Original Article

Synovial Sarcoma: A Comparative Study of Immunohistochemical Markers (Bcl2 and CD99) versus Fluorescence In Situ Hybridization

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DOI: 10.21276/APALM.3340

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Submitted: 30-Mar-2024
Final Revision: 18-May-2024
Acceptance: 20-May-2024
Publication: 01-June-2024

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Abstract

Background

Synovial Sarcoma (SS) is a mesenchymal tumor, which displays a variable degree of epithelial differentiation including gland formation, and has a specific chromosomal translocation t(x;18)(p11;q11) that leads to the formation of an SS18-SSX fusion gene. The diagnostic gold standard for Synovial Sarcoma is the demonstration of the characteristic translocation between the SS18 (SYT) gene on chromosome 18 and one of the three SSX genes (SSX1, SSX2, or rarely SSX4) on chromosome X {t(x;18)(p11.2;q11.2)} [1, 2]. The use of these techniques is limited by many practical issues like cost and specialized equipment availability. Thus, in practice, the diagnosis of SS is usually based on histological examination and immunohistochemistry (IHC) [3, 4]. Our objective is to study the expression of Bcl2 and CD99 in Synovial Sarcoma and to compare the results with fluorescence in situ hybridization (FISH) to evaluate the sensitivity of Bcl2 and CD99 in the diagnosis of Synovial Sarcoma.

Material and Methods

Tissue was processed and microtomy was done. Hematoxylin and eosin (H&E) staining was performed. IHC was carried out on all sections using CD99, Bcl2, EMA, CK, and all other markers which aid in differentiating SS from its mimics. All the formalin-fixed paraffin-embedded (FFPE) tissue samples from 50 histologically diagnosed Synovial Sarcoma cases, which showed Bcl2 and CD99 positivity, were submitted for FISH..

Results

Forty cases out of fifty histologically and Bcl2 & CD99 positive Synovial Sarcoma cases were positive for FISH, showing the t(x;18)(p11.2;q11.2) translocation.

Conclusion

Bcl2, CD99, Vimentin, EMA, and PCK are routinely used markers in the diagnosis of Synovial Sarcoma. It is found that Bcl2 and CD99 are highly sensitive markers in the initial diagnosis and management of Synovial Sarcoma in patients who cannot afford cytogenetic studies.

Keywords:

Synovial Sarcoma, Immunohistochemistry, Bcl2, CD99, Fluorescent in situ hybridization

Introduction

Synovial Sarcoma (SS) is a mesenchymal tumor that displays a variable degree of epithelial differentiation, including gland formation, and has a specific chromosomal translocation t(x;18)(p11;q11) that leads to the formation of an SS18-SSX fusion gene. It is the fourth most common high-grade soft tissue sarcoma, after Malignant Fibrous Histiocytoma (MFH), Leiomyosarcoma, and Rhabdomyosarcoma (RMS). It accounts for 5-10% of all soft tissue sarcomas and is accompanied by an often poor prognosis with a high chance of metastasis despite surgical resection. Local control requires wide local excision and radiation therapy, causing significant morbidity in relatively young patients.

Synovial Sarcoma is most prevalent in adolescents and young adults aged between 15-40 years. Males are more often affected than females, with an M:F ratio of 2:1.

Synovial Sarcoma is a well-characterized malignant soft tissue sarcoma that often occurs in close proximity to large joints of the extremities. SS is known to occur at various other sites, such as the head and neck, thorax, and abdomen. Although the name arises owing to its resemblance to synovium, SS is thought to originate from primitive mesenchymal cells (uncommitted) that undergo differentiation to resemble synovial cells.

Three main histological types are described: a Classic biphasic type consisting of spindle cells and epithelial cells (forming glandular structures), a Monophasic type made up of only spindle cells or epithelial cells, and a Poorly Differentiated type consisting of cells that resemble those of small round blue cell tumors. Although Biphasic SS was the first variant to be recognized, Monophasic SS is much more common.

The diagnostic gold standard for Synovial Sarcoma is the demonstration of the characteristic translocation between the SS18 (SYT) gene on chromosome 18 and one of the three SSX genes (SSX1, SSX2, or rarely SSX4) on chromosome X {t(x;18)(p11.2;q11.2)} [1,2]. Detection of t(x;18) can be accomplished by cytogenetic karyotyping, fluorescent in situ hybridization (FISH), or reverse-transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC). The use of these techniques is limited by many practical issues like cost, specialized equipment, and availability. Thus, in practice, the diagnosis of SS is usually based on histological pathological examination (HPE) and immunohistochemistry [3][4].

Immunohistochemical markers like cytokeratin, Epithelial Membrane Antigen (EMA), Bcl-2, CD99, and TLE1 are used to identify Synovial Sarcoma. Many attempts have been made to identify highly specific and sensitive markers in the diagnosis of SS [5][6].

The diagnosis of SS may pose a considerable challenge to pathologists owing to the lack of an epithelial component. The chromosomal translocation t(x;18) was first reported in SS by Limon et al., and subsequently reported in several cases of SS as a specific chromosomal abnormality by karyotypic analysis. In 1993, Lee et al. reported the identification of translocation t(x;18) in SS by FISH. FISH is a cytogenetic method using fluorescent probes that bind to parts of chromosomes showing a high degree of sequence complementarity. It was developed by biomedical researchers in the 1980s and can be used to detect and localize the presence or absence of specific DNA sequences.

The gold standard for the diagnosis of SS is the demonstration of SS18-SSX gene translocation through FISH. However, the use of this technique is limited by many practical issues like cost, specialized technicians, and specialized equipment, which is practically not possible in most hospital settings. Thus, in practice, the diagnosis of SS is usually based on HPE and IHC. The aim of this study is to identify specific and sensitive IHC markers in the diagnosis of SS so that patients who cannot afford the FISH

technique can be diagnosed with HPE and IHC accurately.

Materials and Methods

It is a prospective study conducted for a period of two years. Due importance was given to record a brief clinical history with age, in-patient registration number, biopsy number, presenting symptoms and signs, computed tomography (CT) and magnetic resonance imaging (MRI) findings. Trucut biopsy or core needle biopsy of the growth was done, followed by wide excision.

The specimens were received in 10% formalin. Measurements of the specimens were recorded. Thorough gross examination was carried out and salient features like haemorrhage, necrosis, calcification, and cyst formation were recorded (Figure 1).

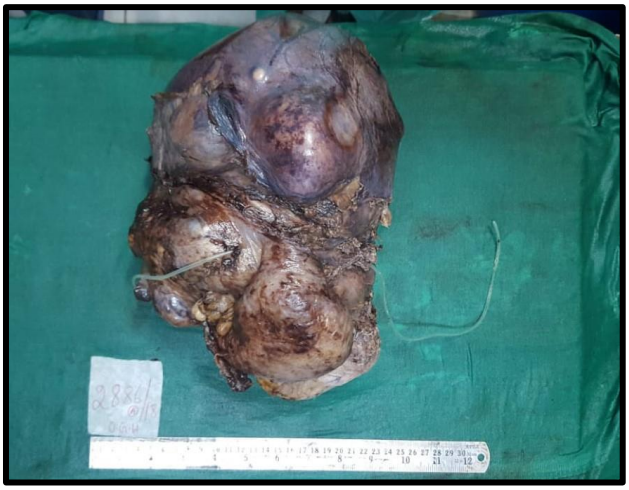

Figure 1: Macroscopic image of Synovial Sarcoma

Parallel transverse sections were given through each half, about 1 cm apart. Depending on the size of the tumour, an adequate number of sections were given. Tissue was processed and microtomy done. H&E staining was done. IHC was carried out on all sections using CD99, Bcl2, EMA, CK, and all other markers which aid in differentiating SS from its mimics, using positive and negative controls.

Inclusion Criteria: This study is approved by the Institutional Ethics Committee. The study includes 50 cases of Synovial Sarcoma. The original H&E and IHC slides of all the cases were reviewed to confirm the diagnosis.

Fluorescent In Situ Hybridisation: All the formalin-fixed paraffin-embedded tissue samples from 50 histologically diagnosed Synovial Sarcoma which showed Bcl2 and CD99 positivity were submitted for FISH. The original H&E slides of the tumour were reviewed, and representative areas were marked.

FISH Procedure: Probe: The probe used is a commercially available Vysis LS1 SS18 Dual Color Break Apart Probe. It has two probes. The first probe - a 650 KB probe labelled in spectrum orange - extends distally from the SS18 gene. The second probe - green - lies 3’ or proximal to the SS18 gene and is ~1040 KB in length.

The FISH analysis was performed on 3 microns thick tissue sections, which are initially deparaffinized in xylene (3x30 min) at 45 degrees Celsius. Then dehydration of the sections was done in 100% fresh ethanol (3x5 min). Pre-treatment of the tissue was done by using HCL for 20-30 minutes and 1M Sodium Isothiocyanate for 20-30 minutes. The tissue sections were then subjected to protein digestion using Pepsin at 37 degrees Celsius for 15 minutes, followed by neutralisation with formaldehyde for 5-10

minutes and serial dehydration in 70%, 85%, and 100% alcohol (5 min each) and air dried at room temperature.

Now, 2-3 microlitres of probe are added to each section and sealed with rubber cement and slides were kept in a Thermobrite and programmed to denature the tissue section at 78 degrees Celsius for 5 minutes followed by hybridisation at 37 degrees Celsius overnight. Post-hybridisation, tissue is washed with 0.3% NP40 at 73 degrees Celsius for 1 minute and air dried at room temperature. 4',6-diamidino-2-phenylindole is added to the sections and a cover slip placed over it and sealed properly with nail polish.

Interpretation of Results: The sections were viewed under a fluorescent microscope. A minimum of 50 cells were examined. Green and orange signal fusion is considered normal and a split signal pattern is considered positive for gene rearrangements. Cases were considered positive for gene rearrangement when 15% of the cells exhibit a split signal pattern.

Results

Out of a total of 50 cases of synovial sarcoma, 33 were male and 17 were female. Most of the cases were among the age group of 20-50 years, although the age distribution is between 12-70 years, and the median age of presentation is 35 years (Table 1).

Table 1: Age wise distribution of Synovial sarcoma cases

S. NO.	Age group in years	No. Of cases (n=50)	Percentage
1.	12 - 20 years	09	18%
2.	20 - 50 years	34	68%
3.	> 50 years	07	14%

Out of 50 cases of synovial sarcoma, 32 cases (64%) presented in the lower limb, 11 cases (22%) presented in the upper limb, 2 cases were noted in the mediastinum, and one case each was noted in the axillary, inguinal, gluteal, scapular regions, and one in the retroperitoneum (Table 2).

Table 2: Site of involvement of synovial sarcoma

S.NO.	Site of involvement	No. Of cases (n)	Percentage
1.	Lower Limb	32	64%
2.	Upper Limb	11	22%
3.	Mediastinum	02	4%
4.	Inguinal Region	01	2%
5.	Gluteal Region	01	2%
6.	Axillary Region	01	2%
7.	Scapular Region	01	2%
8.	Retroperitoneum	01	2%

Most of the cases presented as painless swelling in the limbs. Limping was the presenting feature in 8 cases. Joint effusion was seen in 7 cases. Pain was seen in a few cases. Pressure symptoms like numbness were noted in a few cases.

Out of 50 cases of synovial sarcoma, 34 cases (68%) were monophasic type (Figure 2). Some of the monophasic type also showed squamous differentiation (Figure 3). Four cases (8%) were biphasic type (Figure 4), and 12 cases (24%) were poorly differentiated type (Figure 5) (Table 3).

Table 3: Histological types of Synovial sarcoma

Histological Type	No. Of Cases (Total - 50)	Percentage (%)
Monophasic	34	68%
Biphasic	04	8%
Poorly Differentiated	12	24%

Table 4: Summary of Basic Immunohistochemical Panel of Synovial Sarcoma

IHC Marker	No. Of Cases Done	No. Of Cases Positive	Percentage (%)
BCL 2	50	46	92%
CD99	50	46	92%
Vimentin	29	28	97%
EMA	38	28	74%
PCK	07	02	28%
SMA	08	00	0%
Desmin	07	00	0%
S100	07	00	0%
CD34	05	00	0%

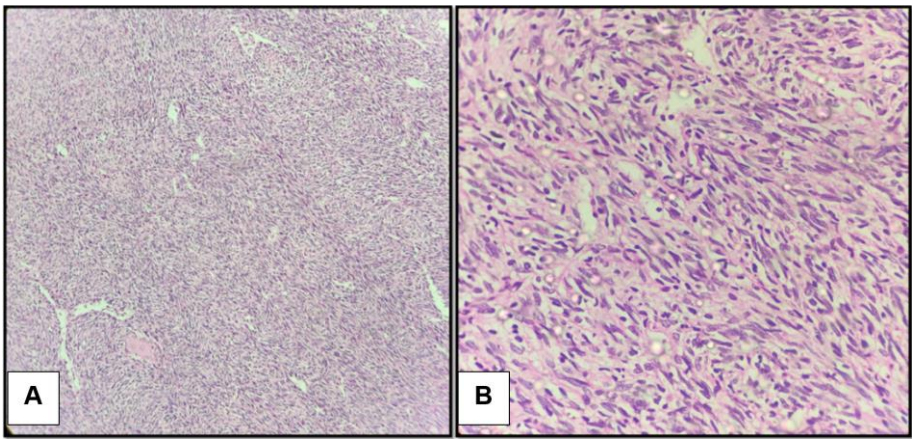

Figure 2: H&E Stained Sections of Monophasic SS A) scanner view B) low power

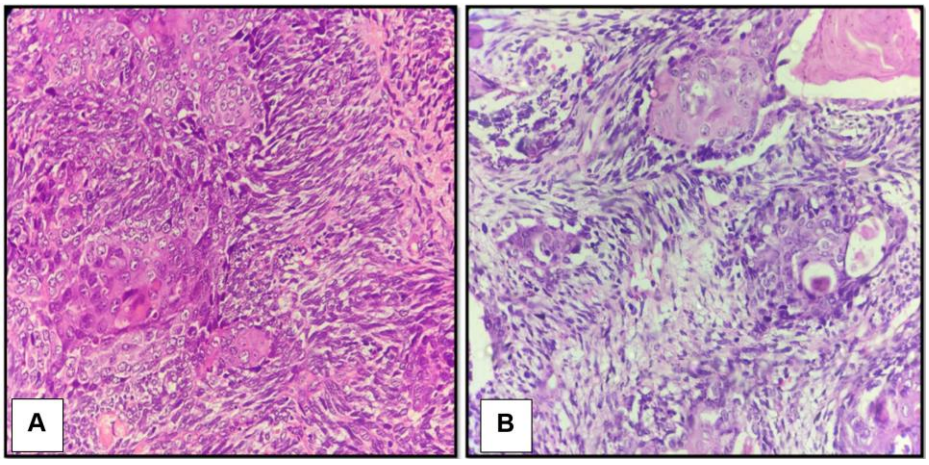

Figure 3: Monophasic SS with squamous differentiation A and B – H&E stain

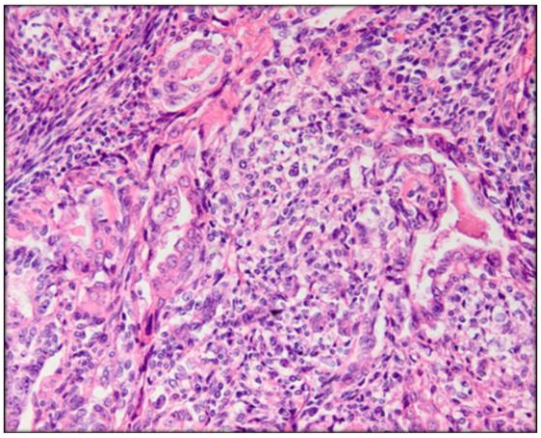

Figure 4: H&E Stained Sections of Biphasic SS low power view

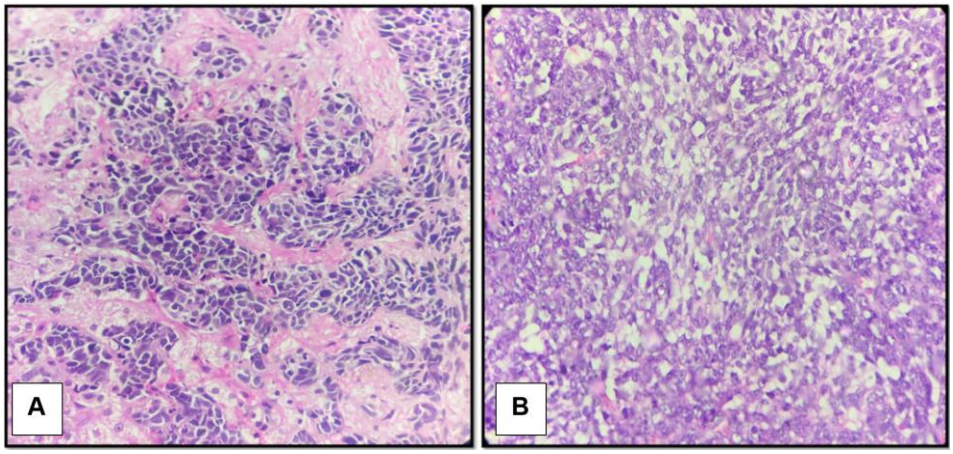

Figure 5: Poorly Differentiated SS A) Low power B) High power – H&E stain

Interpretation of FISH Results: In normal cells, a two-fusion signal pattern will be observed, reflecting the two intact copies of the SS18 gene. In an abnormal cell with t(18q11.2), one fusion, one orange, and one green signal will be expected. Abnormal cells are positive for FISH (Figure 7). Normal cells are negative for FISH (Figure 8)

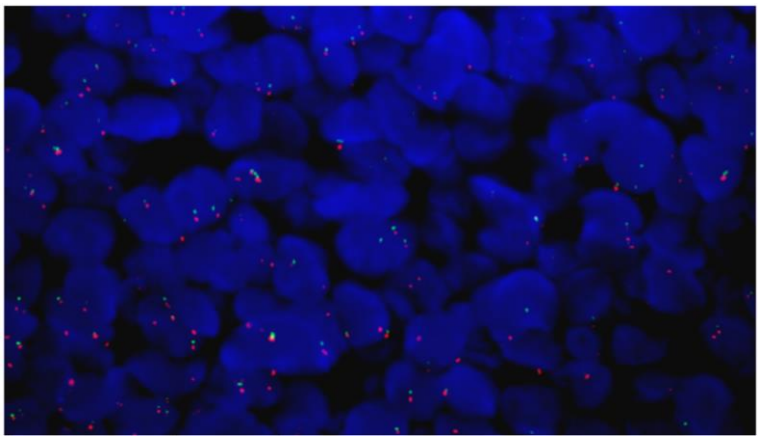

Figure 6: Synovial sarcoma showing positive FISH results

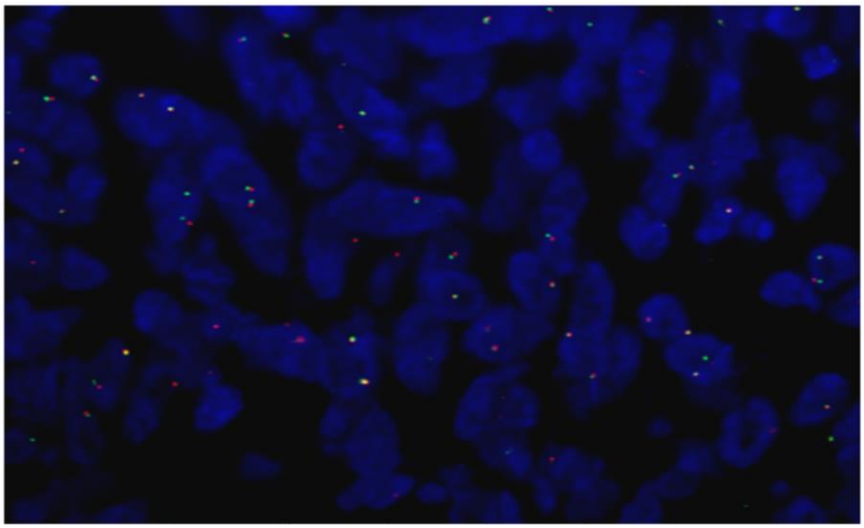

Figure 7: Synovial sarcoma showing negative FISH results

No result means no signals are seen on the microscopy. This may be due to errors in fixation and processing of tissue or old tissue.

This shows that 80% of cases are positive for FISH, 12% negative, and 8% of cases showed no signals (Table 4).

Table 5: Results of FISH in synovial sarcoma

Synovial Sarcoma		No. of Cases Positive	No. of Cases Negative	No. of Cases with No Result
Monophasic	34	30	02	02
Biphasic	04	04	00	00
Poorly Differentiated	12	06	04	02
Total	50	40	06	04

Discussion

Synovial sarcoma is a distinct soft tissue tumor that shows evidence of both mesenchymal and epithelial differentiation at the light microscopic, immunohistochemical, or ultrastructural level. It has become increasingly important to make a definitive diagnosis of synovial sarcoma because more effective chemotherapeutic agents, such as Ifosfamide, have become available to treat patients with these tumors.

Synovial sarcoma is most prevalent in adolescents and young adults between 15 and 40 years of age. In this study, we found that the majority of patients were between the ages of 20 and 40 years. Males were more commonly affected than females. Our patients presented at a relatively young age compared to Western studies. The most common site is the lower extremity, followed by the upper extremity, which is in concordance with several other studies. However, inguinal, retroperitoneal, axillary, gluteal, scapular region, and mediastinal locations were also seen in a few patients.

The diagnosis of biphasic synovial sarcoma poses no difficulty as it exhibits both spindle and glandular components. However, monophasic fibrous and epithelial types can be easily confused. Monophasic SS can be difficult to distinguish from its histological mimics, which include other spindle cell sarcomas such as MPNST, cellular schwannoma, solitary fibrous tumor (SFT), fibrosarcoma, and leiomyosarcoma. Poorly differentiated SS closely mimics other round cell tumors, which include Ewing's sarcoma/PNET, myxoid chondrosarcoma, and various other tumors with predominant round cell and undifferentiated morphology.

The specific diagnosis and subtype of SS is further complicated in small biopsy samples in which epithelial elements may be sparse or may not be sampled. Hence, histomorphology should be coupled with a carefully selected IHC panel and cytogenetics to arrive at a specific diagnosis and to rule out histological mimics.

Monophasic histology was the most common type (66% of cases) in our study, followed by poorly differentiated (24% of cases), and the rest were biphasic (10% of cases). Deepti B et al. [9] found more patients with monophasic histology (83.3% of cases), which is similar to our study (Table 5).

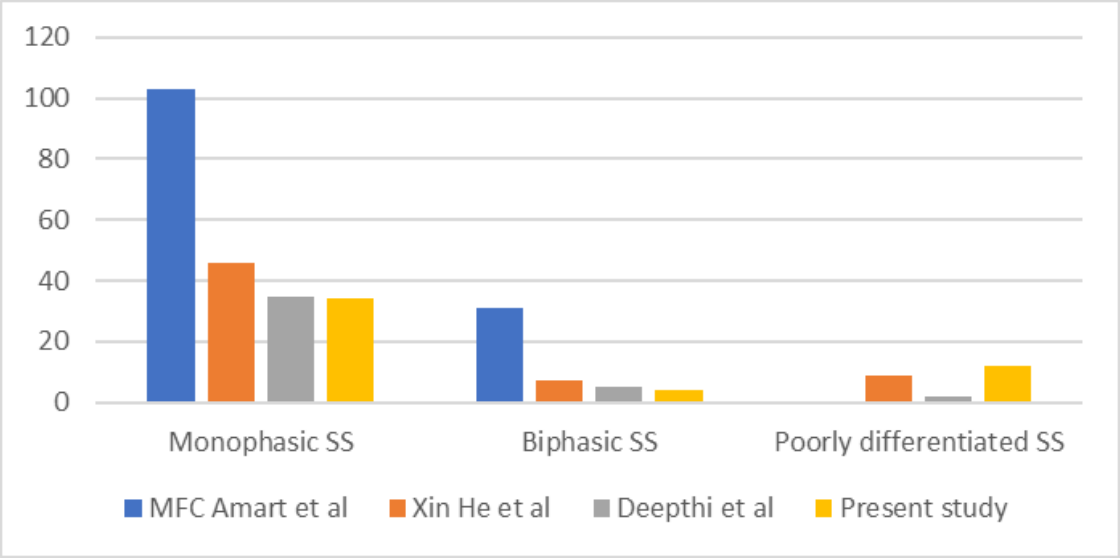

Figure 8: Comparison of histological types of SS with other studies

Though there are an array of markers for SS, no single marker is considered specific for its diagnosis. Therefore, a combination of markers that can delineate mesenchymal and epithelial differentiation are used. In this study, the most sensitive IHC markers are BCL2, CD99, and vimentin, which are positive in all the cases performed. EMA is more sensitive (74% of cases) in staining the focal tiny clusters compared to PCK (28% of cases) (Table 6).

Cytogenetic studies have revealed that most cases of SS contain a chromosomal translocation t(x;18)(p11.2;q11.2) [10]. This translocation is highly specific to SS because about 70% to 90% of all SS have been reported to show this translocation in the literature. Our study also showed 80% of cases positive for the same translocation (Table 7).

Sensitivity of Bcl2 & CD99 in the diagnosis of SS : 82.6%, specificity of Bcl2 & CD99 in the diagnosis of SS : 50%, Positive predictive value: 95%, Negative predictive value: 80%, Accuracy:80%, P value: Not significant (>0.05)

Table 6: Comparison of expression of basic IHC markers in SS

IHC Marker	MFC Amary et al (2007) [4]	Ten Heuvel SE et al (2009) [7]	Xin He et al (2016) [8]	PRESENT STUDY
Bcl2	98%	62%	85%	92%
CD99	56%	-	24%	92%
Vimentin	-	8%	-	97%
EMA	98%	12%	87%	74%
PCK	80%	20%	35%	28%
S100	22%	8%	-	-

Table 7: Comparison of FISH results of present study with few other studies

Studies	Total No. Of Cases	No. Of cases FISH Positive	Percentage (%)
MFC Amary et al., (2007) [4]	101	87	86%
Ten Heuvel SE et al., (2009) [7]	50	41	82%
Xin He et al ., (2016)[8]	65	62	95%
PRESENT STUDY	50	40	80%

Limitations: To increase the sensitivity and specificity of these markers, this study should be carried out in a larger population. Due to financial constraints, we limited our study to only 50 cases as there was no financial support from any source. More studies should be carried out in Indian settings as we are seeing an increase in the number of cases.

Conclusion

The diagnosis of Synovial Sarcoma is based on clinical, radiological, histopathological, and immunohistochemical features. All spindle cell lesions should be kept in mind as differential diagnoses while diagnosing Monophasic Spindle Cell Synovial Sarcoma. All small round blue cell tumors should be kept in mind while diagnosing poorly differentiated Synovial Sarcoma. Bcl-2, CD99, vimentin, EMA, and PCK are routinely used markers in the diagnosis of Synovial Sarcoma. It is found that Bcl-2 and CD99 are highly sensitive markers in the initial diagnosis and management of Synovial Sarcoma in patients who cannot afford cytogenetic studies. SYT gene rearrangement through FISH is specific and the gold standard in the diagnosis of Synovial Sarcoma. However, FISH-negative results do not entirely exclude the presence of SS18 rearrangement as some translocations are cryptic. FISH-negative results do not entirely exclude the diagnosis of Synovial Sarcoma. FISH-negative cases should be thoroughly evaluated with history, and other considered differential diagnoses should be ruled out.

There are always financial and technical constraints in using several IHC markers and further confirmation with FISH studies in Indian settings. The search for limited sensitive markers for Synovial Sarcoma is on the rise, and a few such markers are Bcl-2 and CD99. The present study highlights their utility as fairly sensitive compared to other markers.

Funding: *None*

Competing Interests: *There are no conflicts of interest in this study.*

Acknowledgements: *None*

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