The use of BRAF V600E mutation-specific immunohistochemistry in pediatric Langerhans cell histiocytosis

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Abstract

BRAF p.V600E mutations are detected in greater than 50% of pediatric Langerhans cell histiocytosis (LCH) lesions. However, the use of mutation-specific BRAF V600E immunohistochemistry (IHC) as a surrogate for molecular testing in pediatric LCH is unknown. We tested the mutation-specific BRAF V600E monoclonal antibody (clone VE1) in formalin-fixed, paraffin-embedded LCH samples from 26 pediatric patients (14 males and 12 females, ages 7 mo–17 y) using allele-specific real-time polymerase chain reaction (PCR) with a limit of detection of 0.5% as the comparative gold standard. BRAF VE1 staining was scored for both intensity (0–3+) and percentage of immunoreactive tumor cells (0%–100%). BRAF VE1 immunoreactivity was determined using both lenient (1+, 1%) and stringent (2+, 10%) scoring criteria. Using lenient-scoring criteria, we found that the sensitivity and specificity of IHC compared with allele-specific real-time PCR were 100.0% and 18.2%, respectively. The poor specificity of lenient IHC analysis was attributable to weak, 1+ staining in both *BRAF*-mutated and wild-type LCH. Using stringent-scoring criteria, we found that specificity improved to 100.0% at the expense of sensitivity that decreased to 80.0%. Stringent scoring generated 3 false-negative results, but in all cases, neoplastic tissue comprised less than 5% of the stained section and/or the specimen was decalcified. In conclusion, highly sensitive molecular assays remain the gold standard for *BRAF* mutation analysis in LCH paraffin-embedded lesions. To avoid false-positive results, unequivocal VE1 staining of 2+ intensity in greater than or equal to 10% neoplastic histiocytes is required. However, negative VE1 results require additional studies to exclude false-negatives, and stringent-scoring criteria may not be optimal for scant or decalcified specimens.

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1 | INTRODUCTION

Langerhans cell histiocytosis (LCH) is a proliferative disorder that arises from the myeloid dendritic cell precursors.[1,2] Langerhans cell histiocytosis commonly presents in childhood and has a highly variable clinical presentation ranging from isolated skin or bone lesions that typically follow an indolent course, to potentially lethal-disseminated lesions in the bone marrow, the liver, or the spleen.[3–5] Histologically, LCH lesions are characterized by an aberrant proliferation of neoplastic histiocytes with large reniform and grooved nuclei and a mixed inflammatory infiltrate with abundant eosinophils and occasional multinucleated giant cells. The neoplastic Langerhans cell histiocytes typically express CD1a and CD207 (Langerin), and this characteristic immunophenotype helps distinguish LCH from other histiocyte-rich lesions.[2,6]

The *BRAF* p.V600E mutation is detected in greater than 50% of pediatric LCH cases,[7–9] and other V600 codon mutations such asp.V600K, p.V600D, or p.V600R are typically absent.[10] Recurrent MAP2K1 alterations have also been reported in approximately one-third of *BRAF* wild-type LCH cases highlighting the importance of extracellular signal-regulated kinase (ERK) activation in this neoplastic disease.[11–13] In children, *BRAF* p.V600E mutations have been associated with an increased risk of recurrence[9,14] and high-risk LCH in 1 series.[14] Thus, *BRAF* mutation analysis of the blood and bone marrow samples is an effective modality to monitor disease and can assist with oncologic therapeutic decision making.[5,9]

BRAF V600E–specific immunohistochemistry (IHC) using a monoclonal antibody specific to the mutated V600E epitope (clone VE1) has been applied to formalin-fixed, paraffin-embedded (FFPE) tissue sections from multiple *BRAF* p.V600E–positive tumor types.[15] However, the requisite sensitivity and specificity of BRAF VE1 when compared with gold-standard DNA-diagnostic methods are often lacking, and the performance varies considerably among tumor types.[16–21] The variability of BRAF VE1 sensitivity and specificity is partly attributable to tissue and tumor heterogeneity, nonstandardized scoring criteria, and the chosen comparative molecular method.[22–26]

BRAF mutations are typically detected clinically by DNA-based sequencing assays (eg, Sanger sequencing, pyrosequencing, or next generation sequencing) or allele-specific real-time polymerase chain reaction (AS-qPCR). However, the sensitivities of these assays can vary widely; Sanger sequencing has a limit of detection of approximately 15%, pyrosequencing of approximately 5%, and next generation sequencing of approximately 1% to 2% for detecting *BRAF* missense mutations[27,28] while AS-qPCR and digital droplet PCR can achieve detection limits[29] of less than 1%. There is a known association between neoplasia and inflammation,[30,31] and LCH lesions harbor prominent inflammatory components via hypothesized cell autonomous and nonautonomous etiologies.[8,9] Therefore, highly sensitive molecular assays such as AS-qPCR or digital droplet PCR are needed to

detect BRAF mutations in LCH tissue sections, since *BRAF*-positive cells may comprise a small fraction of the inflammatory cellular lesion.

At our institution, all LCH FFPE patient samples are tested for *BRAF* p.V600E mutations using a sensitive AS-qPCR assay that can detect 1 mutant allele in 200 wild-type alleles (a limit of detection of 0.5%). On occasion, if molecular studies are inconclusive because of insufficient tissue, decalcification, or PCR inhibition, BRAF VE1 IHC is requested to determine the mutation status. However, there is limited literature regarding the use and performance of BRAF VE1 in pediatric LCH tissue lesions, and of the few studies in the literature that have investigated VE1 in LCH, none have detailed the performance of VE1 relative to its molecular comparison method.[32–34] Moreover, the use of VE1 IHC as a surrogate for molecular testing is not well established. Therefore, we tested the commercially available VE1 monoclonal antibody on 26 pediatric FFPE LCH tissue samples and compared the IHC results to the corresponding highly sensitive clinical AS-qPCR molecular results to address its use in pediatric LCH.

2 | METHODS

2.1 | Patient samples

The study was conducted in accordance with protocols approved by the Institutional Review Board at Baylor College of Medicine and Texas Children's Hospital (TCH). From the TCH pathology archives, we retrieved 28 FFPE tissue blocks from 15 male and 13 female LCH patients that had been clinically tested for *BRAF* p.V600E mutations in theTCH Molecular Oncology laboratory using AS-qPCR. One hematoxylin and eosin (H&E) and 2 unstained slides were cut for tissue adequacy and IHC analysis, respectively. After assessment of H&E section adequacy by a board-certified pathologist (MJH or KEF), 2 patient samples (1 female and 1 male) were excluded from analysis because of insufficient remaining neoplastic tissue.

In total, 26 patients were included in the study: 14 males and 12 females, ages 7 months to 17 years at time of diagnosis. Seven of 11 bone specimens were decalcified using a formic acid/formalin solution (Cal-Ex II Fixative/Decalcifier, ThermoFisher, Waltham, Massachusetts) for a maximum of 2 hours maximum prior to sectioning. Single system or multisystem disease was determined from retrospective review of provided clinical history on the laboratory test requisitions.

2.2 | *BRAF* p.V600E AS-qPCR

Molecular *BRAF* p.V600E testing was performed using a clinically validated *BRAF* RGQ AS-qPCR assay (Qiagen, Valencia, California) that preferentially amplifies the single nucleotide c.1799T > A transversion in the *BRAF* (NM_004334.4) gene. Genomic DNA was extracted from FFPE tissue samples using the QIAamp DNA mini kit (Qiagen) per the manufacturer's recommendations. Input DNA was quantified using the NanoDrop spectrophotometer (ThermoFisher), and thermal cycling was performed using the Roche LightCycler 480 instrument and associated software (Roche Diagnostics, Indianapolis, Indiana).

Mutant BRAF amplification, detection, and interpretation were performed using established laboratory protocols.[35] Briefly, a control, nonpolymorphic region of *BRAF* exon 3 was amplified in separate wells to ensure DNA integrity and normalize the *BRAF* p.V600E results. Tissue sample Ct values were calculated as the difference between the Ct (crossing threshold) of the p.V600E mutation reaction and the exon 3 control reaction. Samples were determined to be *BRAF* p.V600E mutation positive if the Ct was less than 10, where a Ct of greater of equal to 10 was interpreted as negative for the p.V600E mutation. The lower limit of detection was established as 0.5% (eg, 1 *BRAF* homozygous mutant cell in approximately 200 *BRAF* wild-type cells) per cell-mixing studies.

2.3 | BRAF V600E (VE1) immunohistochemistry

Immunohistochemistry was performed using the BOND-III automated IHC/ISH stainer (Leica Biosystems, Buffalo Grove, Illinois) and a commercially available BRAF V600E monoclonal antibody (clone VE1; Ventana, Tucson, Arizona) at a 1:45 dilution in Ventana antibody diluent, antigen retrieval with ER2 solution for 40 minutes, and diaminobenzidine as a chromogen. Three *BRAF* p.V600E–negative cases (2 papillary thyroid carcinomas, 1 melanoma) and 3 BRAF p.V600E–positive cases (2 papillary thyroid carcinomas, 1 sessile-serrated adenoma) were used for antibody optimization and included as IHC controls.

Slides were evaluated and scored for VE1-staining intensity (0–3+) and percentage of tumor cells positive for VE1 staining (0%–100% scored in 5% increments) to produce a composite score. BRAF VE1 immunoreactivity was determined by both lenient (1+, 1%) and stringent (2+, 10%) scoring criteria as described.[24] VE1-staining intensity was determined as follows: 0+, absence of staining; 1+, faint staining appreciable only at 400× magnification; 2+, moderate staining apparent at 100× magnification with minimal variability in intensity; and 3+, strong staining apparent at 40× magnification (Figure 1). Three board-certified pathologists blinded to molecular status interpreted and scored VE1 IHC (LYB, KEF, and MJH), and consensus scoring was achieved for all cases by concomitant review.

3 | RESULTS

Twenty-six pediatric LCH samples (14 males and 12 females; 7 mo–17 y) with corresponding *BRAF* p.V600E AS-qPCR results (15 molecular-positive and 11 molecular-negative) were included in the study. The pertinent characteristics of each patient sample are summarized in Table 1. Samples were obtained from bone (n = 11), soft tissue (n = 6), lymph nodes (n = 5), skin (n = 2), the liver (n = 1), and the spleen (n = 1). Fourteen patients had multisystem LCH, and 12 patients had single system LCH. Clinical data were limited to the clinical information provided on pathology test requisitions that precluded additional clinical characterization.

Seven bone specimens were subjected to decalcification prior to sectioning. Two decalcified FFPE tissue samples failed AS-qPCR initially, so corresponding nondecalcified sections from the same specimen were tested to determine molecular *BRAF* positivity (Table 1, denoted by *). Hematoxylin and eosin slides and immunohistochemical stains for CD207 and CD1a were reviewed concurrently to confirm both the diagnosis of LCH and amount of

lesional tissue available for scoring. The estimated amount of neoplastic LCH contained in each FFPE section ranged from less than 5% to 95% by H&E light microscopic examination.

The original report,[18] a recent white paper,[36] and the manufacturer's package insert recommend that cytoplasmic staining of 1+ or greater intensity in tumor cells with the VE1 monoclonal antibody be interpreted as positive although no consensus requirement for percent of immunoreactive tumor cells is given (referred to hereafter as lenient scoring criteria: 1+, 1%). Using these criteria, we found that VE1 staining was absent in 2 cases, and the remaining 24 cases exhibited at least weak (1+) staining in greater than or equal to 10% of neoplastic cells (Table 1). Thus, the sensitivity and specificity of IHC compared with AS-qPCR using lenient-scoring criteria were 100.0% and 18.2%, respectively (Table 2). The poor specificity of lenient IHC analysis was directly attributable to the weak cytoplasmic staining detected in lesional Langerhans cell histiocytes regardless of BRAF mutation status (Figure 2A).

To control for the lack of specificity secondary to weak staining, we elected to reanalyze the data using previously established stringent criteria (scored as IHC positive if 2+ or 3+ VE1 staining was detected in 10% of neoplastic histiocytes).[24] Using these scoring criteria, we found that 12 cases were scored as VE1 positive, and 14 cases were scored as VE1 negative. Stringent-scoring criteria improved specificity (100.0% vs 18.2%) at the expense of sensitivity (80.0% vs 100.0%, Table 2). Notably, 3 VE1 false-negative results were observed (Figure 2B), and all contained less than 5% neoplastic tissue and/or were decalcified (Tables 1 and 3).

Langerhans cell histiocytosis involves the bone in approximately 90% of pediatric cases,[37] and bone specimens may undergo decalcification prior to histological processing. Importantly, decalcification has been shown to affect DNA quality and interferes with nucleic acid–based assays,[38] although some PCR applications can be optimized for decalcified specimens.[39–41] We reasoned that if decalcified samples might produce inconclusive molecular results, VE1 may have a certain degree of use in such cases. Therefore, we also analyzed the performance of the VE1 antibody in the 7 Cal-Ex II-decalcified LCH samples included in our study (3 molecular-positive and 4 molecular-negative). The results are summarized in Table 3.

Both cases with complete absence of staining had been decalcified and demonstrated concordance with corresponding negative molecular studies (Table 3). One VE1-positive case (2+, 60%) was also molecular-positive and concordant. The remaining 4 decalcified cases demonstrated weak, 1+ staining in variable percentages of neoplastic cells with VE1 immunoreactivity (range, 10%–50%), and were scored VE1-negative using stringent criteria. However, 2 decalcified VE1-negative cases harbored *BRAF* mutations by molecular analysis (2 false-negatives). Lastly, the 2 decalcified specimens that required repeat molecular testing due to inconclusive initial results demonstrated very weak VE1 staining (absent and 1+, 10%, respectively), but were concordant with negative molecular results seen in nondecalcified sections from the same specimen.

4 | DISCUSSION

In this study, we tested FFPE samples from 26 pediatric patients (18 y of age) with a tissue diagnosis of LCH for BRAF p.V600E mutations using a BRAF V600E-specific monoclonal antibody (clone VE1). We compared VE1 results to highly sensitive AS-qPCR results (lower limit of detection of 0.5%) using 2 separate IHC-scoring criteria and assessed the staining properties of this antibody in decalcified LCH FFPE samples or samples with scant neoplastic infiltrate. The study highlights some important considerations to avoid *BRAF* false-negative and false-positive results when using molecular or VE1 IHC analyses in pediatric LCH tissue sections.

4.1 | *BRAF* p.V600E mutation detection by AS-qPCR

The reported prevalence of *BRAF* p.V600E mutations in pediatric LCH varies widely from 21%[42] to 69%[43] with an average prevalence of approximately 50%[44]. We observed *BRAF* mutations in 57.7% of samples using an AS-qPCR assay capable of detecting *BRAF* mutations with high sensitivity (0.5%, 1 mutated allele in 200 wild-type alleles). For comparison, published sensitivities for *BRAF* mutation detection using Sanger sequencing, pyrosequencing, and next generation sequencing are approximately 10% to 20%, 5%, and 1% to 2%, respectively.[27,28] Interestingly, the frequency of 57% is identical to the original publication by Badalian-Very et al who used next generation sequencing methods on CD1a + cells purified from FFPE specimens.[7]

BRAF wild-type inflammatory cells are recruited to the LCH lesional milieu via several proposed cell autonomous and nonautonomous mechanisms.[8,9] Therefore, unlike other *BRAF*-positive malignancies such as melanoma, colorectal carcinoma, or papillary thyroid carcinoma, mutated dendritic cells may comprise only a minor fraction of the composite neoplastic lesion and are typically not amenable to microdissection. Using Sanger sequencing, lower *BRAF* mutation rates (<30%) were detected in several adult and pediatric LCH studies raising the distinct possibility of false-negative results.[34,45,46] Therefore, we recommend that LCH *BRAF* mutation testing be performed with highly sensitive molecular assays to account for the low-mutant allele fractions intrinsic to LCH and limit potential false-negative molecular results. Additional studies are needed to define the optimal method(s) and requisite lower limit of detection for LCH *BRAF* mutation testing.

4.2 | Immunohistochemistry scoring algorithm: lenient vs stringent scoring

Although thorough technical optimization of the BRAF antibody using control tissue occurred prior to the study, it was clear that optimization of the IHC interpretation for LCH was also required; optimized scoring criteria and highly sensitive comparative molecular gold standards are required to reliably assess the sensitivity and specificity of VE1.[24,25] In 2 previous studies of BRAF VE1 IHC in LCH, Roden et al considered any cytoplasmic staining as positive staining,[33] and Mehes and colleagues used a semiquantitative (0–2+) intensity scale.[32] In both studies, false-positive VE1 results were observed. Likewise in our study, weak, nonspecific cytoplasmic staining was observed in 24 of 26 cases and yielded 9 false-positive VE1 results (Table 2). This nonspecific staining and corresponding suboptimal

specificity precluded any meaningful comparisons using lenient IHC-scoring criteria (1+, 1%).

Using a stringent scoring algorithm devised for melanoma samples that combined staining intensity (0–3+) and percentage staining of tumor cells (0%−100%), we found that unequivocal IHC staining (2+, 10%) was 100.0% specific for an underlying *BRAF* mutation. However, sensitivity suffered with these criteria, as false-negative results were seen in approximately 20% of cases. False-negative IHC results can be seen in tumors harboring non-p.V600E codon mutations (eg, p.V600K) that cross-react in molecular assays, but not with the mutant antibody epitope.[47] However, non-p.V600E codon mutations are rarely detected in LCH; in-frame *BRAF* exon 12 deletions[48] or mutations in other MAP kinase pathway genes such as *MAP2K1*, *MAP3K1*, and *ARAF* are far more common.[12,13,49] Thus, our data and the data of others highlight that VE1 negative samples require additional testing to exclude false-negative results. Also, consensus BRAF VE1-scoring algorithms are needed to standardize IHC results across laboratories for LCH and other tumor types.

4.3 | Low neoplastic cell content and decalcification impact BRAF immunohistochemistry results

Although molecular assays are highly sensitive, sufficient concentrations of extracted, intact nucleic acid from neoplastic nuclei are required for valid results. Langerhans cell histiocytosis biopsies with rare neoplastic cells and/or decalcified specimens may be acceptable for histopathologic and immunophenotypic diagnoses, but are often inadequate for molecular testing. Thus, we evaluated the ability to use VE1 as a complement to molecular testing in these specimen types.

In the 3 IHC false-negative cases by stringent-scoring criteria, 2 cases harbored very low-LCH content (<5%, Table 1). In our experience, stringent IHC-scoring criteria are best suited for FFPE sections with abundant tumor to make an aggregate assessment of staining intensity and percentage of positive tumor cells; scant neoplastic tissue may underestimate IHC intensity and/or percentage of neoplastic cells because of the inherent heterogeneity of the tumor and or FFPE sections.[23,26] In such cases, a binary positive-scoring/negative-scoring assessment would be favored, but the high false-positive rate nullifies any potential benefit.

In addition to the detrimental effects on nucleic acid–based molecular assays,[38] decalcification has been associated with decreased IHC-staining intensity of breast cancer markers.[50] Interestingly, VE1-staining intensity was attenuated in almost all decalcified samples. Only 1 of three (33%) AS-qPCR–positive decalcified bone samples demonstrated greater than 1+ VE1 staining (Table 3). Both samples with complete absence of VE1 staining showed concurrent PCR negative results, including 1 sample that initially failed molecular analysis due to insufficient DNA integrity. The other decalcified sample that failed initial testing and was repeated with nondecalcified material (LCH-55) also demonstrated 1+ staining and yielded negative AS-PCR results. Additionally, 2 AS-qPCR–positive bone samples, LCH-33 and LCH-6 with 30% and less than 5% LCH, respectively, demonstrated false-negative VE1 results by stringent criteria (Table 3).

Collectively, these data suggest that scant and decalcified tissue specimens predispose to false-negative IHC results secondary to scoring inaccuracies and compromised VE1-staining intensity, respectively. For these reasons, VE1 offered no definitive advantage over molecular testing in decalcified LCH tissue sections in our study. We use a formic acid/ formalin solution–based chelation (Cal-Ex II) solution for short duration to minimize nucleic acid hydrolysis and acknowledge that the type of decalcification reagent may have influenced the IHC and molecular results presented here. Therefore, a more extensive study is needed to definitely assess the effects of low-neoplastic tissue content and the effects of various decalcification solutions on the performance of VE1 in LCH using a semiquantitative scoring algorithm.

5 | CONCLUSION

In this study, we assessed the performance of mutation-specific VE1 using optimized stringent-scoring criteria and compared results to a highly sensitive AS-qPCR molecular method in FFPE tissue samples from 26 pediatric LCH patients. Nonspecific, 1+ cytoplasmic staining in both *BRAF*-mutated and wild-type LCH initially hindered IHC analysis, but stringent-scoring criteria improved specificity to 100.0%. Stringent-scoring criteria yielded false-negative results in 20% of cases, and false-negative results were enriched in both decalcified samples and samples with scant neoplastic LCH content. We conclude that strong, 2+, staining in greater than or equal to 10% neoplastic histiocytes is indicative of an underlying *BRAF* p.V600E mutation, but all negative VE1 results require additional testing to exclude false-positives. Also, caution should be used when interpreting either decalcified specimens or specimens with scant neoplastic tissue content. Importantly, highly sensitive molecular assays capable of detecting low-level mutations remain the gold standard for *BRAF* mutation analysis in LCH lesion.

ACKNOWLEDGEMENTS

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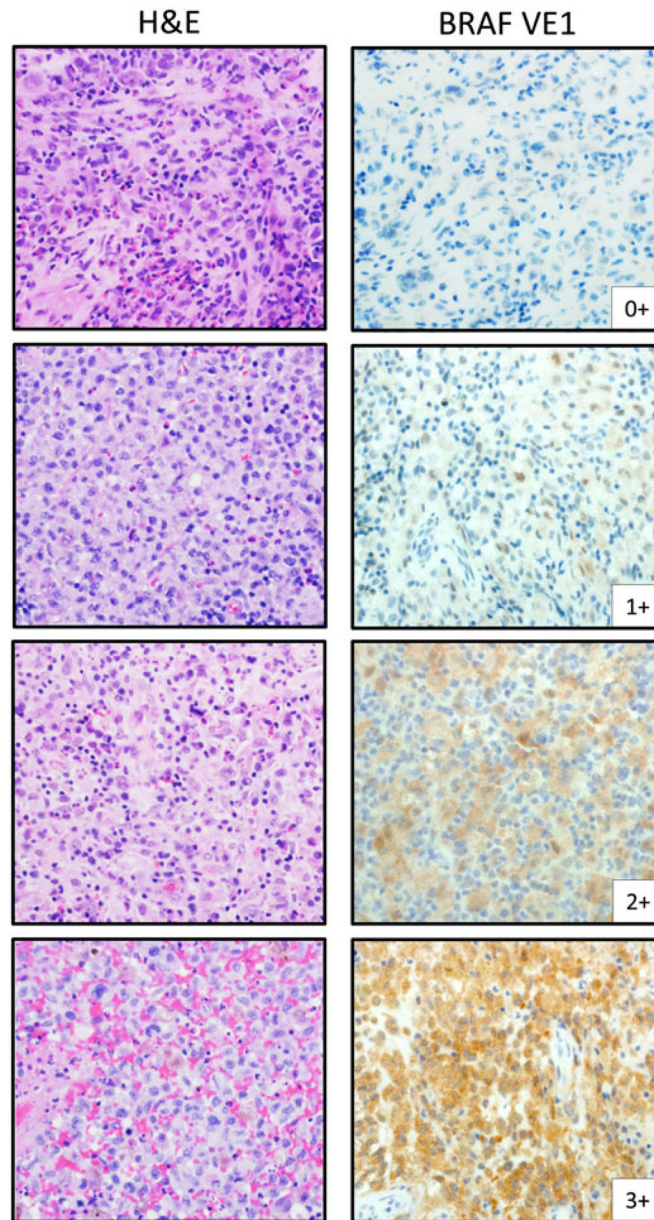
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FIGURE 1.

Semiquantitative IHC-scoring assessment for BRAF VE1 in pediatric LCH. A semiquantitative IHC-scoring scale (0–3+) was combined with percent staining of neoplastic cell cells (0%–100%) to deduce a composite score (see Methods for additional details). Lenient-scoring criteria considered any cytoplasmic staining of 1+ or greater intensity in any amount of neoplastic tissue (1%) as positive. Stringent-scoring criteria required greater than or equal to 2+ staining intensity in greater than or equal to 10% neoplastic cells to be considered positive. Light microscopic images acquired at 400× magnification to highlight 1+ VE1 staining. H&E, hematoxylin and eosin, IHC, immunohistochemistry
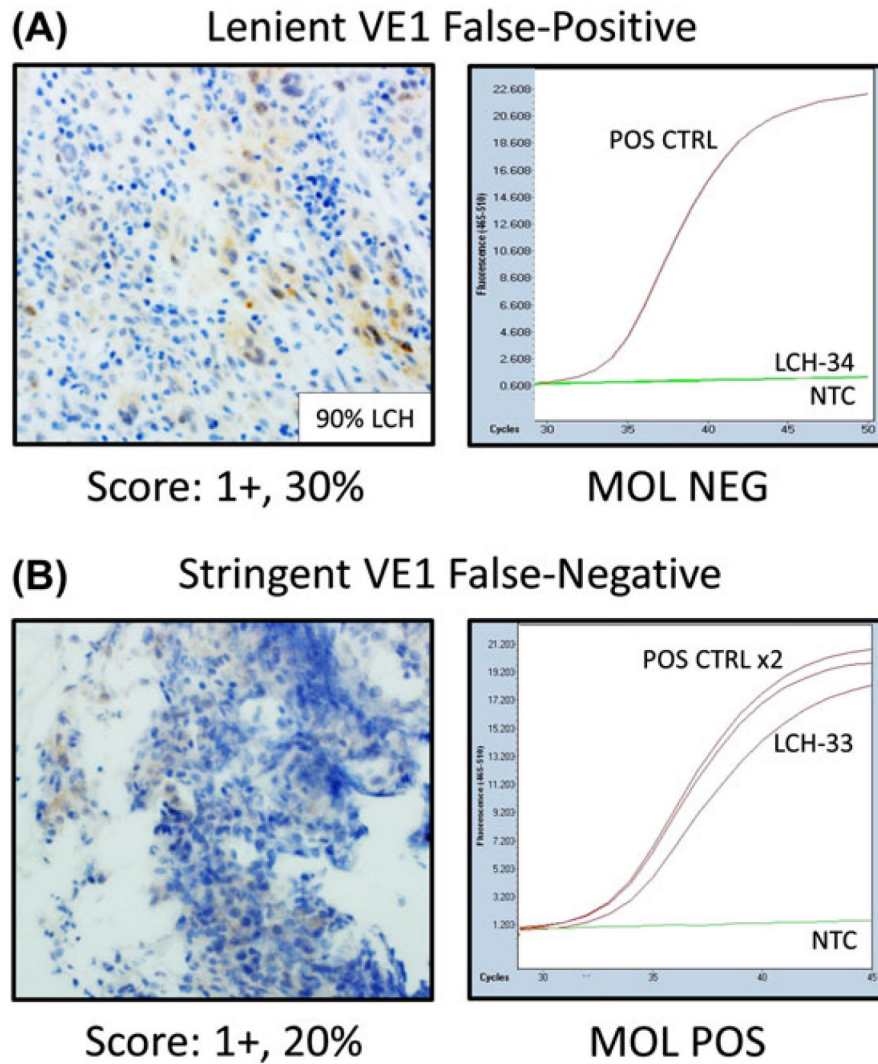
FIGURE 2.
VE1-scoring criteria can influence comparison results. A, A lenient VE1 false-positive sample (LCH-34) showing weak, 1+ staining in 30% of neoplastic histiocytes but no amplification in the AS-qPCR molecular assay. B, A stringent VE1 false-negative sample (LCH-33) showing weak, 1+ staining in 20% of neoplastic histiocytes and amplification in the AS-qPCR molecular assay. Light microscopic images acquired at 400× magnification to highlight 1+ VE1 staining. CTRL, control; IHC, immunohistochemistry; LCH, Langerhans cell histiocytosis; MOL, molecular; NEG, negative; NTC, no template control; POS, positive; VE1, BRAF p.V600E immunohistochemistry

TABLE 1

Clinicopathologic characteristics of pediatric LCH study cohort (n = 26)

Sample	Age/Gender	Clinical Classification	LCH,% of section	Specimen	Decal	VE1 0–3+	VE1 0%–100%	AS-qPCR	IHC/MOL Concordance	
									Lenient IHC(1+, 1%)	Stringent IHC(2+, 10%)
LCH-51	11/M	MS-LCH	40	Bone	Yes	0+	0	NEG	NEG	NEG
LCH-31	1/F	SS-LCH	80	Bone	Yes	0+	0	NEG*	NEG	NEG
LCH-55	10/M	SS-LCH	30	Bone	Yes	1+	10	NEG*	POS-FP	NEG
LCH-6	11/M	SS-LCH	5	Bone	Yes	1+	50	NEG	POS-FP	NEG
LCH-16	2/M	MS-LCH	95	Bone	No	1+	70	NEG	POS-FP	NEG
LCH-32	3/M	SS-LCH	90	Bone	No	1+	80	NEG	POS-FP	NEG
LCH-34	1/M	SS-LCH	90	Bone	No	1+	30	NEG	POS-FP	NEG
LCH-35	1/F	MS-LCH	20	Skin	No	1+	60	NEG	POS-FP	NEG
LCH-57	7 mo/F	MS-LCH	70	LN	No	1+	70	NEG	POS-FP	NEG
LCH-58	9 mo/F	SS-LCH	50	LN	No	1+	90	NEG	POS-FP	NEG
LCH-59	10/M	SS-LCH	80	Bone	No	1+	70	NEG	POS-FP	NEG
LCH-2	1/M	MS-LCH	<5	Soft tissue	No	1+	30	POS	POS	NEG-FN
LCH-33	9 mo/M	MS-LCH	30	Bone	Yes	1+	20	POS	POS	NEG-FN
LCH-13	8/F	SS-LCH	<5	Bone	Yes	1+	50	POS	POS	NEG-FN
LCH-53	4/M	SS-LCH	25	Bone	Yes	2+	60	POS	POS	POS
LCH-9	1/M	MS-LCH	85	LN	No	2+	80	POS	POS	POS
LCH-36	2/F	MS-LCH	40	Skin	No	2+	50	POS	POS	POS
LCH-47	2/F	MS-LCH	70	LN	No	2+	90	POS	POS	POS
LCH-3	9 mo/F	MS-LCH	75	Liver	No	3+	40	POS	POS	POS
LCH-5	2/F	MS-LCH	70	Soft tissue	No	3+	80	POS	POS	POS
LCH-7	11 mo/M	SS-LCH	60	Soft tissue	No	3+	50	POS	POS	POS
LCH-30	17/M	SS-LCH	60	Soft tissue	No	3+	80	POS	POS	POS
LCH-48	5/F	MS-LCH	70	LN	No	3+	30	POS	POS	POS
LCH-54	1/F	SS-LCH	80	Soft tissue	No	3+	80	POS	POS	POS
LCH-60	10/M	MS-LCH	40	Soft tissue	No	3+	60	POS	POS	POS
LCH-62	3/F	MS-LCH	90	Spleen	No	3+	60	POS	POS	POS

The asterisks (*) denote decalcified samples that failed AS-qPCR in FFPE sections. Molecular results for these 2 specimens were obtained from nondecalcified sections from the same specimen.

Abbreviations: AS-qPCR, allele-specific real-time PCR; Decal, decalcification; F, female; FN, false-negative; FP, false-positive; IHC, immunohistochemistry; LCH, Langerhans cell histiocytosis; LN, lymph node; M, male; mo, month; MOL, molecular; MS-LCH, multisystem LCH; NEG, negative; POS, positive; SS-LCH, single system LCH.

TABLE 2

Sensitivity and specificity of lenient and stringent IHC-scoring criteria

Lenient IHC Scoring (1+, 1%)

	MOL NEG	MOL POS
IHC NEG	2	0
IHC POS	9	15
Sensitivity	100.0%	
Specificity	18.2%	

Stringent IHC Scoring (2+, 10%)

	MOL NEG	MOL POS
IHC NEG	11	3
IHC POS	0	12
Sensitivity	80.0%	
Specificity	100.0%	

Abbreviations: IHC, immunohistochemistry; MOL, molecular; NEG, negative POS, positive.

TABLE 3

VE1 IHC in decalcified LCH bone samples (n = 7)

Decalcified Bone Samples	LCH, % of section	Composite VE1 Score, %	AS-qPCR	IHC/MOL Concordance		
				Lenient IHC (1+, 1%)	Stringent IHC (2+, 10%)	
LCH-51	40	0+, 0	NEG	NEG	NEG
LCH-31	80	0+, 0	NEG*	NEG	NEG
LCH-55	30	1+, 10	NEG*	POS-FP	NEG
LCH-6	5	1+, 50	NEG	POS-FP	NEG
LCH-33	30	1+, 20	POS	POS	NEG-FN
LCH-13	<5	1+, 50	POS	POS	NEG-FN
LCH-53	25	2+, 60	POS	POS	POS

The asterisks (*) denote decalcified samples that failed AS-qPCR in FFPE sections. Molecular results for these 2 specimens were obtained from nondecalcified sections from the same specimen.

Abbreviations: AS-qPCR, allele-specific real-time PCR; FN, false-negative; FP, false-positive; IHC, immunohistochemistry; MOL, molecular; NEG, negative; POS, positive.