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Prospective evaluation of TLE1 as a diagnostic immunohistochemical marker in synovial sarcoma

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Abstract:

Synovial sarcoma is a high-grade soft tissue sarcoma that can be challenging to diagnose on the basis of histology alone. It is defined by a characteristic translocation t(X;18) that produces the fusion oncogene *SYT-SSX*. The current diagnostic gold standard for synovial sarcoma is demonstration of the translocation by fluorescence in situ hybridization, reverse transcriptase polymerase chain reaction, or cytogenetics, in an appropriate histologic context. *TLE1* encodes a transcriptional corepressor that is overexpressed in synovial sarcomas. Gene and tissue microarray studies have identified TLE1 as an excellent bio-marker for distinguishing synovial sarcoma from other soft tissue malignancies. We prospectively evaluated incoming soft tissue tumor cases where the histology and clinical setting made synovial sarcoma a real consideration in the differential diagnosis. TLE1, Bcl2, EMA and cytokeratin expression were assessed using commercially available antibodies. TLE1 gave intense, diffuse nuclear staining in 35 of 35 molecularly-confirmed synovial sarcoma cases, and was rare to absent in the 73 other soft tissue tumors examined (positive staining was found in only 1 of 43 malignant peripheral nerve sheath tumors, the one tested fibrosarcoma, and 1 pleomorphic sarcoma). TLE1 was more sensitive and specific for synovial sarcoma than other currently available immunohistochemical markers including Bcl2, EMA and cytokeratins, and had a positive predictive value of 92% and a negative predictive value of 100% in this clinical setting. Our findings confirm, in a prospective diagnostic context, that TLE1 is more sensitive and specific for synovial sarcoma than any

other currently available immunohistochemical stains, and in some cases may preclude the need for molecular testing.

Key words: synovial sarcoma, TLE1, immunohistochemistry

Introduction:

Synovial sarcoma is a high-grade malignancy that accounts for 5-10% of all adult soft tissue sarcomas. It can occur at any age and anatomic site, but is most commonly found in the extremities of young adults[8]. Synovial sarcoma is classified into three histological subtypes: monophasic, (the most common) consisting of monomorphic spindle cells; biphasic, consisting of a mixture of spindle cells and epithelial elements; and poorly-differentiated, usually consisting of sheets of blue cells with prominent nucleoli[8,9]. Synovial sarcoma can be challenging to diagnose by histology as the monophasic subtype resembles other spindle cell tumors including malignant peripheral nerve sheath tumor, dermatofibrosarcoma protuberans with fibrosarcomatous transformation, and hemangiopericytoma/solitary fibrous tumor[23]. Poorly differentiated synovial sarcoma can resemble small round blue cell tumors such as Ewing's sarcoma and rhabdomyosarcoma, among others[10,23].

Synovial sarcoma is characterized by the presence of an (X;18) translocation[2,29], fusing an *SSX* gene on chromosome X to the *SYT* gene (also known as *SS18*) on chromosome 18. The resultant SYT-SSX fusion oncoprotein brings together transcriptional activation (SYT) and repression (SSX) activities, and appears to be involved in the development of synovial sarcoma through the dysregulation of transcription[6,17,18], inducing epigenetic changes that silence key tumor suppressor genes[6,20]. The current gold standard for diagnosis of synovial

sarcoma is demonstration, in an appropriate histologic context, of the characteristic translocation t(X;18) by fluorescence in situ hybridization (FISH), reverse transcriptase polymerase chain reaction (RT-PCR), or cytogenetics. The use of these techniques is limited by practical constraints such as cost, time, and availability of special equipment and personnel[2,5,29]. Recently, SYT has been suggested to work as an immunohistochemical marker for synovial sarcoma[12], but this component of the fusion oncoprotein is actually very widely expressed in its native form[7,33] and so might be expected to lack specificity. To our knowledge, there are no existing anti-SSX antibodies suitable for immunohistochemistry. Thus antibodies directed against other proteins have been evaluated, rather than the fusion oncoprotein itself. Cytokeratins, epithelial membrane antigen (EMA), and Bcl-2 are used clinically to identify synovial sarcomas, but have limited specificity and sensitivity[10,23]. Epithelial markers may be focally expressed or even absent in monophasic or poorly-differentiated synovial sarcoma, posing a particular challenge to diagnosis in small core biopsy samples.

TLE1 is another potential biomarker for synovial sarcoma. TLE1 is an important protein in the Wnt/beta-catenin pathway, a signalling pathway that is strongly associated with synovial sarcoma[3,24]. In both gene and tissue microarray studies, TLE1 has been identified as an excellent bio-marker for distinguishing synovial sarcomas from other soft tissue tumors[1,3,26,32]. There are, in fact, 4 *TLE* genes (Transducin-Like Enhancer of split), which are normally expressed during embryonic neurogenesis, organogenesis and haematopoiesis[14,27,30,35]. All 4 *TLE*

genes have been shown to be highly expressed in synovial sarcoma[1,3,21,26]; the *TLE* genes encode transcriptional corepressors, homologous to the *Drosophila* corepressor *groucho*[27]. The repressor function of TLE1 is regulated by phosphorylation status and histone deacetylase (HDAC) activity[4,22,36]; its overexpression is consistent with SYT-SSX conferring a state of transcriptional repression that can be reversed using histone deacetylase inhibitors[20].

Retrospective analysis of soft tissue tumor tissue microarrays by Terry *et al*. demonstrated that a commercially available antibody for TLE1 was sensitive and specific in distinguishing synovial sarcoma from other soft tissue malignancies[32]. Recently, a whole section study by Kosemehmetoglu *et al.* concluded that TLE1 expression was sensitive but not specific for synovial sarcoma[16]. By contrast, in this study we present the first prospective evaluation of the commercially available antibody for TLE1, in comparison to other currently used immunohistochemical markers and gold standard molecular tests. We assess the value of TLE1 as a diagnostic marker in synovial sarcoma on whole sections from new, incoming cases where the initial histopathologic evaluation led to a consideration of synovial sarcoma as a possible diagnosis.

Materials & Methods

Tumor Samples

During the study period (August 2006-March 2009), standard H&E sections from incoming sarcoma biopsies and soft tissue tumor consultation cases received at Vancouver General Hospital (VGH) (Vancouver, Canada) and the Cleveland Clinic (Cleveland, OH) were assessed by a soft tissue pathologist (BPR or TON) as per the usual surgical pathology approach. Tissue collection was performed in accordance with protocols approved by ethics committees at each institution. In 50 in-house and 58 consultation cases, synovial sarcoma was considered in the differential diagnosis, and these cases were further tested by immunohistochemistry. Morphologic features considered to be suggestive of synovial sarcoma included: cellular tumors, consisting of tight fascicles of spindle shaped cells arranged in a classic herringbone pattern, with nuclei typically containing fine chromatin without conspicuous nucleoli, and minimal cytologic pleomorphism. Other, less common, features included a wiry collagenous background and hemangiopericytoma-like vascular pattern.

Immunohistochemistry

Tissue sections from all cases with sufficient material underwent immunohistochemical staining for TLE1, Bcl-2, pan-cytokeratins and EMA. Additional immunohistochemical markers were also ordered in many cases,

depending on what other entities were considered in the differential diagnosis based on the clinical and morphologic features. Following deparaffinization, antigen retrieval was performed using a Ventana automated immunostainer (Tucson, AZ) with mild CC1 reagent; exceptions were the samples used for cytokeratin, which were treated with proteases. Tissue sections were hybridized with dilutions of commercially available antibodies as described in Table 1, according to the manufacturer's instructions, and optimized locally at each institution. This was followed by washing and detection with the i-View DAB kit from Ventana, except for sections stained with TLE1 at VGH where detection was performed with the DABMap kit from Ventana.

Interpretation of immunohistochemistry

For TLE1, bcl-2, EMA and cytokeratin, immunoreactivity was graded as 3+ (strong) if over 50% of the tumor cells exhibited strong staining obvious at 4x objective power, 2+ (moderate) if 26%-50% showed strong staining visible at 4x objective power or if over 50% of cells clearly stained positive above background at 10x objective power, 1+ (weak or focal) if staining was present below these thresholds, and 0 if staining was not visible above background. These criteria were altered slightly from those used in our previous study so that we could employ the same scoring system for all assessed biomarkers, and represent standard thresholds in use at our institutions for immunohistochemistry analysis. The extremely high sensitivity seen previously for TLE1 allowed us to raise the

scoring threshold for a calling a case 2+/positive (previously 10%, now > 25% strong positive nuclear staining).

The correct subcellular distribution was nuclear for TLE1, membranous for EMA, and cytoplasmic for bcl-2 and cytokeratin, with other distributions and immunoreactivity in non-neoplastic cells scored as background (negative). TLE1 and bcl-2 were positive if scored at the 2+ or 3+ level. Due to the focal staining patterns for cytokeratin and EMA typically observed in synovial sarcoma, samples scored as 1+ or above were considered positive for these two markers.

Molecular Confirmation of Synovial Sarcoma

Following assessment of the initial immunohistochemical panel, fluorescence in situ hybridization analysis for split-apart of the *SYT* gene, and/or reverse transcriptase polymerase chain reaction (RT-PCR) for *SYT-SSX* gene fusion was performed in cases that were positive for TLE1 or where a prior diagnosis of synovial sarcoma had been rendered. As per our standard diagnostic approach, in cases where a definitive diagnosis other than synovial sarcoma could be confidently assigned following assessment of clinical, morphologic and immunohistochemical features, molecular testing was not performed. For example, a diagnosis of malignant peripheral nerve sheath tumor was rendered without molecular testing, where the biopsy showed high mitotic activity, subendothelial infiltration, and a light-dark growth pattern due to stromal edema, in the context of clinical association with a major nerve and/or neurofibromatosis.

If any case was positive for TLE1, molecular testing was performed to verify the presence of an *SYT* gene region rearrangement or presence of *SYT-SSX* fusion gene.

Fluorescence in-situ hybridization analysis was performed at either VGH or Cleveland Clinic using a previously described *SYT* break-apart probe method[31]. To summarize, 6-micron sections underwent deparaffinization, and subsequent demasking. Sections were hybridized overnight to the Vysis LSI *SYT* (18q11.2) dual colour break apart rearrangement probe from Abbott Molecular (Des Plains, IL) followed by counterstaining with 4',6-diamidino-2-phenylindole (DAPI-I). In cases that were *SYT* gene-break negative, but synovial sarcoma was still considered the most appropriate diagnosis, RT-PCR for the presence of *SYT-SSX* gene fusion was performed using a previously described method[15]. Briefly, 10-micron sections were deparaffinized, followed by laser capture microdissection. Total RNA was extracted from the cells collected using TRIzol reagent (Life Technologies, Inc., Grand Island, NY). Reverse transcription for first-strand cDNA synthesis was performed with the ProSTARTM First Strand RT-PCR Kit (Stratagene, La Jolla, CA), according to the manufacturer's instructions, and subsequent PCR amplification was carried out using the *SYT* sense primer, and SSX1-a, SSX1-b or SSX2 antisense primers. Amplified PCR products were detected by Southern blot hybridization overnight with an internal probe for *SYT-SSX*.

Results

TLE1

The commercially available polyclonal anti-TLE1 antibody used produced intense, readily interpretable nuclear immunoreactivity in positive cases. Background or cytoplasmic staining was rarely present. Examples of the four immunohistochemical markers applied to the same case of poorly differentiated synovial sarcoma, as described in the Materials and Methods section, are displayed in Figure 1. Further examples of the scoring interpretation for TLE1, from 0 to 2+, are presented in Figure 2 for three different tumor types.

A summary of the immunohistochemical results is presented in Table 2. Detailed immunohistochemistry results for synovial sarcoma, stratified by histologic subtype and TLE1 immunoreactivity, are shown in Table 3. TLE1 was positive (2+ or 3+ intense nuclear immunoreactivity) in all 35 synovial sarcomas, where the diagnosis was subsequently confirmed by molecular testing. Importantly, TLE1 was strongly positive (3+) in all four poorly-differentiated synovial sarcomas. In the one case of biphasic synovial sarcoma in this study, TLE1 stained strongly positive (3+) in both the epithelial and spindle cell components.

Using the scoring system specified above, TLE1 staining was minimal or absent in the other malignant and benign soft tissue tumors examined in this study.

TLE1 was positive in one of 43 malignant peripheral nerve sheath tumors, the one pleomorphic sarcoma, NOS and the one tested fibrosarcoma. In the remaining tumors examined, where synovial sarcoma had been a diagnostic consideration based on the initial H&E section, TLE1 was negative (Table 2).

Other immunohistochemical markers

Bcl-2 exhibited intense positive (i.e. 2+ or 3+) cytoplasmic immunoreactivity in 30 of 31 synovial sarcomas. However, Bcl-2 was also positive in 11/42 malignant peripheral nerve sheath tumors, in both assessed cellular solitary fibrous tumors / hemangiopericytomas, and in occasional other tumor types that were assessed (Table 2). Due to the lack of sufficient extra tissue sections on some consultation cases, it was not possible to perform Bcl-2 immunohistochemistry on four synovial sarcomas, two mesotheliomas, nor on single examples of some of the other entities in the differential diagnosis.

EMA displayed positive membranous immunoreactivity (at the 1+ or above threshold) in 18 of 28 (64%) synovial sarcoma cases that were examined. Where present, EMA generally exhibited a focal or patchy distribution in synovial sarcoma sections. EMA was also positive in 3/41 malignant peripheral nerve sheath tumors, and occasional other tumor types (Table 2). It was not possible to perform EMA staining on seven synovial sarcomas, two mesotheliomas, two malignant peripheral nerve sheath tumors, and single cases of some of the other soft tissue tumors in the differential diagnosis.

Cytokeratins displayed positive cytoplasmic immunoreactivity (1+ or above) in 24 of 34 (71%) synovial sarcomas. Cytokeratin staining also typically exhibited a focal or patchy distribution pattern in the synovial sarcoma sections examined. Cytokeratin staining was positive in 7/42 malignant peripheral nerve sheath tumors, and occasional other tumors. It was not possible to perform cytokeratin staining for one synovial sarcoma, and single examples of some of the other tumors in the differential diagnosis.

Sensitivity, specificity and predictive values of immunohistochemical markers for synovial sarcoma

The comparative performance of TLE1, Bcl-2, EMA and cytokeratin for synovial sarcoma in this prospective diagnostic setting is presented in Table 4. The final diagnosis was synovial sarcoma in 33 cases and another soft tissue tumor type in 75. TLE1 was highly sensitive (100%) and specific (96%) for synovial sarcoma in comparison to other currently available immunohistochemical markers. Accordingly, TLE1 displayed a strong positive predictive value (92%) and 100% negative predictive value. Bcl-2 was also highly sensitive (97 %), but lacked specificity (71%) in the clinical and morphological context of suspected synovial sarcoma, which is reflected in its mediocre positive (63%) but high negative (98%) predictive value. In contrast, EMA and cytokeratin displayed lower sensitivity (64% and 71%, respectively) but somewhat higher specificity (91% and 85%, respectively). The performance of EMA and cytokeratin in this

diagnostic setting was similar, with positive predictive values of 75% and 71%, respectively, and negative predictive values of 86% and 85%, respectively.

Comparison with results of molecular testing for t(X;18) *SYT-SSX*

Use of fluorescence in-situ hybridization alone for detection of t(X;18) has a sensitivity ranging from 86-97% and a specificity of up to 100%, depending on the cut-off ratios and individual probes used[2,29,31]. Two cases in the study were strongly positive (3+) for TLE1, but were negative or indeterminate for t(X;18) by FISH. Subsequent analysis by RT-PCR revealed the presence of a cryptic *SYT-SSX2* translocation, confirming the diagnosis of synovial sarcoma in both cases. Cryptic X;18 rearrangements have been reported in occasional cases of synovial sarcoma and can mask the typical X;18 translocation, making molecular diagnosis problematic[19,25,34].

At our institution, the turnaround time for SYT FISH results is approximately 4-7 days, whereas TLE1 immunohistochemistry results are available within one day. There was also considerable cost difference between SYT FISH ($145-$785 per case) and TLE1 immunohistochemistry ($47 per case).

In one case with a particularly small sample containing only a few tumor cells, TLE1 immunohistochemistry was critical in identifying the population of cells for FISH analysis (Fig. 3). This sample would otherwise have been considered

uninterpretable, and thus TLE1 immunohistochemistry prevented the need for an open biopsy.

Discussion

TLE1 as a diagnostic immunohistochemical marker for synovial sarcoma

Synovial sarcoma can be a challenging tumor to distinguish by histology alone as it often resembles other spindle cell tumors. Immunohistochemistry is therefore a valuable tool in confirming a diagnosis of synovial sarcoma. However, the current immunohistochemical markers used for synovial sarcoma lack sensitivity and specificity[10,23]. Gene expression studies identified *TLE1*, a gene that is consistently overexpressed in synovial sarcoma, as a potentially important biomarker[1,3,21,26]. Our previous retrospective tissue microarray analysis found that TLE1 was sensitive and specific for synovial sarcoma, supporting its potential for use as a diagnostic immunostain[32]. However, a recent whole section study from Kosemehmetoglu *et al*., found TLE1 immunoreactivity in other mesenchymal tumors, suggesting that TLE1 expression is not specific to synovial sarcoma[16].

The results of this study, the first prospective whole section study evaluating TLE1 as an immunohistochemical marker for synovial sarcoma, confirm that TLE1 immunohistochemistry is indeed sensitive and specific for synovial sarcoma in a diagnostic setting. In contrast to Kosemehmetoglu *et al*., this study found TLE1 immunohistochemistry to be highly specific for synovial sarcoma

compared to other soft tissue tumors examined, in the context where an experienced surgical pathologist is considering synovial sarcoma in the differential diagnosis. Importantly, TLE1 immunohistochemistry reliably distinguished synovial sarcoma (positive in 100%) from malignant peripheral nerve sheath tumor (positive in 2.3%), entities which can occur in a similar clinical context and have overlapping morphologic features. We also directly compared TLE1 with the other most commonly employed immunohistochemical markers for synovial sarcoma, and found TLE1 was, in fact, superior in sensitivity, specificity, positive and negative predictive value to bcl-2, EMA and cytokeratin. Although bcl-2 is also highly sensitive for synovial sarcoma, TLE1 is much more specific in this diagnostic context, and retains the highest positive predictive value (92.1%) of any immunohistochemical marker for synovial sarcoma.

One previous report suggested that immunostaining for SYT is useful in the diagnosis of synovial sarcoma[12]. That study reported 41/47 synovial sarcomas to be strongly positive for nuclear immunoreactivity, versus 13/99 soft tissue tumors of various types with mostly weaker nuclear immunoreactivity. However, it is worth noting that SYT expression is widespread in human tissues[7,33]. We tested SYT immunohistochemistry under the published conditions on our synovial sarcoma and malignant peripheral nerve sheath tumor tissue microarrays[32], and found the intensity of staining to be weak and background high, making interpretation much more difficult than TLE1. In the context of tissue microarray

cores representing the differential diagnosis of synovial sarcoma, SYT had a sensitivity of 51% and specificity of 81%. These results were so much poorer than TLE1, Bcl-2, EMA and pan-cytokeratin that we chose not to evaluate SYT in this prospective series.

Although our previous developmental work was done on tissue microarrays and the current study used whole sections, the results were very similar. This is consistent with a body of literature supporting near equivalence of results obtained with tissue microarrays versus whole sections[11,13,28,37]. An advantage of the current study, is its prospective design, meaning that TLE1 immunohistochemical studies were interpreted prior to the rendering of a final diagnosis and without knowledge of a molecular testing result. Such knowledge can potentially create a conscious or unconscious bias in the interpretation of immunohistochemistry results. The study design also ensured that previous findings were validated on a completely independent new set of cases, in the exact setting where TLE1 immunohistochemistry was thought most likely to be of diagnostic value (making it possible to calculate the clinically-relevant positive and negative predictive values).

In contrast to the focal staining pattern of EMA and cytokeratin in synovial sarcoma, TLE1 produces intense nuclear immunoreactivity in a high proportion of cells. This provides more readily interpretable results on small tissue samples such as core biopsies. With our methods, the commercially available anti-TLE1

antibody gave clear results and rarely displayed background or cytoplasmic staining. Because the sensitivity for synovial sarcoma is so high, there is no need to enhance sensitivity further with aggressive antigen retrieval, signal-amplifying detection systems, or by accepting 1+ immunoreactivity as positive. In validating our methods, we noted that using heat for antigen retrieval increased background staining in MPNST; eliminating the heat reduced background staining while maintaining robust TLE1 staining in synovial sarcoma. The methods used by Kosemehmetoglu *et al.* included heat-induced antigen retrieval and Dako Dual Envision + detection[16]. Use of such approaches likely contributed to the observed reduction in specificity TLE1, without appreciable gain in sensitivity (which approaches 100% even in the absence of such signal-enhancing techniques).

While TLE1 immunohistochemistry appears to distinguish synovial sarcoma from other entities in its differential diagnosis in the great majority of cases, we acknowledge that it is not perfectly specific. In this study, 1 of 43 malignant peripheral nerve sheath tumors, one pleomorphic sarcoma NOS and the one tested fibrosarcoma were positive. In our previous tissue microarray study, occasional positivity was also seen in schwannoma and solitary fibrous tumor/hemangiopericytoma[32]. It is highly likely that TLE1 immunostaining may be positive in some other normal and neoplastic tissues, as noted by Kosemehmetoglu *et al*[16]. However, this prospective study was limited to addressing real-world differential diagnostic situations guided by the histology

and clinical features (patient age, site, and sometimes imaging features) listed on the requisition submitted to the surgical pathology laboratory. If TLE1 were to be applied with a less rigorous approach, then its imperfect specificity could lead to an increase in false positive results, conceivably reducing the positive predictive value below our observed 92%.

Due to its extremely high negative predictive value (100%) in the examined context of nonpleomorphic spindle cell tumors, a negative TLE1 result can probably obviate the need for molecular testing unless no other alternative diagnosis can be rendered. It is possible that a subset of synovial sarcomas does not overexpress TLE1 and definitive diagnosis of such tumors will depend on molecular testing. It was confirmed that, rarely, other tumors can be positive for TLE1. If clinical or morphologic features are unusual for synovial sarcoma, molecular testing should be used to confirm the diagnosis. Reducing the need for molecular testing is of practical significance. While molecular testing is available at our institutions, immunohistochemistry requires no specialized equipment or personnel, is three to sixteen-fold lower in cost and several days faster in turnaround time than current molecular tests.

Several cases in the series illustrate the value of TLE1 immunohistochemistry, even when FISH testing for SYT gene breaks is readily available. Two cases displaying strong (3+) TLE1 staining were negative or indeterminate for SYT by FISH. This unexpected discrepancy prompted further examination with RT-PCR,

which revealed cryptic SYT-SSX2 rearrangements and confirmed the diagnosis of synovial sarcoma. Cryptic X;18 rearrangements are a rare but documented phenomenon[19,25,34] and TLE1 can clarify the diagnosis in the setting of confusing, indeterminate or unavailable molecular results. In another case, TLE1 immunoreactivity changed the diagnosis from MPNST to synovial sarcoma in a patient with a metastatic lesion and a longstanding diagnosis of MPNST. The 3+ positive TLE1 staining in this sample was considered a possible false positive result. However, the subsequent FISH was positive for SYT split-apart, verifying the change in diagnosis. A fourth case had a very poor sampling, with so few tumor cells present (in a fibrous background with chronic inflammation) that the molecular pathologist reviewing the H&E thought the biopsy would be uninterpretable by FISH and an additional diagnostic procedure would likely be required. However, the TLE1 stain not only convinced the molecular laboratory to run the test, but also guided the FISH technologist to the right cell population to score.

In conclusion, TLE1 is a sensitive and specific immunohistochemical marker for synovial sarcoma. It performs better than other currently employed immunohistochemical markers for synovial sarcoma in a prospectively-tested diagnostic context, and in some cases may preclude the need for more costly and time-consuming molecular testing.

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Figure Legends

Figure 1. Sections from a single case of poorly-differentiated synovial sarcoma, illustrating the immunohistochemical scoring of TLE1, Bcl-2, cytokeratins and EMA. Original magnification x200. A) TLE1, 3+ score, B) Bcl-2, 3+ score, C) Cytokeratin, 1+/focal staining, D) EMA, 1+/focal staining.

Figure 2. Sections from representative soft tissue tumors, illustrating the immunohistochemical scoring of TLE1 from 0 to 2+. Original magnification x200. A) monophasic synovial sarcoma, score 2+, B) MPNST, score 1+, C) mesothelioma, score=0.

Figure 3. A small population of cells shows intense TLE1 immunoreactivity in a core needle biopsy taken from a thigh mass in an 18-year-old female. TLE1 identified the proper cell population for FISH scoring, facilitating a correct diagnosis and averting the need for an open biopsy.

Tables

Table 1. Antibodies, dilutions, and conditions used for immunohistochemistry

	VANCOUVER GENERAL HOSPITAL	CLEVELAND CLINIC
TLE1	1:20 polyclonal rabbit anti-TLE1 (M-101), from Santa Cruz Biotechnology (Santa Cruz, CA) for 32 minutes with heat	1:40 polyclonal rabbit anti-TLE 1 (M-101), from Santa Cruz Biotechnology for 60 minutes with no heat
Keratin	1:200 monoclonal mouse anti-human cytokeratin (AE1/AE3), 1:1000 polyclonal rabbit anti-cow cytokeratin (wide spectrum screening) from DAKO (Glostrup, Denmark) for 32 minutes	1:200 monoclonal mouse anti-cytokeratin (AE1/AE3) from Millipore(Billerica, MA) for 12 minutes
EMA	1:200 monoclonal mouse anti-EMA antibody (E29) from DAKO for 32 minutes	1:200 monoclonal mouse anti-EMA antibody (E29) purchased from DAKO for 32 minutes
Bcl-2	1:20 dilution of monoclonal mouse anti-human Bcl-2 antibody (clone 124) from DAKO for 32 minutes	Prediluted anti-Bcl-2 antibody (clone 124) from Ventana for 32 minutes

Table 2. Summary of prospective immunohistochemistry results for soft tissue tumour specimens

FINAL DIAGNOSIS	N	TLE1		KERATIN		EMA		BCL-2	
		No. Positive	% Positive	No. Positive	% Positive	No. Positive	% Positive	No. Positive	% Positive
Synovial Sarcoma	35	35	100	24	70.6	18	64.3	30	96.8
MPNST[a,b]	43	1	2.3	7	16.6	3	7.3	11	26.2
FS-DFSP[c]	5	0	0	0	0	0	0	1	20
HPC/SFT[d]	3	0	0	0	0	1	33	2	100
Ewing Sarcoma	2	0	0	0	0	1	50	1	50
Mesothelioma	2	0	0	1	100	--[e]	--	--	--
Myxofibrosarcoma	2	0	0	1	100	0	0	1	50
Spindle cell carcinoma	2	0	0	1	100	0	0	0	0
Spindle cell sarcoma NOS[f]	2	0	0	0	0	1	100	--	--
Fibromatosis	1	0	0	--	--	--	--	--	--
Fibrosarcoma	1	1	100	0	0	0	0	0	0
High grade sarcoma with epithelioid features	1	0	0	0	0	0	0	--	--
Low grade myofibroblastic sarcoma	1	0	0	0	0	0	0	0	0
MFH[g]	1	0	0	0	0	0	0	0	0
Nodular fasciitis	1	0	0	0	0	0	0	0	0
Pleomorphic sarcoma NOS	1	1	100	0	0	0	0	1	100
Poorly diff malignant epithelioid neoplasm	1	0	0	0	0	0	0	--	--
Rhabdomyosarcoma	1	0	0	0	0	0	0	--	--
Schwannoma	1	0	0	0	0	0	0	1	100
Small blue round cell tumour, consistent with ARMS[h]	1	0	0	0	0	0	0	1	100
Small blue round cell tumour, consistent with mesenchymal chondrosarcoma	1	0	0	0	0	0	0	--	--

[a]MPNST indicates Malignant Peripheral Nerve Sheath Tumour.
[b]Included one case of glandular MPNST, negative for TLE and bcl-2, but positive for cytokeratin and EMA.
[c]FS-DFSP indicates Dermatofibrosarcoma protuberans with fibrosarcomatous change
[d]HPC-SFT indicates Hemangiopericytoma/Solitary Fibrous Tumour
[e]--indicates test was not performed
[f]NOS indicates Not Otherwise Specified
[g]MFH indicates Malignant Fibrous Histiocytoma
[h]ARMS indicates Alveolar Rhabdomyosarcoma

Table 3. Detailed Summary of Immunohistochemistry results for Synovial Sarcoma

SUBTYPE	SCORE	TLE1	KERATIN	EMA	BCL-2	SYT-FISH
Monophasic N=30	0	-	10	9	-	28 Positive 1 Indeterminate* 1 Negative*
	1+	-	16	8	1	
	2+	4	3	7	4	
	3+	26	-	-	22	
Biphasic N=1	0	-	-	-	-	1/1 Positive
	1+	-	-	-	-	
	2+	-	1	1	-	
	3+	1	-	-	1	
Poorly-differentiated N=4	0	-	-	1	-	4 Positive
	1+	-	4	1	-	
	2+	-	-	1	-	
	3+	4	-	-	3	

* RT-PCR in these two cases was positive for the presence of an *SYT-SSX* fusion transcript

Table 4. Sensitivity, specificity, positive and negative predictive value for TLE1 in comparison to other immunohistochemical stains for the diagnosis of synovial sarcoma

IHC	SENSITIVITY	SPECIFICITY	POS PREDICTIVE VALUE	NEG PREDICTIVE VALUE
TLE1	100	96	92	100
Bcl2	97	71	63	98
EMA	64	91	75	86
Keratin	71	85	71	85

Figure 1

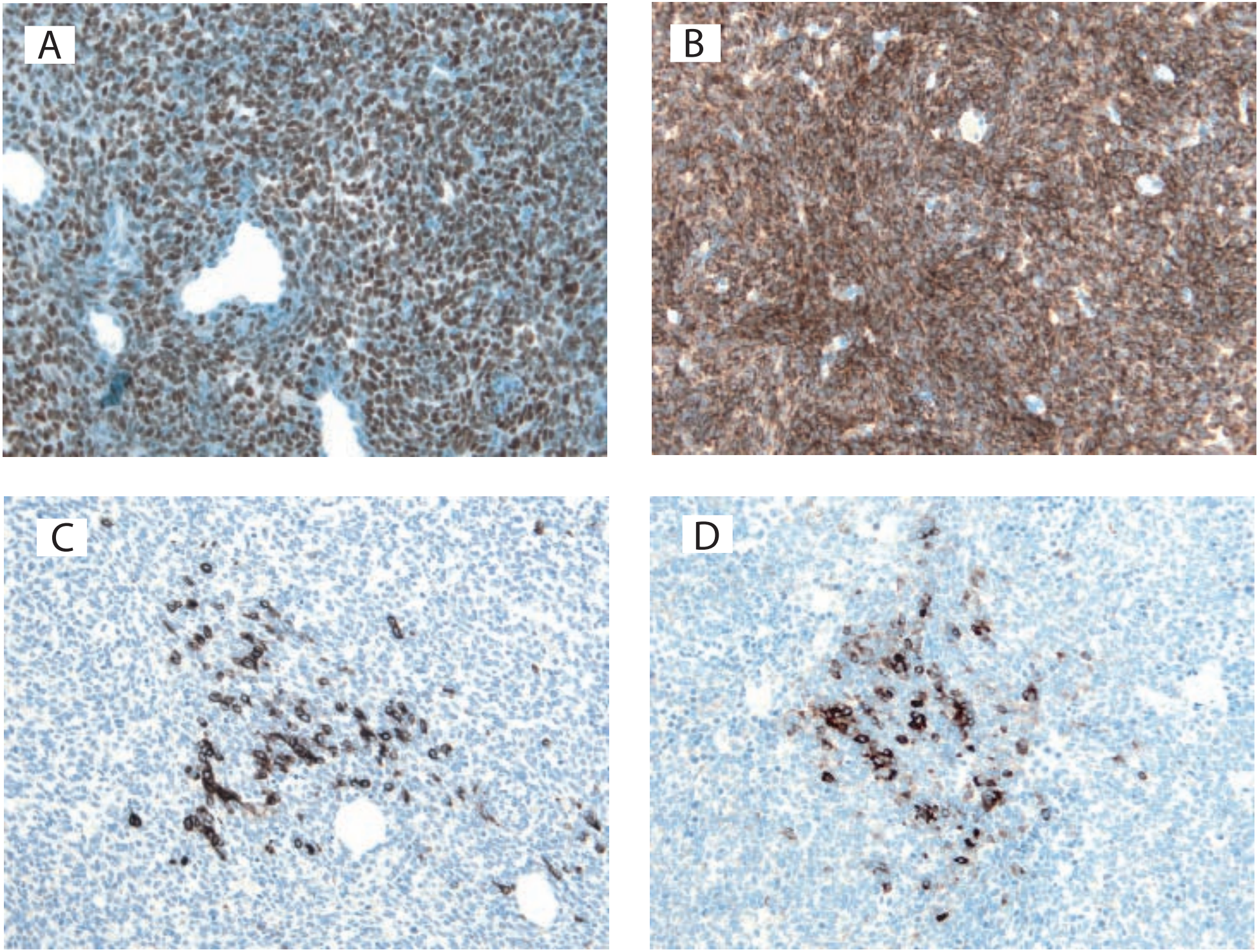

Figure 2.

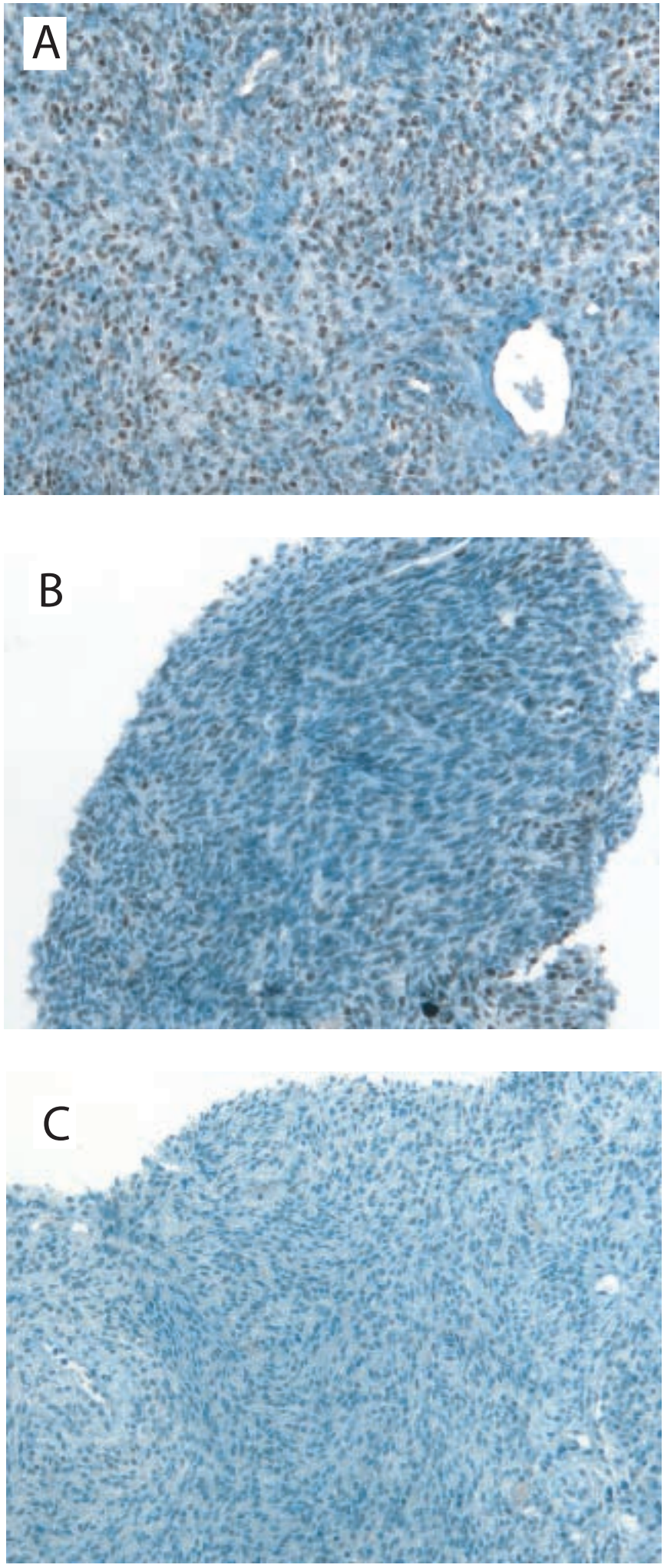

Figure 3