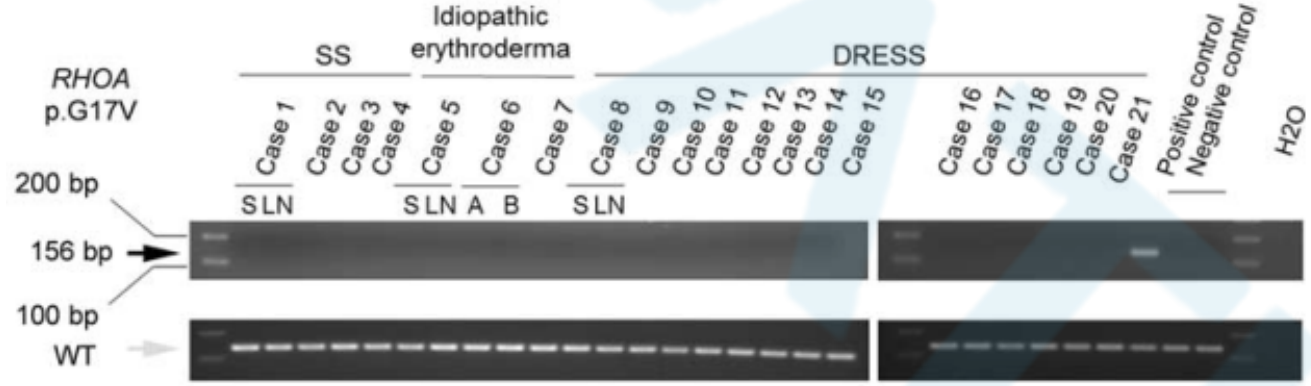
TABLE 5. RHOA pG17v and IDH2 pR172K Mutational Status and T-Cell Clonality Results in Skin and LN Samples of 21 AITL Patients

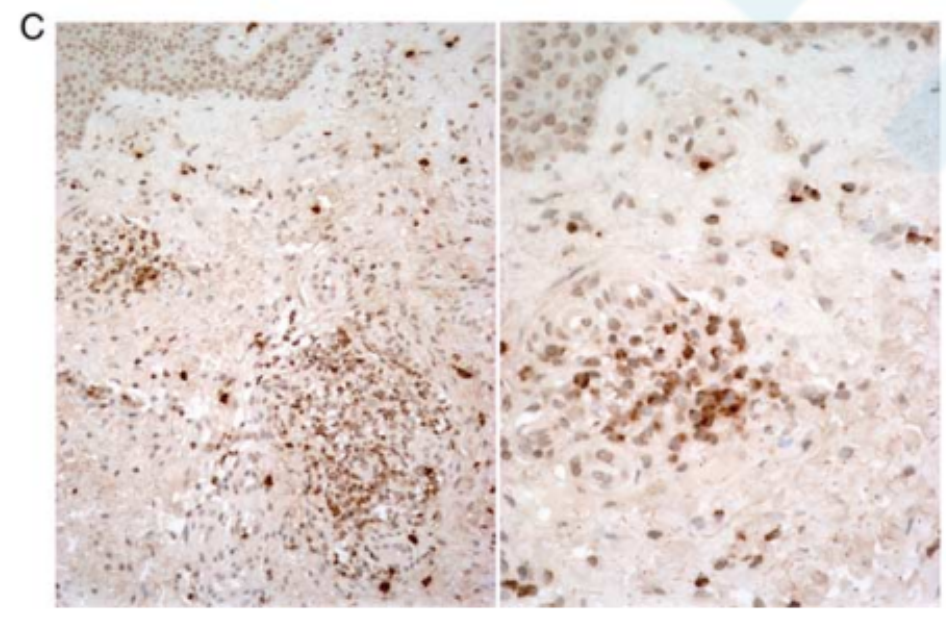
		RHOA p.G17V		*IDH2* p. R172K/S		T-Cell Clonality	
Case	**Pattern**	**S**	**LN**	**S**	**LN**	**S**	**LN**
1	1	WT	M	WT	WT	+	+
2	1	M	WT	WT	WT		
3	1	M	M	WT	WT	+	+
4	1	M	M	WT	WT	+	+
5	1	M	M	WT	WT		
6	2	M	M	WT	WT	+	+
7	2	M	NI	WT	NI		
8	2	M	M	NI	WT	+	+
9	2	NI	M	NI	WT	+	+
10		WT	WT	M	M	+	
11	3	WT	WT	M	WT		
12	Other*	M	M	M	M	+	
13	Other†	M	M	WT	WT		+
14	2	M		WT			
15	2	M		WT			
16	2	M		NI		+	
17	3	WT		WT			
18	3	M		WT			
19	3	NI		NI			
20	Other‡	M		WT			
21	Other§	NI		NI			

*Neutrophilic urticarial.
†Mycosis fongoide-like pattern.
‡Rich plasma cells' infiltrate.
§Diffuse infiltrate.
M indicates mutated; NI, not interpretable; S, skin; WT, wild type.


A
RHOA
p.G17V
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9
Patient 10
Patient 11
Patient 12
Patient 13
Patient 14
Patient 15
Patient 16
Patient 17
Patient 18
Patient 19
Patient 20
RHOA WT cell line
RHOA G17V cell line
H2O
200 bp
156 bp
100 bp
WT
200 bp
100 bp
200 bp
100 bp


B
RHOA
p.G17V
Control 1
Case 1
Case 2
Case 3
Case 11
Case 12
negative control
positive control
G17V RHOA cell line
H2O
LN S LN S LN S LN S LN S LN S LN LN
200 bp
156 bp
100 bp


SS
Idiopathic erythroderma
DRESS
RHOA
p.G17V
Case 1
Case 2
Case 3
Case 4
Case 5
Case 6
Case 7
Case 8
Case 9
Case 10
Case 11
Case 12
Case 13
Case 14
Case 15
Case 16
Case 17
Case 18
Case 19
Case 20
Case 21
Positive control
Negative control
H2O
S LN S LN A B S LN
200 bp
156 bp
100 bp
WT


C

DISCUSSION

- A TFH phenotype was identified in 82% and 73%, respectively, of cases with the most challenging patterns 1 and 2. TFH markers and EBV can thus help for diagnosis and are detected in samples with low-density infiltrates
- The RHOA G17V mutation was identified in a proportion of biopsies with patterns 1 and 2, which represent a diagnostic challenge
- The frequency of RHOA G17V mutation was similar to that reported in LNs. It may represent a sensitive diagnostic marker in the skin, helpful in cases with low-density infiltrates

CONCLUSION

- In light of the frequent subtle and nonspecific appearance of the skin infiltrates in AITL, the identification of TFH markers and *RHOA* mutations are diagnostic tools for the diagnosis of AITL, especially in patients presenting with skin lesions as a first manifestation

Thank You !