

Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is a highly sensitive marker for intravascular large B-cell lymphoma: immunohistochemical analysis of 152 pathology specimens from 88 patients

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Abstract

Intravascular large B-cell lymphoma (IVLBCL) is a rare type of aggressive extranodal large B-cell lymphoma characterized by selective growth of lymphoma cells within the lumina of blood vessels, particularly capillaries. IVLBCL lacks mass formation, and its diagnosis can be challenging. We analyzed the utility of IMP3 immunohistochemistry (IHC) for IVLBCL diagnosis in various organs. Double staining with PAX5 was performed for validation. Overall, 152 pathological specimens (111 positive and 41 negative for IVLBCL) obtained from 88 patients with a diagnosis of IVLBCL were stained for IMP3 and IMP3/PAX5. As negative controls, 40 pathology specimens from 38 patients with no history of IVLBCL or other B-cell lymphomas were stained for IMP3, which comprised 31 benign pathological specimens from 29 patients in whom malignancy was suspected, seven cases of appendicitis with intravascular and/or intralymphatic lymphoid proliferations, and two cases of intravascular natural killer/T-cell lymphoma. All mononuclear cells with cytoplasmic staining were considered positive for IMP3 expression, but expression restricted to germinal center B (GCB) cells was excluded from evaluation. All 111 IVLBCL pathological specimens were positive for IMP3 and IMP3/PAX5. In addition, 11 of the 41 specimens originally diagnosed as IVLBCL-negative showed IMP3/PAX5 double-positive cells, raising the suspicion of

IVLBCL. However, of the 40 negative control samples, IMP3-positive non-GCB cells were detected in only two samples ($p=0.0131$) and no intravascular IMP3-positive B cells suspicious for IVLBCL were identified. Altogether, IMP3 IHC is a highly sensitive marker of IVLBCL and can be a helpful adjunct for IVLBCL diagnosis.

Keywords: insulin-like growth factor II mRNA-binding protein 3 (IMP3), intravascular large B-cell lymphoma, immunohistochemistry, double staining

Introduction

Intravascular large B-cell lymphoma (IVLBCL) is a rare type of aggressive extranodal large B-cell lymphoma characterized by selective growth of lymphoma cells within the lumina of vessels, particularly capillaries, with the exception of larger arteries and veins.(1) First reported in 1959 as “angioendotheliomatosis proliferans systemisata,” a term now obsolete, it was originally thought to be endothelial in nature(2) and later consolidated as a lymphoreticular disease entity.(3) IVLBCL is a disseminated disease that primarily affects older patients with a median age of 67 years (range: 13–85).(4, 5) Patients present clinically with nonspecific systemic symptoms, such as pyrexia of unknown origin and/or B symptoms, general fatigue, performance deterioration, and symptoms related to the specific organ of involvement.(5, 6) Generally, skin and/or central nervous system (CNS) involvement is observed in 60–80% of patients, while liver, spleen, or bone marrow involvement is observed in over a third of patients.(7) This lack of specificity leads to delayed diagnosis, which is considered one of the main reasons for the poor prognosis of this disease.(8) As a result, reports of post-mortem diagnosis are not uncommon.(9) In patients diagnosed with IVLBCL, treatments with rituximab-based immunochemotherapies have improved survival, highlighting the necessity of timely and accurate diagnosis of the disease.(5, 10, 11) Organ biopsies,

commonly random skin and bone marrow biopsies, are standard diagnostic procedures for patients with suspected IVLBCL.(12) However, these procedures are limited by a significant proportion of false-negative results, since IVLBCL typically lacks mass formation, with only a few tumor cells present in a biopsy specimen.(1) Furthermore, the lack of sufficient tumor cells for adjunctive tests such as gene analysis and flow cytometry is another significant limitation. Genetic analysis using cell free DNA is showing great promise to address these challenges.(13, 14) Similarly, the development of widely accessible and commonly employed diagnostic markers to overcome the challenges in IVLBCL diagnosis is desirable.

Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is a member of the IMP family, which plays a role in the early stages of embryogenesis.(15) Despite its absence in most normal adult tissues,(16) variable IMP3 expression is observed in human neoplasms.(17) Notably, its expression has been detected in classic Hodgkin lymphoma and most high-grade non-Hodgkin B-cell lymphomas.(18-21) In our practice, we use IMP3 as a useful marker for these lymphomas, especially in small biopsy specimens with low numbers of tumor cells. Using a single institutional cohort, we performed immunohistochemistry for IMP3 on skin biopsies and found IMP3 to be a highly sensitive marker for IVLBCL in skin biopsies.(22)

Considering that the clinical presentation and diagnosis of IVLBCL are not limited to the skin, we sought to expand on these findings to capture the full clinical and pathological presentation of IVLBCL. Here, we used a multi-institutional cohort to analyze the utility of IMP3 immunohistochemistry (IHC) in the diagnosis of IVLBCL in various organs and validated it using double staining with paired box 5 (PAX5).

Materials and Methods

Patient selection

We searched for positive and negative biopsies from patients with IVLBCL diagnosed at 11 institutions between 2001 and 2022. The participating institutions were Kyoto University Hospital, Kyoto Katsura Hospital, National Hospital Organization Kyoto Medical Center, Osaka Red Cross Hospital, Otsu Red Cross Hospital, Kitano Hospital, Uji-Tokushukai Medical Center, Toyooka Hospital, Tenri Hospital, Takatsuki Red Cross Hospital, and Japanese Red Cross Wakayama Medical Center. As controls, negative biopsies from patients without IVLBCL in Kyoto University Hospital were collected; we included patients who were initially suspected to have IVLBCL and excluded those with other B-cell lymphomas. In addition, histological mimics of

IVLBCL, such as intravascular natural killer (NK)/T-cell lymphoma(23) and reactive intravascular and intralymphatic lymphocyte proliferation,(24, 25) were searched as negative controls. Clinical and pathological information was obtained from pathology reports and patient charts. This study was approved by our institutional review board (approval code: R3843) and conducted in accordance with the Declaration of Helsinki.

Immunohistochemistry

IHC for IMP3 and cluster of differentiation (CD) 20 was performed on 4- μ m formalin-fixed and paraffin-embedded tissue sections prepared from all the pathology samples from patients with and without IVLBCL using anti-IMP3 (Clone 69.1, Agilent, Santa Clara, CA, USA) and anti-CD20 (Clone L26, Agilent) antibodies with an automatic immunohistochemical staining device BOND-RX (Leica Biosystems, Wetzlar, Germany). The following additional primary antibodies were used for immunophenotypic analysis: B-cell lymphoma 6 (BCL-6) (Clone G1191E/A8, Roche diagnostics, Indianapolis, IN, USA); CD3 (Clone 2GV6, Roche Diagnostics); CD5 (Clone SP19, Roche Diagnostics); CD10 (Clone 56C6, Leica Biosystems); multiple myeloma oncogene 1 (MUM1) (Clone MUM1p, Agilent); and Epstein-Bar virus encoded small RNAs (EBER) in situ hybridization (PB0589, Leica Biosystems).

IMP3 and PAX5 (Clone EPR3730(2), Abcam, Cambridge, UK) double staining was also performed using the BOND-RX as described previously(26) in all pathological specimens from patients with IVLBCL and in selected specimens from patients without IVLBCL.

Pathological evaluation

Histological diagnosis for IVLBCL was performed according to the World Health Organization criteria.(1) Cytological atypia of intravascular lymphoma cells was identified using hematoxylin and eosin (H&E) staining. CD20 immunostaining was performed to confirm B-cell lineage. All mononuclear cells with cytoplasmic staining were considered positive for IMP3 expression, but IMP3 expression limited to germinal center B (GCB) cells was excluded from evaluation. IMP3 expression in non-mononuclear cells, namely, megakaryocytes and glial cells, was considered negative.(27, 28) IMP3/PAX5 double staining was considered positive when cells showed both nuclear staining for PAX5 and cytoplasmic staining for IMP3. IMP3 staining intensity was graded as negative, 1+ (weak), 2+ (moderate), or 3+ (strong).(18) Two authors (M.S and M.F) jointly evaluated the cases using a multi-head microscope. Cases with differing interpretations were discussed until consensus was reached.

Statistical analyses

Categorical variables between the two groups were compared with Fisher's exact test using GraphPad Prism (GraphPad software; San Diego, California, USA). The results were considered significant when the two-sided p-value was <0.05.

Results

Clinicopathological features

A total of 152 pathological specimens from 88 patients with IVLBCL (46 males and 42 females; median age, 72 [age range, 51–95] years) were collected. The clinicopathological characteristics, including immunophenotypes extracted from patient pathology reports, are presented in Table 1. All pathological specimens were obtained from the following organs and tissues: skin (n=80), bone marrow (n=46), lung (n=10), brain (n=4), nasal/paranasal mucosa (n=3), kidney (n=2), liver (n=2), artery, colon, lymph node, testis, and stomach. Among the 88 patients, complete IHC results for the Hans classifier(29) were available in 27 specimens obtained from 26 patients. Twenty-three specimens from 22 patients were classified as non-GCB subtype and four specimens from four patients were classified as GCB subtype. The original diagnosis

was positive for IVLBCL in 111 samples and negative for IVLBCL in 41 samples (Table 2).

As negative controls, 31 pathological specimens (brain [n=13], bone marrow [n=4], skin [n=4], stomach [n=2], ileum [n=2], appendix, duodenum, lacrimal gland, lung, nasopharynx, pleura) obtained from 29 patients without IVLBCL and other B-cell lymphoma (22 males and 7 females; median age, 61 [range, 4–81] years) who were suspected for malignancy, including 19 patients who were clinically suspected of having IVLBCL, were analyzed. All 31 pathological specimens from the 29 patients were diagnosed as benign conditions and included the following final diagnosis: acute gastric mucosal injury; amyloid angiopathy; appendicitis; autoimmune gastritis; benign lymphoid hyperplasia; chronic pleuritis; CNS graft-versus-host disease; demyelinating disease; duodenitis; fever of unknown origin (n=7); hemophagocytic syndrome; IgG4-related disease; ileal ulcer; ileitis; Japanese encephalitis; mitochondrial encephalomyopathy; multiple sclerosis; progressive multifocal leukoencephalopathy; primary angiitis of the CNS (n=3); Rasmussen's encephalitis; and varicella zoster virus encephalitis. Additionally, we analyzed two cases of intravascular NK/T-cell lymphoma (skin biopsy [male, 62 years old] and spleen biopsy [female, unknown age]) and seven cases of appendicitis with intravascular and intralymphatic lymphoid proliferations (two

males and five females; median age, 32 [range, 23–73] years) identified in our pathology archives.

Immunohistochemical results of IMP3, CD20, and IMP3/PAX5

All IVLBCL tumor cells were positive for CD20. IMP3 expression was observed in the majority (>50%) of IVLBCL cells in all 111 specimens, most cases showing moderate to strong intensity (81.1%) (Figure 1). Furthermore, IMP3-positive IVLBCL tumor cells in all 111 specimens were positive for IMP3/PAX5 double staining. Among the 41 pathological specimens from 21 patients with IVLBCL that were originally diagnosed as negative for IVLBCL, 14 (34.1%) were positive for IMP3, but only 11 (26.8%) co-expressed CD20, as confirmed by IMP3/PAX5 double staining. The three specimens with IMP3-positive mononuclear cells lacking CD20 and PAX5 staining were all bone marrow specimens. The IMP3-positive mononuclear cells were scattered in the interstitium rather than in the intrasinusoid, had eccentric nuclei with coarse chromatin, and were considered plasma cells.

Of the 31 pathological specimens from patients without IVLBCL, four harbored IMP3-positive cells. In three bone marrow biopsies, weak IMP3 staining was observed in the interstitial plasma cells. In one case of varicella zoster encephalitis, IMP3 staining was

observed in extravascular mononuclear non B-cells. In all four specimens, IMP3-positive mononuclear cells were not observed within the vascular lumen. Among these 31 pathological specimens, IMP3/PAX5 double staining was performed on 15 biopsies in which IVLBCL was clinically suspected, or IMP3-positive cells could not be determined to be CD20-positive or -negative. All IMP3-positive cells did not co-express CD20 or PAX5. Thus, in contrast to the negative biopsies from patients with IVLBCL in which 26.8% (11/41) were positive for IMP3-positive non-GCB lymphocytes, none were positive in patients without IVLBCL (0/31; $p=0.0017$).

Among the seven appendicitis specimens with intralymphovascular lymphoid proliferations, four harbored a few IMP3-positive cells (Figure 2 A-D). IMP3/PAX5 double staining was positive in two of these four cases, one of which showed a few IMP3/PAX5 double positive intralymphatic B-lymphocytes (Figure 2 D, E). No IMP3-positive B lymphocytes were found in the vascular lumens. Two intravascular NK/T-cell lymphomas, which were positive for CD3 and EBER, exhibited negative staining for CD20, IMP3, and PAX5 (Figure 2E–H).

In summary, IMP3-positive non-GCB lymphocytes were found in only 5% (2/40) of non-IVLBCL cases, while 26.8% (11/41) were positive in the negative biopsies from IVLBCL cases ($p=0.0131$). Furthermore, no intravascular IMP-positive B cells were

identified in non-IVLBCL cases that would raise suspicion of IVLBCL. The results of IMP3 and IMP3/PAX5 IHC in 152 pathological specimens from 88 patients with IVLBCL and 40 specimens from 38 patients without IVLBCL are summarized in Table 3.

Re-evaluation of negative biopsies from patients with IVLBCL with IMP3-positive B lymphocytes

The clinical and pathological findings of the 11 specimens from 11 patients with IVLBCL (cases 1-11) that were originally diagnosed as negative but positive for IMP3/PAX5-positive lymphocytes are summarized in Table 4. Coupled with the rarity of IMP3 expression in reactive non-GCB cells, these IMP3-positive B lymphocytes were retrospectively suspected to be IVLBCL cells. Except for cases 7-9, IVLBCL or other hematolymphoid malignancy was clinically suspected at the time of biopsy. In the two patients (cases 1 and 5) who underwent flow cytometry at initial diagnosis, one showed a small number (0.4%) of abnormal B cell populations with light chain restriction, while the other showed no abnormal B cells. In another three patients (cases 2-4), CD20 IHC was performed at the time of initial diagnosis. In the set of 11 specimens, six were bone marrow aspirations or biopsies (cases 1–6). Within these six bone marrow specimens, cells positive for IMP3/PAX5 were noticeably larger and more

atypical than IMP3-negative B cells. These cells did not form large clusters and were dispersed and intermixed with hematopoietic cells (Figure 3A–C). Due to the frequent scattered/interstitial extravasated pattern associated with IVLBCL in the bone marrow, IVLBCL was suspected.(30) Case 7 was a perforated diverticulitis that harbored only a few intravascular IMP3/PAX5-positive large atypical B-cells which was resected more than a year before the diagnosis of IVLBCL was established. The patient had a long history of systemic symptoms associated with rheumatoid arthritis, which may have delayed the diagnosis of IVLBCL. In case 8, a lung biopsy was performed with an initial clinical suspicion of interstitial pneumonia that retrospectively revealed intravascular IMP3/PAX5-positive cells with crush artifacts, which limited cytomorphological evaluation (Figure 3D–F). Definitive diagnosis of IVLBCL was made 69 days later following a bone marrow biopsy when the patient had persistent fever and elevated lactate dehydrogenase levels. The patient in case 9 underwent lung resection for pulmonary adenocarcinoma; retrospective evaluation by IMP3/PAX5 staining highlighted intravascular large B-cells; however, intense background tumor-associated inflammation initially obscured these cells. Although IVLBCL was not suspected at the time of surgery, the diagnosis was confirmed by random skin biopsy 42 days later due to postoperative unconsciousness and blood tests showing atypical cells.

Cases 10 and 11 were skin biopsies that harbored only a few intravascular IMP-positive B-cells suspicious for IVLBCL (Figure 3G–I).

Discussion

IVLBCL cells express mature B-cell-associated antigens and are routinely identified using pan-B cell markers, including CD20, CD79a, and PAX5.(1, 4) Moreover, recent studies revealed programmed cell death ligand 1 (PD-L1) expression in IVLBCL, which can be a potential target for immunotherapy.(14, 31) However, despite its diagnostic difficulty, no IHC marker has been previously reported to differentiate IVLBCL from reactive B-cells. The present study demonstrated that all 111 samples of IVLBCL expressed IMP3, and combined with the results of the previous study,(22) all 136 pathology specimens diagnosed with IVLBCL were positive for IMP3. Moreover, IMP3 highlighted cells suspicious for IVLBCL in 26.8% (11/41) of pathological specimens obtained from IVLBCL patients, which were diagnosed as negative for IVLBCL in routine practice. In contrast, IMP3-positive B-cells were found in only two cases of appendicitis, accounting for 5% (2/40) of the negative control samples ($p=0.0131$), and these cells did not involve the vessels. Consequently, IMP-positive B-cells suspicious

for IVLCBL were not found in the patients without IVLBCL. This study further suggests that IMP3 IHC is a useful adjunct for the diagnosis of IVLBCL.

Although IMP3 expression in B lymphocytes is mostly restricted to germinal centers in normal tissues, IMP3 is expressed in various B-cell neoplasms of both GCB and non-GCB origins.(18) In our cohort, 84.6% (22/26) of patients with IVLBCL with available immunophenotypic data were classified as non-GCB type , which is similar to the previously reported proportion.(4) Because IMP3 expression was observed in all cases, irrespective of GCB or non-GCB origin, IMP3 expression in IVLBCL is not related to GCB or non-GCB origin, as expected from previous studies.

Further research is required to reveal the mechanisms underlying IMP3 expression in IVLBCL. Nuclear factor kappa B (NF- κ B) pathway activation has been implicated in the expression of IMP3 in non-hematolymphoid malignancies.(32, 33) Gene expression profiling of IMP3 was correlated with an upregulated NF- κ B activated gene signature in human renal cell carcinoma lines.(32) In gliomas, IMP3 knockdown experiments showed downregulation of p65/Rela, a subunit of NF- κ B transcription factors, which was further collaborated by demonstration of IMP3 binding to the 3'UTR of p65 transcript, resulting in an increase in its translation.(33) Because activation of NF- κ B

plays a central role in the pathogenesis of some B-cell malignancies, including IVLBCL,(34) it may also correlate with IMP3 overexpression in IVLBCL.

In contrast to B-cell lymphomas, the expression of IMP3 in T-cell lymphomas is low. In a previous study by King et al., 90% of anaplastic large T-cell lymphomas were negative for IMP3 expression.(18) Furthermore, IMP3 expression in reactive T-cells is low, as shown by Tang et al., who found that IMP3 expression was limited to large malignant B-cells in T-cell-rich large B-cell lymphoma.(20) Using quantitative reverse transcription polymerase chain reaction, Natkunam et al. revealed the predominance of IMP3 in B-cell lymphoid cell lines, whereas IMP1 instead of IMP3 predominated in T-cell and nonlymphoid cell lines.(35) Accordingly, intravascular NK/T-cell lymphoma was negative for IMP3, although only two cases were tested in this study.

Previously, the expression of IMP family members in normal human bone marrow has been demonstrated in megakaryocytes, myeloid precursors, and occasional lymphocytes and plasma cells using a pan-VICKZ antibody that recognizes all three human isoforms of IMP, namely, IMP1, IMP2, and IMP3.(35) However, our data shows that the co-expression of IMP3 and CD20 or PAX5 in reactive non-GCB cells is rare, including in the bone marrow. Furthermore, no intravascular IMP3-positive B-cells were identified

in non-IVLBCL patients. Thus, with clinical suspicion and morphological correlation, the presence of intravascular IMP3-positive B-lymphocyte would be a strong indicator of IVLBCL.

Eleven negative pathology samples from IVLBCL patients were retrospectively suspicious for IVLBCL. Notably, definitive diagnosis of IVLBCL was made between months to more than a year after initial biopsy or surgery in 36.4% (4/11) of the cases. In three cases, the clinical differential diagnosis did not include hematolymphoid malignancy, but all patients were symptomatic at the time of biopsy or surgery, suggesting that they had lymphoma at that time rather than being in an early prodromal state. It was assumed that the potential tumor cells were initially missed in these cases because (1) the number of tumor cells was small, (2) tumor cells degenerated, (3) tumor cells were mixed with other cells, or a combination of all these factors. IMP3 IHC highlights a limited number of tumor cells, including deformed tumor cells. Double staining for IMP3 and PAX5 is useful for the identification of tumor cells, especially when IVLBCL cells are masked in association with other cells, such as in bone marrow biopsies.

By definition, IVLBCL is diagnosed when tumor cells are identified in the intravascular

lumen.(1) However, in bone marrow lesions, IVLBCL cells often show a scattered/interstitial extravasated pattern and may not be differentiated from other high-grade B-cell lymphomas.(30) Reviewing our pathology archive, other B-cell lymphomas involving bone marrow, namely Burkitt lymphoma, classic Hodgkin lymphoma, diffuse large B-cell lymphoma, and follicular lymphoma, were also positive for IMP3. Therefore, IMP3 is more useful for the detection of subtle lesions than as a tool to distinguish lymphoma subtypes.

In conclusion, in all 111 pathology specimens from various organs diagnosed with IVLBCL, IMP3 expression was consistently found in IVLBCL cells but rarely in reactive non-GCB cells. While most IVLBCLs are diagnosable using H&E staining and CD20 IHC, 11 pathology specimens that initially tested negative were suspected to be positive for IVLBCL by IMP3 IHC, representing 26.8% (11/41) of negative biopsies and 7.2% (11/152) of all biopsies from IVLBCL patients. IMP3, in combination with CD20, highlights a limited number of tumor cells, including deformed tumor cells, and is useful in cases where IVLBCL is suspected but cannot be identified or confirmed using H&E and CD20. In most cases, conducting double staining for IMP3 and PAX5 is not necessary to rule out overinterpretation of IMP3-positive cells that are not B cells,

but it may be useful when tumor cells are masked because of their association with other hematolymphoid cells.

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Table 1. Clinicopathological characteristics of 88 patients with intravascular large B-cell lymphoma (IVLBCL) included in this study

Number of patients	88
Sex	
Male	46
Female	42
Age (years), median (range)	72 (51–95)
Number of total specimens	152
Number of specimens/patients, median (range)	1 (1–9)
Immunophenotype of IVLBCL	
Number of specimens positive for IVLBCL	111
CD5	
Positive	16
Negative	26
Not available	69
CD10	
Positive	3
Negative	45
Not available	63
BCL6	
Positive	15
Negative	5
Not available	91
MUM1	
Positive	24
Negative	3
Not available	84
Hans classifier	
Non-GCB type	23
GCB type	4
Not available	84

BCL6, B-cell lymphoma 6; GCB, germinal center B cell; MUM1, multiple myeloma oncogene 1

Table 2. Organs and original pathological diagnosis of 152 pathology specimens from 88 patients with intravascular large B-cell lymphoma (IVLBCL)

		IVLBCL		N
		Positive	Negative	
Organ	Skin	65 (81%)	15 (19%)	80
	Bone marrow	28 (61%)	18 (39%)	46
	Lung	8 (80%)	2 (20%)	10
	Brain	4 (100%)	0 (0%)	4
	Nasal/paranasal mucosa	2 (67%)	1 (33%)	3
	Kidney	2 (100%)	0 (0%)	2
	Liver	1 (50%)	1 (50%)	2
	Artery	0 (0%)	1 (100%)	1
	Colon	0 (0%)	1 (100%)	1
	Lymph node	1 (100%)	0 (0%)	1
	Testis	0 (0%)	1 (100%)	1
	Stomach	0 (0%)	1 (100%)	1
Total		111 (73%)	41 (27%)	152

Table 3. Immunohistochemical results of IMP3 and IMP3/PAX5 in 152 pathology specimens from 88 patients with intravascular large B-cell lymphoma (IVLBCL) and 40 biopsies from 38 patients without IVLBCL

	IMP3-positive				IMP3-negative	IMP3-positive cells with CD20 and/or PAX5 co-expression		Total
	IS 1+	IS 2+	IS 3+	Total (any IS)		Positive	Negative	
Positive specimen from IVLBCL	26	31	54	111	0	111	0	111
Negative specimen from IVLBCL	6	8	0	14	27	11	30	41
Negative specimen from non-IVLBCL	8	0	0	8	32	2	38	40

IMP3, IMP3, Insulin-like growth factor II mRNA-binding protein 3; IS, intensity score; PAX5, Paired box 5

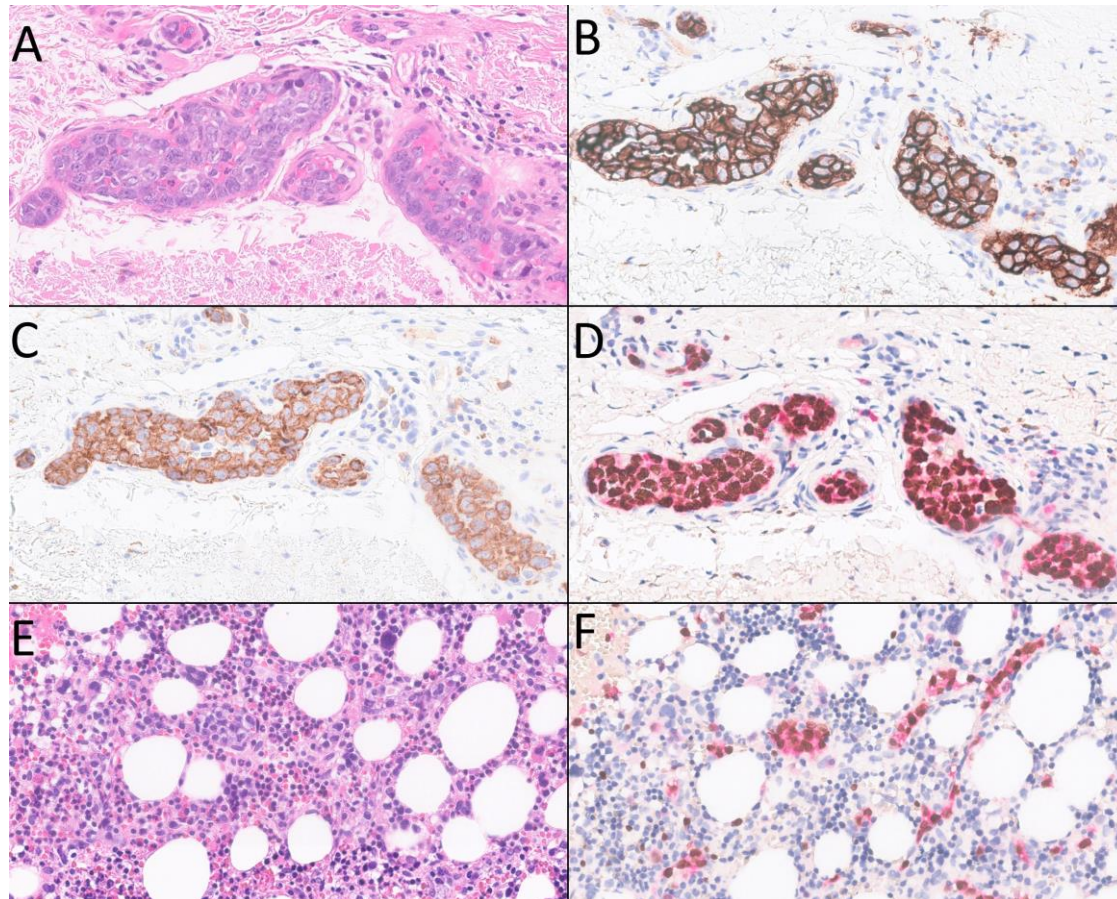
Table 4. Clinicopathological characteristics of 11 patients with IMP3/PAX5 double-positive cells originally diagnosed as negative for intravascular large B-cell lymphoma (IVLBCL)

Case no.	Age (years)	Sex	Organ	Original pathological diagnosis	Number of IMP3/PAX5- positive cells/section (/×400)	Location of IMP3/PAX5- positive cells	Crush artifact	Mixed cell infiltration	Time lag before or after definitive diagnosis of IVLBCL
1	76	M	Bone marrow	Hypercellular marrow	36 (8)	Interstitial	Not present	Present	6 days after
2	63	F	Bone marrow	Hypercellular marrow	101 (10)	Interstitial	Not present	Present	0 days
3	77	M	Bone marrow	Hypercellular marrow	123 (7)	Interstitial	Not present	Present	0 days
4	77	M	Bone marrow	Hypercellular marrow	48 (5)	Interstitial	Not present	Present	3 days before
5	77	F	Bone marrow	Hypercellular marrow	68 (14)	Interstitial	Not present	Present	2 days before
6	72	M	Bone marrow	Hypercellular marrow	136 (21)	Interstitial	Not present	Present	150 days before
7	75	F	Colon	Perforated diverticulitis	4 (4)	Intravascular	Not present	Not present	568 days before
8	63	F	Lung	Pulmonary tissue	26 (13)	Intravascular and extravascular	Present	Present	69 days before
9	87	M	Lung	Adenocarcinoma	85 (25)	Intravascular	Not present	Present	42 days before
10	62	M	Skin	Senile angioma	5 (5)	Intravascular	Not present	Not present	136 days before
11	66	M	Skin	Psoriasiform spongiotic dermatitis	14 (14)	Intravascular and extravascular	Present	Present	381 days after

IMP3, Insulin-like growth factor II mRNA-binding protein 3; PAX5, Paired box 5

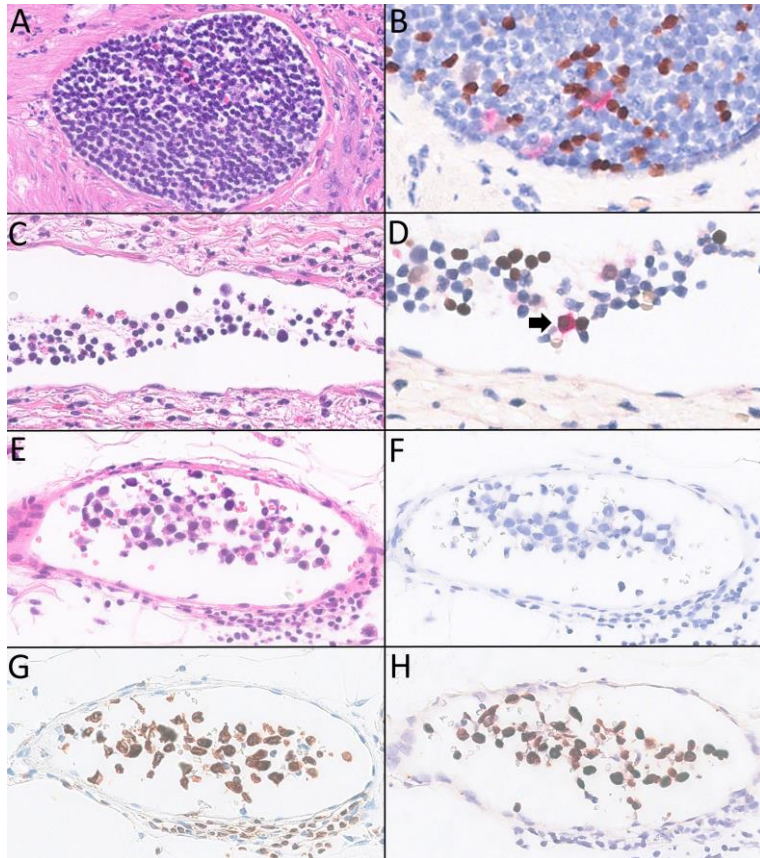
Figure legends

Figure 1.



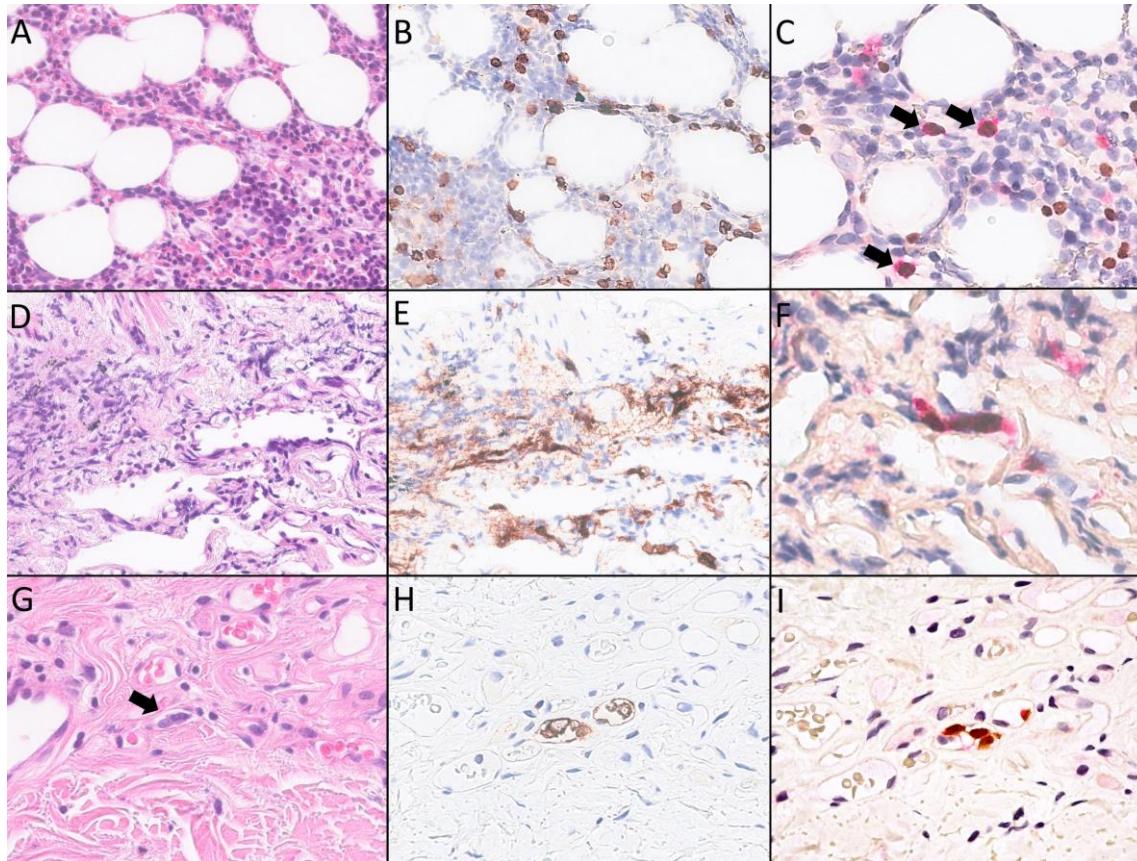
Representative micrographs of intravascular large B-cell lymphoma (IVLBCL) diagnosed based on skin biopsy and bone marrow biopsy. Large, atypical lymphocytes are observed within the vascular lumen in a skin biopsy (A, H&E, ×400). IVLBCL cells are immunohistochemically positive for CD20 (B, CD20, ×400) and IMP3 (C, IMP3, ×400). Double staining for IMP3 (red) and PAX5 (brown) confirms the co-expression of IMP3 and PAX5 (D, IMP3/PAX5, ×400). Clusters of large atypical lymphocytes are observed within a bone marrow biopsy (E, H&E, ×400). Double staining for IMP3 (red) and PAX5 (brown) not only highlights clusters of IVLBCL cells but also individual scattered IVLBCL cells (F, IMP3/PAX5, ×400). H&E, hematoxylin and eosin; IMP3, Insulin-like growth factor II mRNA-binding protein 3

Figure 2.



Representative micrographs of cases other than intravascular large B-cell lymphoma. A, B) Uniform small lymphocytes occluding a blood vessel in an appendicitis specimen (A, H&E, ×400). Lymphocytes with IMP3 staining (red) and negative for PAX5 staining (brown) are observed in less than 5% of the cells (B, IMP3/PAX5, ×600). C, D) Mixed small and large mononuclear cells infiltrate a lymphatic vessel in another appendicitis specimen (C, H&E, ×400). A few IMP3-positive (red) intralymphatic lymphocytes co-expressing PAX5 (brown, indicated by an arrow) are observed (D, IMP3/PAX5, ×600). E–H) A case of intravascular natural killer/T-cell lymphoma. Large lymphocytes are observed in the vascular lumen (E, H&E, ×400). The cells are negative for IMP3/PAX5 double staining (F, IMP3/PAX5, ×400) but positive for CD 3 (G, CD3, ×400) and EBER (H, EBER, ×400). H&E, hematoxylin and eosin; IMP3, Insulin-like growth factor II mRNA-binding protein 3

Figure 3.



Representative micrographs of pathological specimens from patients with intravascular large B-cell lymphoma (IVLBCL) initially diagnosed as negative for IVLBCL. A–C) Case 1, bone marrow biopsy. Lymphocytes intermingled with hematopoietic cells are inconspicuous (A, H&E, ×400). Scattered CD20-positive cells are observed (B, CD20, ×400). IMP3 (red)/PAX5 (brown) double-positive staining highlights large lymphocytes (arrows) suspicious for IVLBCL showing a scattered/interstitial pattern (C, IMP3/PAX5, ×600). D–F) Case 8, lung biopsy. Lymphocytes with crush artifacts (D, H&E, ×400) are positive for CD20 (E, CD20, ×400). IMP3 (red)/PAX5 (brown) staining highlights deformed lymphocytes suspicious for IVLBCL. (F, IMP3/PAX5, ×600). G – I) Case 10, skin biopsy. A few intravascular atypical cells (arrow) (G, H&E, ×600) positive for CD20 (H, CD20, ×600) and IMP3 (red)/PAX5 (brown) double staining (I, IMP3/PAX5, ×600). In these cases, IMP3 could have been the basis for recommending earlier re-biopsy. H&E, hematoxylin and eosin; IMP3, Insulin-like growth factor II mRNA-binding protein 3