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Langerhans cell histiocytosis associated with lymphoma: an incidental finding that is not associated with *BRAF* or *MAP2K1* mutations

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Abstract

Langerhans cell histiocytosis (LCH) is characterized by a localized or systemic proliferation of Langerhans cells. *BRAF* mutations have been reported in 40–70% of cases and *MAP2K1* mutations have been found in *BRAF*-negative cases, supporting the interpretation that LCH is a true neoplasm, at least in mutated cases. In a small subset of patients, LCH is detected incidentally in a biopsy specimen involved by lymphoma. These lesions are usually minute and rarely have been assessed for mutations. We assessed for *BRAF* and *MAP2K1* mutations in 7 cases of LCH detected incidentally in biopsy specimens involved by lymphoma. We also performed immunohistochemical analysis for phosphorylated (p)-ERK. The study group included 4 men and 3 women (median age 54 years; range, 28–84). The biopsy specimens were lymph nodes (n=6) and chest wall (n=1). The lymphomas included 5 cases of classical Hodgkin lymphoma, 1 mantle cell lymphoma and 1 angioimmunoblastic T-cell lymphoma. All cases were negative for *BRAF* mutation by pyrosequencing, and *MAP2K1* mutation by Sanger sequencing. Nevertheless, 3 of 7 (42.8%) cases were positive for p-ERK. We then performed mutation analysis of a panel of 134 commonly mutated genes (including *BRAF* and *MAP2K1)* by next-generation sequencing on 3 cases including the 2 cases positive for p-ERK by immunohistochemistry. No mutations were detected in any of the three cases assessed. Six patients were treated for lymphoma with chemotherapy; 2 also received radiotherapy, and 2 underwent stem cell transplantation. With a median follow-up of 21 months (range, 6–89), no patients developed recurrent LCH. We conclude that lymphoma-associated LCH is a clinically benign process that is not associated with *BRAF* or *MAP2K1* mutations. Nevertheless, the expression of p-ERK in 2 cases suggests that the RAS-BRAF-MAP2K1-ERK pathway may be activated by non-mutational mechanisms, perhaps induced by the presence of lymphoma or lymphoma-microenvironment interaction.

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Disclosure/Conflict of Interest

The authors have no conflicts of interest to disclose.

Keywords

Langerhans cell histiocytosis; concurrent lymphoma; *BRAF* mutation; *MAP2K1* mutation; p-ERK

Introduction

Langerhans cell histiocytosis (LCH) is a rare disease characterized histologically by a proliferation of Langerhans cells (LCs) admixed with eosinophils, lymphocytes, macrophages and multinucleated giant cells, with or without eosinophilic abscesses or necrosis.[1] Virtually any anatomic site can be involved by LCH, and the disease may present as a localized lesion or as multiorgan disease. Prognosis is variable, correlating in part with extent of disease, but morphologic findings of LCH are identical in the localized or multifocal disease and are not an indicator of prognosis. Because of its heterogeneous clinical manifestations and benign morphology, the reactive or neoplastic nature of LCH was controversial for over 50 years.[2] However, in 1994 two independent groups showed that a subset of LCH cases is monoclonal by using a human androgen receptor X-chromosome-inactivation assay (HUMARA).[3,4]

In 2010, Badalian-Very *et al.* described *BRAF* V600E mutation in 40–70% of cases of LCH[5] supporting the interpretation that LCH is a neoplasm, at least in mutated cases. Similar findings also were reported by others.[5–9] BRAF is a member of the rapid accelerating fibrosarcoma (RAF) kinase family that is activated by RAS and RAS-coupled receptor tyrosine kinases. RAS-RAF activated complexes transmit downstream signals to the mitogen activated protein kinase (MAPK) cascade including MEK/ERK kinases via protein phosphorylation.[10–12] The RAS-RAF-MAPK pathway is a key regulator involved in cell proliferation, growth, differentiation and apoptosis by transmitting activating signals to several nuclear, cytoplasmic and cell membrane targets.[10,11] Subsequently, a subset of *BRAF* wild-type LCH cases (30–50%) were shown to carry mutations in the *MAPK-kinase 1* (*MAP2K1* or *MEK1)* gene, further implicating the oncogenic MAPK pathway signaling in LCH pathogenesis.[13,14] Most of these mutations cause constitutive activation of MAP2K1 and activation of the RAS-RAF-MAPK signaling cascade via ERK phosphorylation.[13,14] *BRAF* V600E and *MAP2K1* mutations are not exclusively seen in LCH and also have been reported with variable frequency in other hematopoietic neoplasms, for example, hairy cell leukemia as well as non-hematopoietic malignancies including melanoma, papillary thyroid carcinoma, colorectal carcinoma, and glioneuronal tumors.[10–12,15–22]

In a small subset of patients, LCH has been identified as an incidental finding in biopsy specimens involved by lymphoma. Classical Hodgkin lymphoma is the most common associated lymphoma,[23–26] whereas only sporadic cases of other types of non-Hodgkin lymphoma associated with LCH are reported in the literature.[23,25,27–30] The relationship between LCH and concurrent lymphoma remains unknown. To our knowledge, there are no previous studies that have assessed the status of *BRAF* or *MAP2K1* in cases of LCH associated with concurrent lymphomas.

In this study, we identified 7 patients with concurrent LCH and lymphoma and assessed the LCH component for *BRAF* and *MAP2K1* mutations.

Materials and Methods

Study group and immunohistochemistry

The archives of the Department of Hematopathology at The University of Texas MD Anderson Cancer Center from January 2000 to December 2015 were searched for cases diagnosed as LCH associated with lymphoma involving the same biopsy specimen. Cases with available paraffin-embedded tissue blocks or unstained slides were selected. Clinical and laboratory data were retrieved from the electronic medical record. This study was conducted under an Institutional Review Board-approved protocol.

Routinely prepared hematoxylin-eosin stained slides for all cases were reviewed. Immunohistochemical studies were performed to confirm the diagnosis of lymphoma and LCH using antibodies specific for CD1a and CD21 (Leica Biosystem, Newcastle, UK); CD2, CD3, CD4, CD7, CD8, CD20, CD30, CD45/LCA, BCL-6, MUM1 and TIA-1 (DAKO, Carpinteria, CA); CD5 and cyclin D1 (Labvision/Neomarkers, Fremont, CA); CD10, CD23 and BCL-2 (Novocastra/Vision Biosystem, Benton Lane, Newcastle-upon-Tyne, UK); CD15 (Becton-Dickinson Biosciences, San Jose, CA); PAX-5 (Transduction Labs, San Diego, CA); CXCL-13 (R&D Systems, Minneapolis, MN); S-100 protein (BioGenex, Fremont, CA) and Langerin/CD207 (Novocastra Biosystem, Newcastle, UK). *In situ* hybridization for Epstein-Barr virus-encoded RNA (EBER) was also performed.

ERK phosphorylation analysis by immunohistochemistry

The highly specific antibody phospho-p44/42 MAPK (Thr202/Tyr204) (D13.14.4E) p-ERK (dilution 1:300, Cell Signaling, Danvers, MA) was used to assess for the presence of nuclear and cytoplasmic phosphorylated p44 and p42 MAPK (Erk1 and Erk2).

BRAF mutation analysis by immunohistochemistry

The VE-1 antibody (dilution 1:50, Spring Bioscience, Pleasanton, CA) was used to assess for cytoplasmic staining supportive of the presence of *BRAF* V600E mutation. The antibody is highly specific for this mutation as shown previously by Capper *et al.*[31]

BRAF mutation analysis by pyrosequencing

A polymerase chain reaction (PCR)-based pyrosequencing assay for *BRAF* mutation analysis was employed. This assay was developed at our institution and covers mutation hotspots in exons 11 (codon 468) and 15 (codons 595 to 600).[32] This assay was chosen because all *BRAF* mutations in LCH identified previously have been clustered in exons 11 or 15 of the gene and alter the kinase domain of the protein.[33] The LCH lesion was microdissected from fixed, paraffin-embedded tissue sections (10 μm thick) and DNA was extracted and PCR amplified using either a forward primer, 5′-TCCTGTATCCCTCTCAGGCATAAGGTAA-3′, and a reverse biotinylated primer, 5′-biotin-CGAACAGTGAATATTCCTTTGAT-3′ (for codon 468 of exon 11), or a forward primer, 5′-CATAATGCTTGCTCTGATAGGA-3′, and a reverse biotinylated primer, 5′-biotin-GGCCAAAAATTTAATCAGTGGA-3′ (for codons 595 to 600 of exon 15). PCR amplification was performed in duplicate on an ABI 2720 Thermocycler (Applied Biosystems, Grand Island, NY). The PCR products underwent electrophoresis on agarose

gels to confirm successful amplification. Fifteen (15) μL of the PCR products were then sequenced in duplicate using primers 5′-TTGGATCTGGATCATTT-3′ (for exon 11) or 5′-GAAGACCTCACAGTAAAAATA-3′ (for exon 15) and the pyrosequencing PSQ96 HS System (Biotage AB, Uppsala, Sweden) according to the manufacturer's instructions. The HL-60 cell line, negative for *BRAF* mutation, and A375, a melanoma cell line positive for *BRAF* mutation (exon 15 V600E), were used as negative and positive controls, respectively.

MAP2K1 mutation analysis

We performed Sanger sequencing to assess for mutations in exons 2 and 3 of *MAP2K1*. DNA was subjected to PCR using a pair of M13-tagged forward primer, 5′-TGTAAAACGACGGCC AGTAGTATTGACTTGTGCTCCCCA-3′, and reverse primer, 5′-CAGGAAACAGCTATGACCTGGTCCCCAGGCTTCTAAGT-3′, for exon 2, and a pair of M13-tagged forward primer, 5′-TGTAAAACGACGGCCAGTCATAAAACCTCTCTTTCTTCCACC-3′, and reverse primer, 5′-CAG GAA ACA GCT ATG ACC CCA GAG CCA CCC AAC TCT TA-3′, for exon 3, in a 50 μL reaction containing 10 ng DNA, 0.03 U/μL Go Taq polymerase (Promega, Madison, WI), 0.2 μM each of forward and reverse primers, 2 mM MgCl2, 1 mM dNTP mix and 1x Go Taq buffer. The reaction mix was first heated to 95°C for 10 min, then subjected to 40 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, followed by 72°C for 7 min. The PCR products underwent electrophoresis to confirm successful amplification, were purified using Agencourt AMPure Kit (Beckman Coulter, Indianapolis, IN), and sequenced using the same primer sets as shown above.

Next-generation sequencing (NGS)

We performed amplicon-based NGS targeting the coding regions of a panel of 134 genes that are commonly mutated in hematopoietic neoplasms using Torrent Suite platform (Thermo Fisher Scientific, Waltham, MA) on DNA extracted from paraffin-embedded tissues. For 2 cases, we were able to retrieve tissues that were not involved by LCH to be used as a control. We used 250 ng of DNA to prepare the genomic library. The genes included in the panel are as follows: *ABL1, ACVRL1, AKT1, ALK, APC, APEX1, AR, ARAF, ATM, ATP11B, BAP1, BCL2L1, BCL9, BIRC2, BIRC3, BRAF, BRCA1, BRCA2, BTK, CBL, CCND1, CCNE1, CD274, CD44, CDH1, CDK4, CDK6, CDKN2A, CHEK2, CSF1R, CSNK2A1, CTNNB1, DCUN1D1, DDR2, DNMT3A, EGFR, ERBB2, ERBB3, ERBB4, ESR1, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, FOXL2, GAS6, GATA2, GATA3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, IFITM1, IFITM3, IGF1R, IL6, JAK1, JAK2, JAK3, KDR, KIT, KNSTRN, KRAS, MAGOH, MAP2K1, MAP2K2, MAPK1, MAX, MCL1, MDM2, MDM4, MED12, MET, MLH1, MPL, MSH2, MTOR, MYC, MYCL, MYCN, MYD88, MYO18A, NF1, NF2, NFE2L2, NKX2-1, NKX2-8, NOTCH1, NPM1, NRAS, PAX5, PDCDILG2, PDGFRA, PIK3CA, PIK3R1, PNP, PPARG, PPP2R1A, PTCH1, PTEN, PTPN11, RAC1, RAF1, RB1, RET, RHEB, RHOA, RPS6KB1, SF3B1, SMAD4, SMARCB1, SMO, SOX2, SPOP, SRC, STAT3, STK11, TERT, TET2, TIAF1, TP53, TSC1, TSC2, U2AF1, VHL, WT1, XOP1*, and *ZNF217*. Following successful library generation and purification, DNA was used for multiplex paired-end sequencing on the Ion Proton platform, and analyzed using the Torrent Suite and OncoSeek data pipeline.

Results

Clinical features

The study group included 7 patients, 4 men and 3 women, with a median age of 54 years (range, 28–84 years). The demographic, clinical, laboratory features at diagnosis, and pathology and molecular results are summarized in Table 1. One patient presented with mild leukocytosis (median white blood cell count, 5.6 $\times 10^3/\mu l$; range, 4–13.2 $\times 10^3/\mu l$; reference range, 4–11 $\times 10^3/\mu l$); 3 patients had normocytic anemia (median hemoglobin, 13.3 g/dL; range, 9.8–16.2 g/dL; reference range, 14–18 g/dL for men and 12–16 g/dL for women), and 1 patient had thrombocytopenia (median platelet count, 158 $\times 10^3/\mu l$; range, 41–368 $\times 10^3/\mu l$; reference range, 140–440 $\times 10^3/\mu l$). Serum lactate dehydrogenase was slightly elevated in 1 patient (median, 434 IU/L; range, 390–668 IU/L; reference range, 313–618 IU/L) and β2-microglobulin levels were elevated in 5 patients (median, 2.3 mg/L; range, 1.8–4.3 mg/L; reference range, 0.7–1.8 mg/L). Laboratory data were not available for 1 patient. The lymphomas include 5 cases of classical Hodgkin lymphoma and 1 case each of mantle cell lymphoma and angioimmunoblastic T-cell lymphoma. The cases of Hodgkin lymphoma were further classified as 3 nodular sclerosis, 1 mixed cellularity, and 1 recurrent disease. Concomitant LCH and lymphoma occurred in lymph nodes in 6 patients: 3 cervical, 1 axillary, 1 retroperitoneal and 1 inguinal. In one patient a biopsy was obtained from the chest wall.

Morphologically, the lymphomas had pathologic and immunophenotypic findings that are typical of classical Hodgkin lymphoma (Figures 1 and 2), mantle cell lymphoma (Figure 3) and angioimmunoblastic T-cell lymphoma (Figure 4), and are not further discussed. In addition to the lymphomas, all biopsy specimens contained foci of LCH characterized by variable amounts of LCs with oval to folded nuclei with nuclear grooves, thin nuclear membranes, vesicular chromatin, inconspicuous nucleoli, and abundant pale to eosinophilic cytoplasm with ill-defined cell borders. The distribution of LCs was variable and ranged from a focal cluster of LCH to multiple foci of LCH intermingled intimately with lymphoma. Interspersed eosinophils, eosinophilic abscesses and areas of necrosis were observed variably (Figures 1–4). No cytologic atypia or increased mitotic activity was found in the LCH component in any case. Immunohistochemical stains for CD1a, langerin and S-100 were positive in the LCH component in all cases (Figures 1–4).

Clinical follow-up data were available for 6 patients. Six patients received chemotherapy for the specific lymphoma (detailed chemotherapy regimens are listed in Table 1); 2 received additional radiation therapy, 2 underwent stem cell transplantation, and 1 patient is on the list in anticipation of stem cell transplantation. No patient received treatment specific for LCH. Treatment and outcome information were not available for 1 patient who was lost to follow up. With a median follow-up of 21 months (range, 6–89 months), 3 patients remained in clinical remission for both LCH and lymphoma, one patient developed recurrent mediastinal lymphoma detected by imaging (biopsy not performed at the time of this study), one patient developed therapy-related myelodysplastic syndrome, and one patient died of Hodgkin lymphoma. No patient developed recurrent LCH.

BRAF and phosphorylated ERK immunohistochemical assessment

The LCH component was negative for BRAF V600E mutant protein by IHC in 6 of 7 cases. The case of LCH positive for BRAF (case 5) is shown in Figure 2D. This result was performed twice with identical results.

The LCH component was positive for p-ERK by IHC in 3 of 7 cases (cases 1, 4, and 6). P-ERK expression was observed in the nucleus and cytoplasm of LCs (Figure 4F). In case 4, 2 small subcapsular LCH nodules were positive for p-ERK whereas the largest focus found in the mid portion of the lymph node was negative (not shown). In all cases, stromal cells, fibroblasts, and endothelial cells were positive for p-ERK (internal controls).

BRAF and *MAP2K1* mutation analysis

None of the 7 cases harbored a *BRAF* or *MAP2K1* mutation by PCR/pyrosequencing and PCR/Sanger sequencing, respectively.

Next-generation sequencing results

To evaluate the discrepancy between immunohistochemistry and Sanger sequencing for *BRAF* mutation on one case, and to explore the mutational status of the three cases positive for p-ERK, we performed NGS analysis to assess mutation status of a panel of 134 genes that are commonly mutated in hematopoietic neoplasms, including *ARAF, BRAF*, *ERBB1, ERBB2, ERBB3, KRAS, MAP2K1, NRAS*, and *PIK3CA*, on 3 cases with DNA available. No mutations in any gene were detected in all 3 cases.

Discussion

We assessed the frequency of LCH-related mutations in biopsy specimens involved by LCH and lymphoma in 7 patients. This form of LCH, virtually always detected incidentally, is rare with most cases reported in the literature as case reports or small case series.[23–30] Most of these studies were published before the discovery of *BRAF* and *MAP2K1* mutations in LCH, and therefore, as far as we are aware, this is the first study to assess *BRAF* V600E and *MAP2K1* mutation status in the LCH component in cases with concomitant LCH and lymphoma.

Using molecular methods, we did not identify *BRAF* V600E or *MAP2K1* mutations in the LCH lesions in this cohort. Nevertheless, we detected p-ERK by IHC in 3 of 7 cases consistent with activation of this pathway. NGS analysis for a panel of 134 genes (including *ARAF, BRAF*, *ERBB1, ERBB2, ERBB3, KRAS, MAP2K1, NRAS*, and *PIK3CA*) on two of these three cases failed to detect any mutation. Our data do not support an explanation for the positive p-ERK result in these 3 cases. One possibility is that the sensitivity of the methods we employed is insufficient to detect mutations. In our opinion, this seems unlikely as the LCH lesions were microdissected and had well above 10% LCs in the specimen analyzed, the lower limit of sensitivity for the methods employed. We considered the possibility of non-V600E BRAF mutations, but the NGS results in two cases exclude this idea. It seems reasonable to suggest that the RAS/RAF/MAPK signaling pathway in some cases of incidental LCH may be activated by non-mutational mechanisms. Possibly, a local

cytokine-mediated process imparting a growth advantage to LCs through activation of p-ERK might be involved, perhaps induced by the presence of lymphoma or a manifestation of lymphoma–tumor microenvrionmental interaction.

Using a commercially available monoclonal BRAF V600E specific antibody (VE-1), the results were negative in 6 cases of LCH, but was positive in 1 case (case 5). The reason for the discrepancy between the IHC result and molecular results in this case is uncertain. Methods do not appear to be an explanation as we used a pyrosequencing-based assay in all our cases to identify *BRAF* mutations similar to most published studies.[8,34,35] This case was also assessed by NGS methods and was negative for mutations in a 134 gene panel that included *BRAF* and *MAP2K1*. The BRAF antibody has been shown to have a high sensitivity and specificity for the *BRAF* mutation and represents an excellent tool for screening tissue samples.[35] It is possible, however, that the BRAF V600E antibody uncommonly may yield false positive results. In a large study of colorectal carcinomas performed at our institution, Estrella and colleagues showed that 7 of 323 (2.2%) carcinomas shown to be wild type for *BRAF* V600E mutation by sequencing analysis were diffusely positive using the VE-1 antibody.[36] It is important to mention that the case of LCH positive for *BRAF* V600E by IHC was negative for p-ERK, indicating that that RAS/RAF/MAPK pathway was not active in this case.

The most frequent lymphoma associated with LCH in this case series was classical Hodgkin lymphoma, in accord with data reported in the literature.[23–26] To our knowledge, we report for the first time an association of LCH with mantle cell lymphoma and angioimmunoblastic T-cell lymphoma. In keeping with the literature, none of the 6 patients with follow-up data developed recurrent LCH or systemic LCH. This behavior, the often focal nature of the LCH, and the absence of *BRAF* and *MAP2K1* mutations suggests that LCH associated with lymphoma is a benign process, at least in most cases, and possibly reactive to the presence of lymphoma. This concept has been supported by others, who have suggested that focal LCH associated with a hematolymphoid tumor is better considered as LC hyperplasia.[2,23–25,29] Christie *et al.* and others have suggested that these lesions be designated as “LC-like lesions”.[29] Furthermore, some authors have suggested that this type of LCH or LC-like lesions may be driven by chemokine/cytokine or other stimuli produced by the associated neoplastic conditions.[2,23–25,29] This hypothesis could be the explanation for why classical Hodgkin lymphoma, a lymphoma that is characterized by the production of a storm of chemokines and cytokines, is the most common lymphoma with associated incidental LCH. Although this suggestion is reasonable for the concurrent findings of these two lesions, we do not have sufficient data to address this idea adequately.

In conclusion, we did not detect *BRAF* V600E or *MAP2K1* mutations in all 7 cases of LCH associated with lymphoma assessed. These data, combined with the incidental nature of LCH detection when associated with lymphoma, the small size of the LCH lesions, and the absence of systemic LCH or recurrence, suggests that LCH associated with lymphoma is benign, as has been suggested by others. Nevertheless, p-ERK was positive in 3 of 7 cases suggesting activation of the RAS/RAF/MAPK pathway via non-genetic mechanisms in a subset of cases. It seems possible that pathway activation in these lesions may be induced by the presence of lymphoma.

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Abbreviations

LC	Langerhans cells
LCH	Langerhans cell histiocytosis
MAPK	mitogen activated protein kinase
NGS	next-generation sequencing
PCR	polymerase chain reaction

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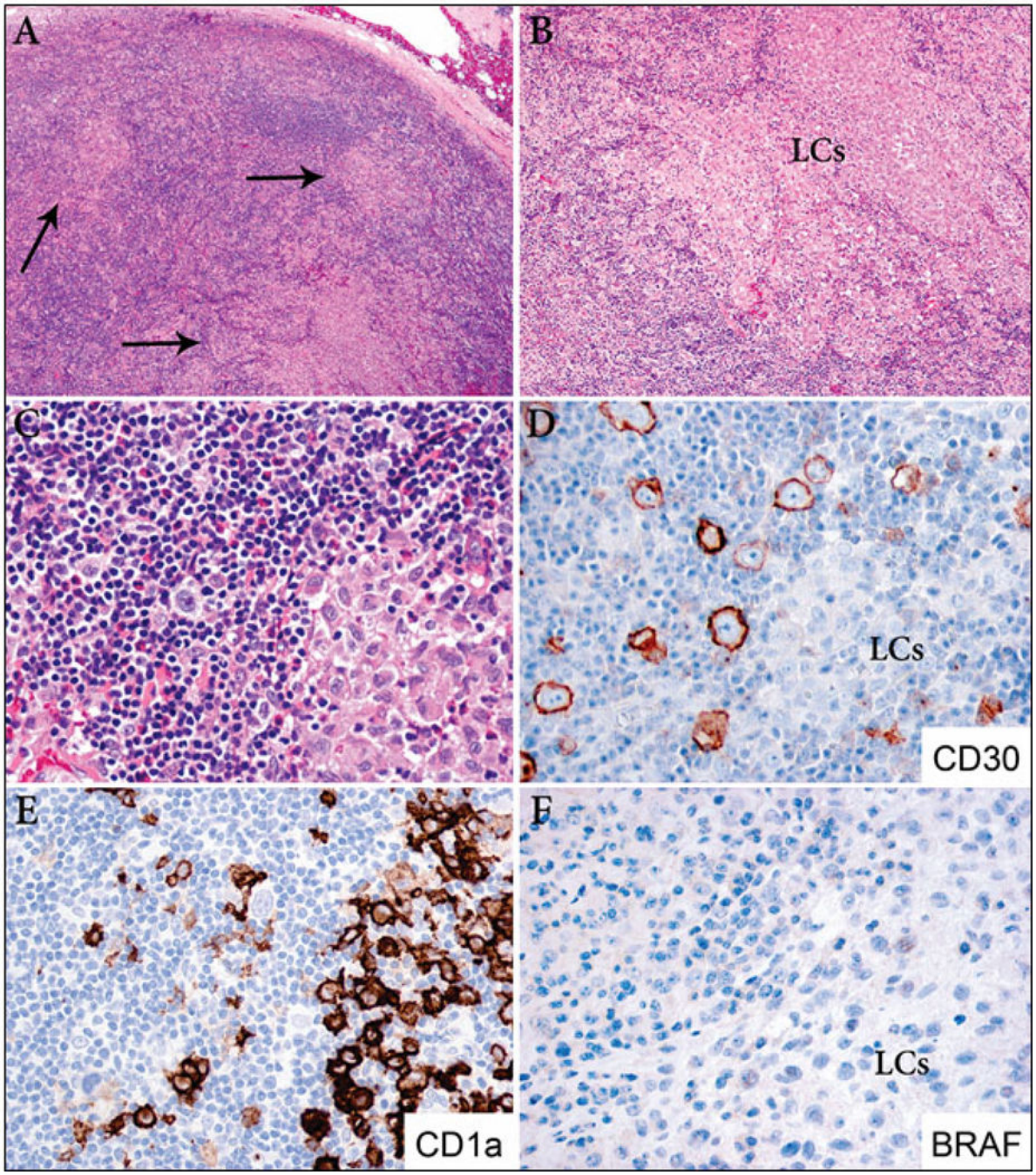

Figure 1. LCH and classical Hodgkin lymphoma, mixed cellularity (case 2)

A. Low power image of a lymph node involved by classical Hodgkin lymphoma, mixed cellularity type, and multiple clusters of LCH of variable sizes (arrows) (40X). B. Largest ill-defined nodule of LCH (200X). C. Area of transition of classical Hodgkin lymphoma with a Reed-Sternberg cell (left) and LCH (right) (400X). D. Immunostain for CD30 highlights the Reed-Sternberg cells and is negative in the Langerhans cells (LCs) (400X). E. Immunostain for CD1a is positive in the LCs and is negative in the Reed-Sternberg cells (400X). F. BRAF is negative in both components (400X).

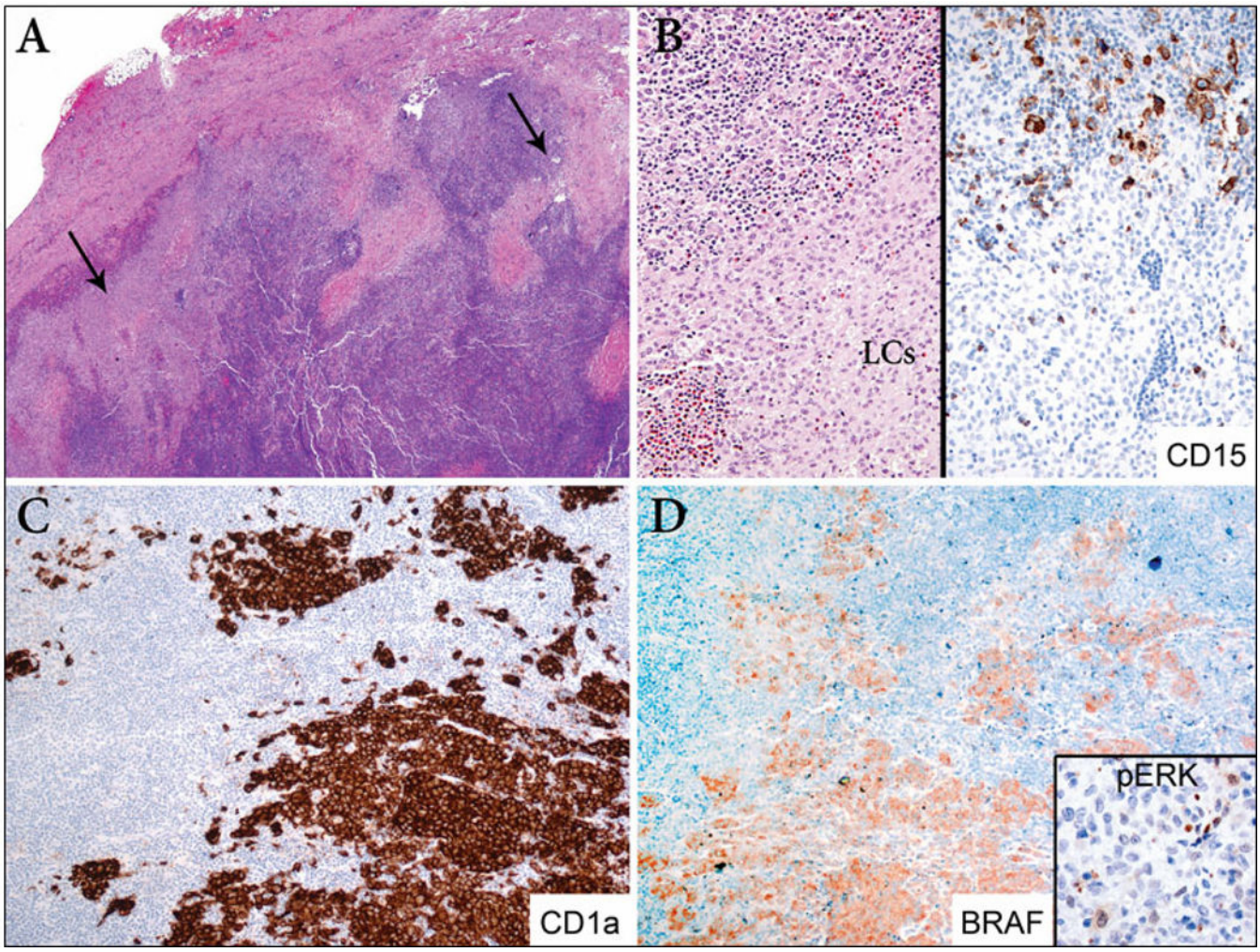

Figure 2. LCH and classical Hodgkin lymphoma, nodular sclerosis (case 5)

A. The lymph node capsule is thickened and contains multiple fibrous bands extending into the parenchyma that incompletely surround cellular areas. In addition, the subcapsular region contains large clusters of LCH (arrows) (40X). B. Area of transition between classical Hodgkin lymphoma (top) and a nodule of Langerhans cells (bottom) (200X). Immunostain for CD15 highlights the Reed-Sternberg cells (top) and is negative in the Langerhans cells (bottom) (200X). C. The widespread involvement of the lymph node by LCH is more evident with immunostain for CD1a (100X). D. Immunostain for BRAF is positive only in the LCH component with a weak cytoplasmic pattern of staining (100X). Despite a positive BRAF V600E immunohistochemical result, *BRAF* pyrosequencing demonstrated a wild type sequence in codons 596 (GGT) and 600 (GTG). This case is negative for pERK by immunohistochemistry (Inset, 100X).

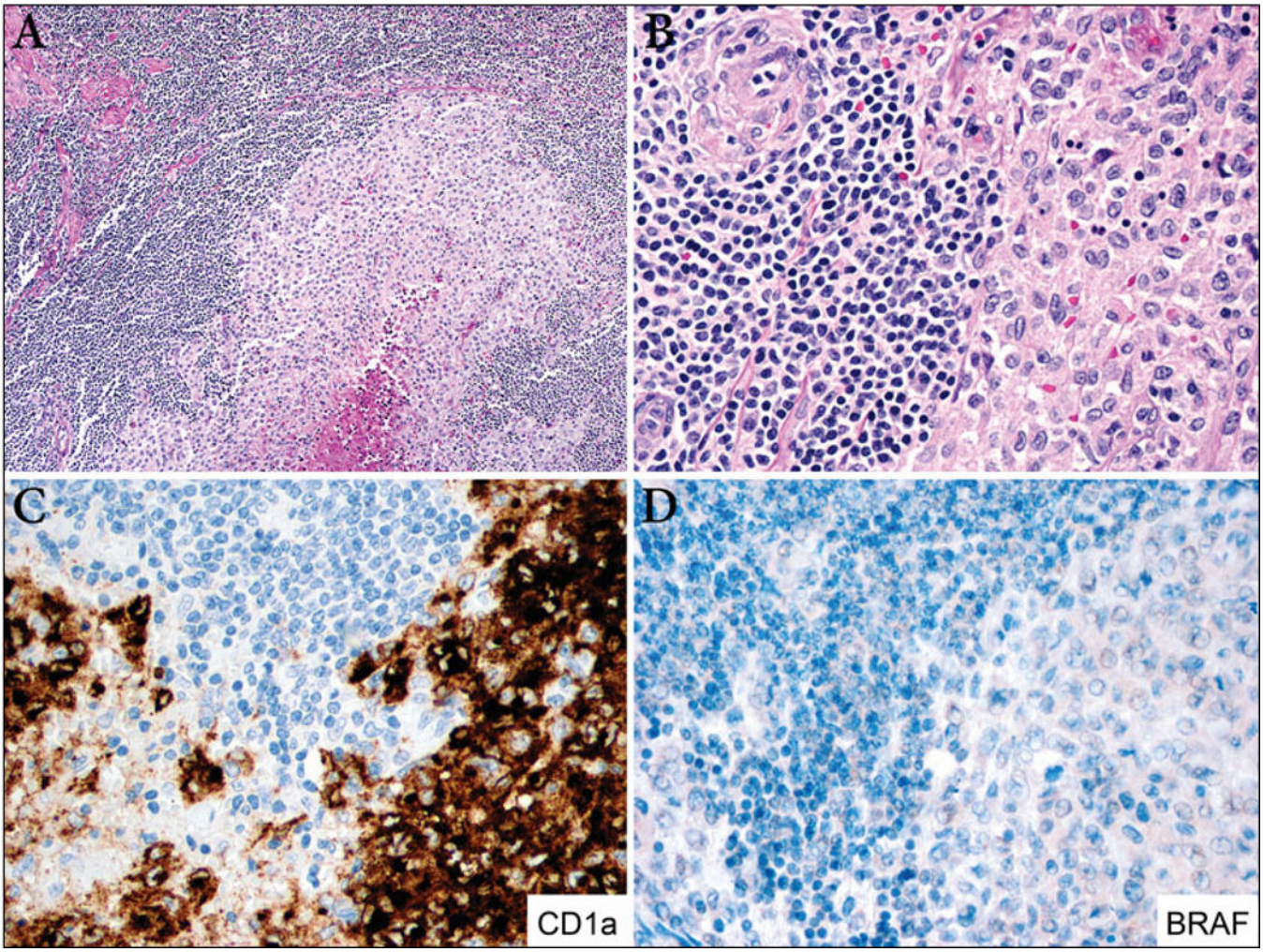

Figure 3. LCH and mantle cell lymphoma (case 7)

A. The lymph node is replaced by a monotonous proliferation of small lymphocytes with focal sclerosis. A single nodule of LCH with central necrosis is identified at the center of the lymph node (100X). B. Left, mantle cell lymphoma composed of small lymphocytes with cleaved nuclei and perivascular fibrosis. Right, LCH module with scattered apoptotic cells (400x). C. Immunostain for CD1a is positive in the Langerhans cells and is negative in mantle cell lymphoma cells (400X). D. BRAF is negative in both components (400X).

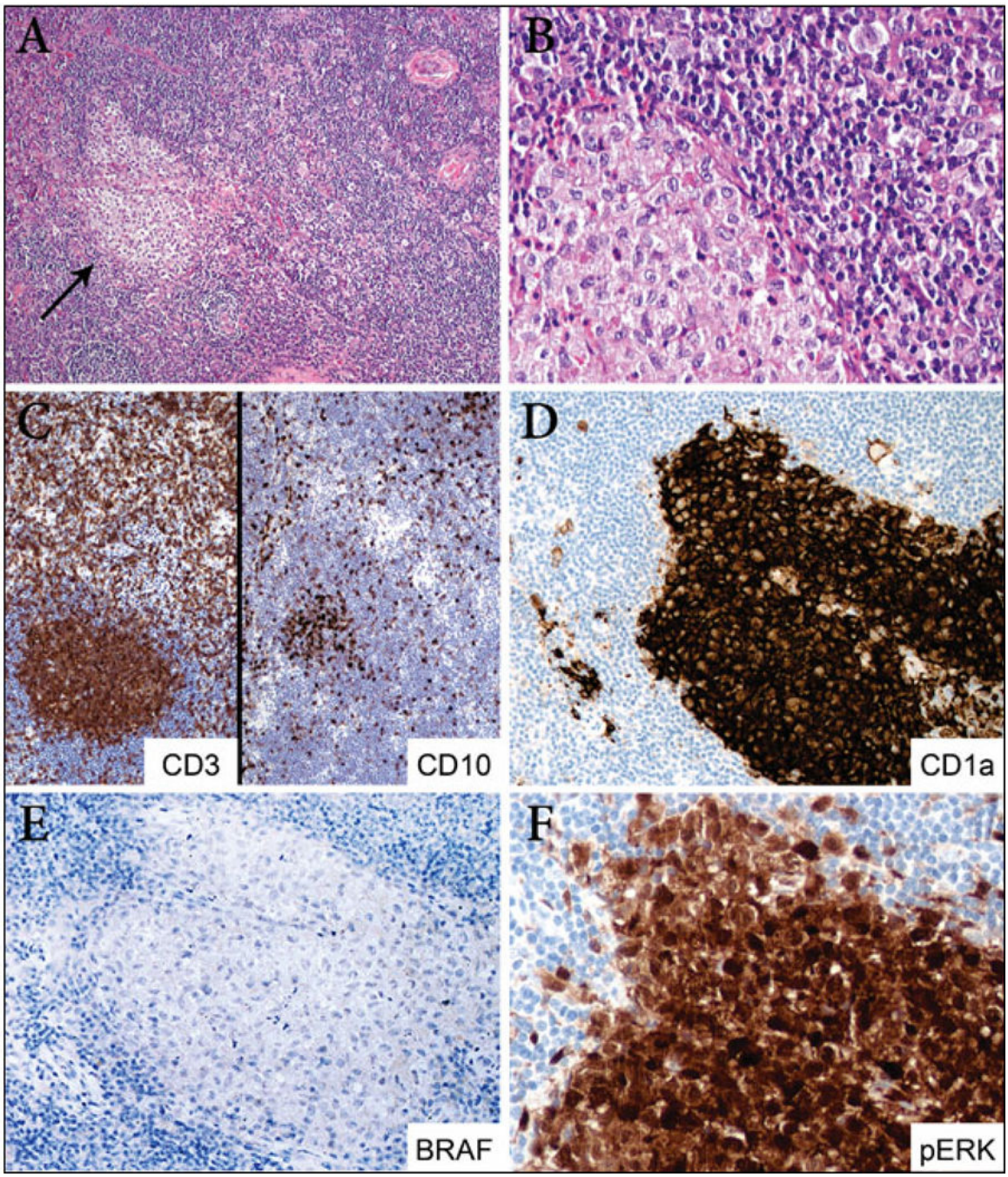

Figure 4. LCH and angioimmunoblastic T-cell lymphoma (AITL) (case 1)

A. At low power, the lymph node is replaced by a diffuse lymphoproliferative process composed of small lymphocytes, clear cells and increased vascularity, characteristic of AITL. A well circumscribed pale nodule of LCH (arrow) is present within the lymphomatous process (100X). B. At higher magnification, the paler nodule (left) is composed of Langerhans cells and scattered eosinophils (400X). C. By immunohistochemistry, the lymphoma cells are positive for CD3 and CD10 (200X). D. The LCH nodule is positive for CD1a (200X). E. BRAF is negative in both AITL and LCH

(200X). F) Immunostain with the anti-pERK antibody is positive in both the nucleus and cytoplasm of Langerhans cells (400X).

TABLE 1

Demographic, clinicopathologic and molecular features, treatment and outcome in patients with LCH and lymphomas

Case	Age	Sex	WBC (x 10^3/µl)	Hgb (g/dL)	Plt (x 10^3/µl)	LDH (IU/L)	B2M (mg/L)	Anatomic location	Lymphoma subtype	LCH distribution	LCH component IHC	pERK IHC	BRAF V600E IHC	*BRAF* V600E PCR	*MAP2K1* PCR	Treatment	Follow up (months)	Outcome
1	68	F	N/A	N/A	N/A	N/A	N/A	Lymph node, cervical	AITL	Multiple small foci	CD1a + langerin + S100 +	Pos	Neg	Neg	Neg	N/A	N/A	N/A
2	84	F	1 0.5	13.2	368	397	2	Lymph node, cervical	CHL, mixed cellularity	Multiple small foci and scattered LCs	CD1a + langerin + S100 +	Neg	Neg	Neg	Neg	AVD XRT	10	CR
3	28	M	6.6	16.2	154	465	2.8	Chest wall	CHL, nodular sclerosis	Few small foci	CD1a + langerin + S100 +	Neg	Neg	Neg	Neg	ABVD	67	CR
4	37	M	4.0	9.8	41	668	2.4	Lymph node, axillary	CHL, nodular sclerosis	Multiple small foci, 2 large focus	CD1a + langerin + S100 +	Pos in subcapsular foci; neg in largest focus	Neg	Neg	Neg	ABVD, VDTPACE, IGEV, thalidomide, revlimid, XRT, auto-SCT	21	DOD (CHL)
5	39	F	5.3	15.2	162	586	2.1	Lymph node, cervical	CHL, nodular sclerosis	Multifocal, large clusters of LCs	CD1a + langerin + S100 +	Neg	Pos	Neg	Neg	ABVD, AVD, ICE 1st cycle, will be followed by SCT	6	Persistent disease in the mediastinum detected by imaging
6	54	M	5.8	12.1	141	402	4.3	Lymph node, retroperitoneal	CHL, recurrent	Small cluster with central necrosis	CD1a + langerin + S100 +	Pos	Neg	Neg	Neg	ABVD, ICE, auto-SCT for CHL Azacitidine, decitabine, MUD-SCT for MDS	21	Therapy-relatedMDS
7	61	M	13.2	13.4	344	390	1.8	Lymph node, inguinal	MCL	Small cluster	CD1a + langerin + S100 +	Neg	Neg	Neg	Neg	Rituximab, hyper CVAD, methotrexate, cytarabine	89	CR

Abbreviations: LCH, Langerhans cell histiocytosis; WBC, white blood cells; Hgb, hemoglobin; Plt, platelets; LDH, lactate dehydrogenase; B2M, β-2 microglobulin; IHC, immunohistochemistry; pERK, phosphorylated ERK; PCR, polymerase chain reaction; F, female; M, male; N/A, not available; AITL, angioimmunoblastic T-cell lymphoma; CHL, classical Hodgkin lymphoma; MCL, mantle cell lymphoma; LCs, Langerhans cells; Pos, positive; Neg, negative; AVD, doxorubicin, vinblastine and dacarbazine; XRT, radiotherapy; ABVD, doxorubicin, bleomycin, vinblastine and dacarbazine; VDTPACE, dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide and etoposide; IGEV, ifosfamide, gemcitabine and vinorelbine; auto-SCT, autologous stem cell transplant; ICE, ifosfamide, carboplatin and etoposide; MUD-SCT, matched unrelated donor stem cell transplant; MDS, myelodysplastic syndrome; hyper CVAD, cyclophosphamide, vincristine, doxorubicin, and dexamethasone; CR, complete remission; DOD, died of disease