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SS18-SSX Rearrangement in Synovial Sarcoma Using Fluorescent in Situ Hybridization: A Report from an Iranian Population and Literature Review

Akbar Safaei, Elham Jansar, Ahmad Monabati, Maral Mokhtari, Mehdi Montazer♦

Department of Pathology, Shiraz University of Medical Sciences, Shiraz, Iran

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Abstract

Background: Synovial sarcoma is an aggressive soft tissue sarcoma. It has a wide spectrum of histopathologic patterns and uncertain immunohistochemistry, rendering it a diagnostic challenge. The objective of this study was to investigate the diagnostic utility and impact of SS18-SSX rearrangement evaluation in an Iranian population previously diagnosed with synovial sarcoma without such molecular tests.

Method: We conducted this cross-sectional study on 44 formalin-fixed paraffin-embedded tissue blocks obtained from 23 synovial sarcoma patients (males, 69%; mean age, 36.4 years) and 11 cases with other neoplasms as negative controls. We assessed these specimens for SSX-SS18 gene rearrangement by break-apart fluorescent in situ hybridization (FISH) probes.

Results: FISH study showed SS18-SSX fusion gene in 17 (73.91%) cases while six (26.09%) cases and negative controls did not show SS18-SSX fusion. Histopathologic type of tumor was significantly related to the presence of rearrangement (P=0.002) (rearranged: 11 biphasic and six monophasic tumors; non-rearranged: three monophasic and three poorly-differentiated tumors).

Conclusion: This study supports the idea that molecular studies contribute to the confirmation of synovial sarcoma diagnosis, particularly in monophasic and poorly-differentiated subtypes, which show vague immunohistochemical results.

Keywords: Synovial sarcoma, Gene fusion, Fluorescent in situ hybridization, SS18-SSX1 fusion protein

♦**Corresponding Author:**
Mehdi Montazer, MD
Department of Pathology, Faculty of Medicine, Setad Sq., Zand St, Shiraz, Iran
Tel/Fax: +98-71-32301784
Email: mehdi.montazer@gmail.com

Introduction

Synovial sarcoma is an aggressive malignant soft tissue tumor of uncertain type accounting for 5-10% of all soft tissue sarcomas.[1,2] Synovial sarcoma is most prevalent in adolescents and young adults aged 15-40 years. This condition impacts males slightly more than females (ratio, 1.2:1) and mainly occurs in the extremities. However, it has been reported in all ages and a wide variety

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of locations. Moreover, synovial sarcoma shows a broad spectrum of histological patterns ranging from the more easily recognizable biphasic type with distinct epithelial and spindle cell parts to the challenging monophasic and poorly-differentiated types.[1, 2] These facts have made synovial sarcoma a usual suspect existing in the differential diagnosis list for almost all soft tissue sarcomas.

Immunohistochemistry, especially the finding of epithelial markers, CD99, and Bcl2 has substantially helped the diagnose of synovial sarcomas; however, it was the identification of reciprocal balanced X;18 translocations (p11.23;q11.2) and the resulting SS18-SSX fusion genes that revolutionized the synovial sarcoma world and opened new horizons into its pathogenesis and diagnosis. This rearrangement can be detected by fluorescent in situ hybridization (FISH) and reverse transcriptase polymerase chain reaction (RT-PCR). The former method is not only simpler to perform but also more practicable and reproducible because the latter requires RNA extraction from formalin fixed paraffin embedded tissue material; this is by itself a challenge in molecular diagnostics laboratory.[1-3]

The function of the SS18-SSX fusion protein is yet to be fully elucidated; however, there are two common break points on Xp11 corresponding to SSX1 (two-thirds of cases) and SSX2 (one-third of cases) genes. These breakpoints join the SS18 gene on 18q11. Interestingly, the SSX1 and SSX2 breakpoints and their representing fusion gene have been associated with biphasic and monophasic synovial sarcomas, respectively. Infrequently, the SSX4 or other more rare breakpoints, also located on Xp11, are involved.[1-2]

We investigated the presence of SS18-SSX rearrangement by FISH method in patients with a prior diagnosis of synovial sarcoma in the referral centers of southern Iran. To our knowledge and based on the Medline and Scopus databases, this is the first report from an Iranian population.

Materials and Methods

Subjects and design

We conducted this cross-sectional study on 23 patients diagnosed with synovial sarcoma and who had undergone resection operation in the main hospitals of the Shiraz University of Medical Sciences (Nemazee, Faghihi, Chamran, Khalili and Madar-va-Kodak) between 2007 and 2016. Besides histomorphology, 16 cases also had immunohistochemistry. We reviewed all archived slides, confirmed the diagnosis based on World Health Organization (WHO) classification, graded the tumors according to French National Federation of Cancer Center (FNLCC), and gathered the relevant histopathologic data. Eleven samples of other types of soft tissue neoplasms were further included in the study as the negative control group. These samples comprised two benign (one neurofibroma, one elastofibroma) and nine borderline or malignant (three malignant mesotheliomas, two leiomyosarcomas, two dermatofibrosarcoma protuberans, one malignant peripheral nerve sheath tumor, and one myxoid fibrosarcoma) cases.

The study was directed in accordance with the declaration of Helsinki and its following revisions and approved by the Vice-Chancellery for Research Affairs of Shiraz University of Medical Sciences (No. 8779-01-01-93).

FISH procedure

We performed FISH on tissue microarrays containing corresponding tissues from all subjects and controls using the Zytovision dual color break apart rearrangement probe (SS18) (Product No. Z-2097, Zytoision company, Germany) according to the manufacturer's instructions.

Briefly, 4-µm thick sections were deparaffinized by placing in a 70°C oven and rinsing in two containers of xylene (10 min for each container). Subsequent hydration was done in consecutive baths of 100%, 100%, 90%, and 70% ethanol, each for 5 min. Pretreatment and proteolysis were carried out by boiling the slides for 15 min in prewarmed heat pretreatment citric solution at 98°C. This was followed by the subsequent incubation of tissue sections with pepsin solution for 10 min at 37°C in a humidity chamber. After dehydration of tissue sections by ethanol series of 70%, 90%, and 100% degrees (1 min for each alcohol step), 10 µl SS18 probe

was applied, and the slides were denatured at 75°C for 10 min on a hot plate. Next, the slides were transferred to a humidity chamber and hybridized overnight at 37°C. The following day, after hydration in graded alcohol, 30 µl of DAPI/antifade-solution was administered onto the slides and incubated in the dark until evaluation.

Interpretation of FISH

SS18 probe mixture contained two probes hybridizing to the distal and proximal of the breakpoint on the 18q11.2 band. These probes were labeled by orange and green fluorochromes, respectively. Normal cells and those tumoral cells which lacked a translocation involving the 18q11.2 band were expected to show two green/yellow/orange fusion signals. On the other hand, interphases harboring a translocation showed one orange/yellow/green fusion signal, one separate green signal, and one separate orange signal. It was expected that the distal probe hybridized to the X chromosome in rearranged cells, such that its corresponding orange signal separated from the green one which stays on the 18 chromosome.

Chromogenic in situ hybridization (CISH) procedure

We performed dual-color break-apart CISH for detecting SSX-SS18 rearrangement; however, it did not lead to interpretable results.

Statistical analysis

Statistical analyses were performed by the Statistical Package for the Social Sciences software version 16.0 (SPSS Inc., Chicago, IL, USA). Group comparisons of categorical variables were analyzed using the Pearson's chi-square test. All tests were two-sided, and *P* values <0.05 were considered to be statistically significant.

Results

14 (69.9%) male and nine (39.1%) female synovial sarcoma patients participated in this study. The mean age (±SD) at diagnosis was 36.4 (±15.08), ranging from 18 to 65 years. Table 1 summarizes the tumor characteristics. In addition, hyalinization, calcification, and prominent mast cell infiltration were present in two (8.7%), eight (34.7%), and five (21.7%) tumors, respectively. Figure 1 shows the different histological patterns of synovial sarcoma in our patients.

Immunohistochemistry

Table 2 summarizes the immunohistochemical

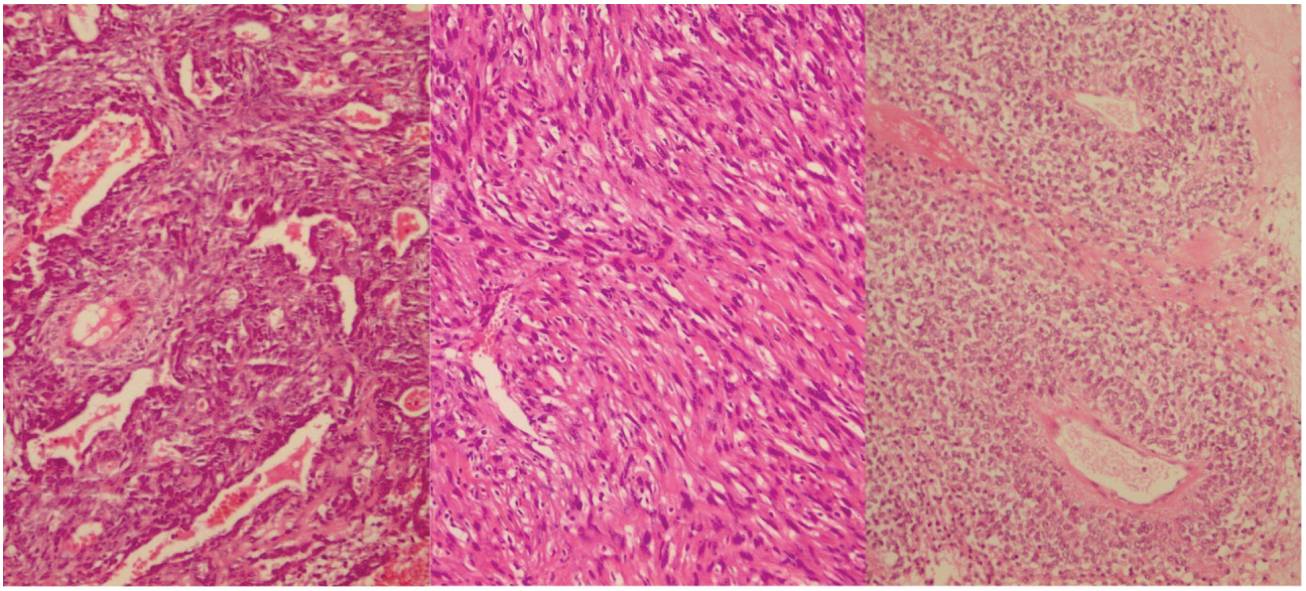

Figure 1. The images show different histological patterns of synovial sarcoma in our patients. Biphasic (left), monophasic (middle), and poorly differentiated (right) (H & E staining, 100×).

Table 1. Summary of tumor characteristics

		Number	Frequency (%)
Tumor Type			
	Biphasic	11	47.8
	Monophasic	9	39.1
	Poorly-differentiated	3	13.0
Tumor Behavior			
	Localized	10	43.5
	Recurrent/Metastatic	13	56.5
Site			
	Lower extremity	11	47.8
	Internal organs	4	17.4
	Head and neck	3	13.0
	Upper extremity	3	13.0
	Chest wall and axillae	2	8.7
Size			
	<5 cm	8	34.8
	≥5 cm	15	65.2
Mitotic activity			
	Score 1 (0-9 per 10 HPF)	3	13.0
	Score 2 (10-19 per 10 HPF)	9	39.1
	Score 3 (≥20 per 10 HPF)	11	47.8
Necrosis			
	Score 0 (no necrosis)	11	47.8
	Score 1 (<50% tumor necrosis)	12	52.2
	Score 2 (≥50% tumor necrosis)	0	0.0
FNCLCC Grading			
	II	16	69.6
	III	7	30.4

characteristics of tumor cells, which were available in medical records for 16 patients. Epithelial markers (cytokeratin and/or EMA) were expressed in about 80% of tumors. CD99, Bcl-2, and S100 were also reactive in 86.7%, 100%, and 37.5%, respectively. All cases revealed high proliferation activity with a mean ki67 index of 35% (range, 30%-80%). Moreover, one case presented focal desmin staining.

FISH results

All cases had bright and interpretable signals. 17 (74%) cases revealed SS18 rearrangement, but the remaining six (26%) cases showed intact signals, thereby failing to show SS18 rearrangement in FISH study. All control specimens were also intact. Most positive specimens showed the expected signal pattern (one orange/yellow/green fusion signal, and two separate green and orange signals); however, one case revealed three separate green and three separate orange signals suggestive of polyploidy; another case showed an unexpected signal pattern consisting of an orange/yellow/green fusion signal and an isolated orange one (Figure 2).

Among the SS18-SSX rearranged cases, 11 (64.7%) were males and six (35.3%) were females. We collected nine (52.94%) specimens from primary tumor sites, seven (41.18%) from the recurrence sites, and one (5.88%) from the metastatic site. 13 (76.47%) and four (23.53%) tumors were grade II and grade III, respectively. These factors, along with size, mitotic activity, and necrosis, were not statistically different from those of tumors without rearrangement. On the other hand, tumor type was significantly associated with the presence of rearrangement (Chi2 test, P=0.002) (11 biphasic and six monophasic tumors in rearranged group versus three monophasic and three poorly differentiated tumors in non-

Table 2. An overview of all cases with the emphasis on the immunohistochemical characteristics of tumor cells

Case	Type	Epithelial markers (CK and/or EMA)	CD99	Bcl2	CD34	Muscle markers (SMA/Desmin)	S100	SS18-SSX Fusion
1	B	NA	NA	NA	NA	NA	NA	P
2	B	NA	NA	NA	NA	NA	NA	P
3	M	+	+	+	-	-	-	P
4	M	-	+	+	-	-	-	P
5	B	+	NA	NA	NA	NA	NA	P
6	B	+	NA	NA	NA	NA	NA	P
7	B	+	NA	+	-	NA	Focal	P
9	M	+	+	+	-	-	-	P
11	M	NA	NA	NA	NA	NA	NA	P
13	M	NA	NA	NA	NA	NA	NA	P
14	B	+	-	-	-	NA	NA	P
15	B	+	+	+	NA	NA	NA	P
16	M	+	+	+	-	-	-	P
19	B	+	+	-	NA	Focal Desmin	NA	P
21	B	+	+	NA	-	-	-	P
22	B	NA	NA	NA	NA	NA	NA	P
23	B	+	+	NA	NA	NA	NA	P
8	M	-	+	NA	NA	NA	NA	N
10	PD	+	-	+	-	NA	NA	N
12	PD	NA	NA	NA	NA	NA	NA	N
17	M	NA	NA	NA	NA	NA	NA	N
18	M	-	+	+	NA	-	Focal	N
20	PD	+	+	+	-	-	Focal	N
Total Percent		81.2%	86.7%	100%	0.0%	4.3%	37.5%	

B, biphasic; CK, cytokeratin; EMA, epithelial membrane antigen; M, monophasic; N, negative; NA, not available; P, positive; PD, poorly differentiated.

rearranged group). Interestingly, all tumors with prominent mast cell infiltration showed the rearrangement.

Discussion

The primary purpose of this study was to investigate the presence of SS18-SSX rearrangement in tumors with diagnoses synovial

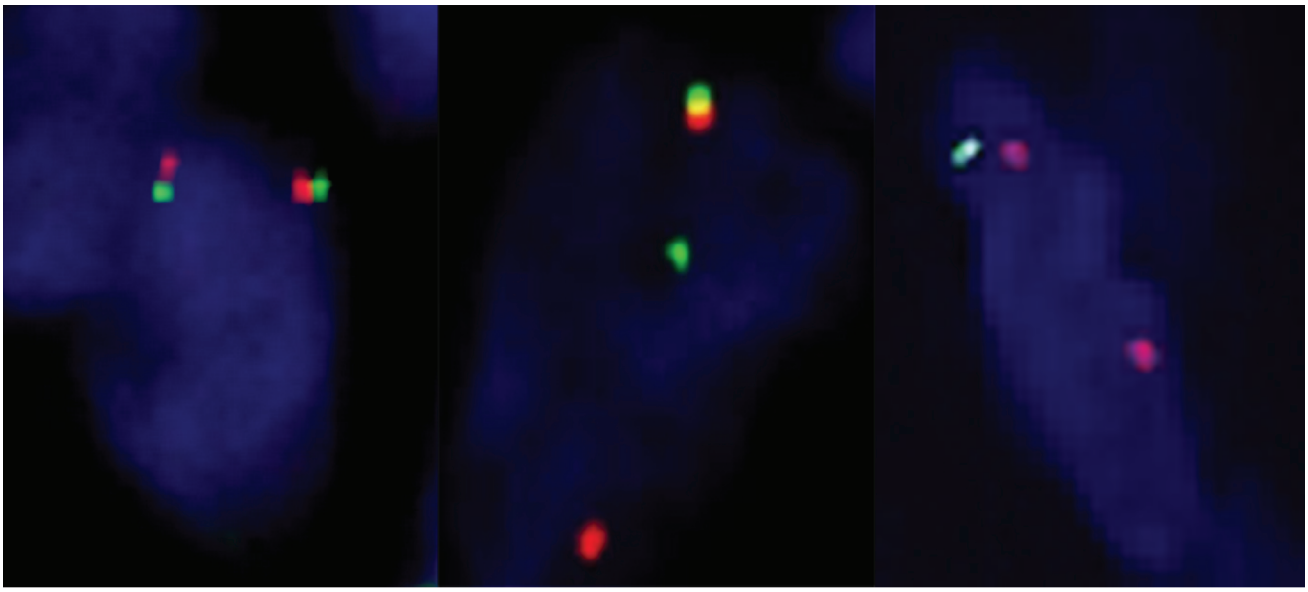

Figure 2. The images show FISH results. Negative (left), positive (middle), and positive with one missed green signal (right).

Table 3. Studies on molecular and conventional cytogenetic diagnosis of synovial sarcoma

	Country, Year	N	FISH	CISH	RT-PCR	Karyotype
Surace C[7]	Sweden and Italy, 2004	28	28/28 (100%)	-	28 (100%)	27/28 (96.42%)
Romeo S[6]	Italy, 2004	15	15/15 (100%)	-	8/8 (100%)	1/1 (100%)
Tvrdik D[5]	Czech Republic, 2005	7	-	-	7/7 (100%)	-
Terry J[11]	Canada, 2005	23	22/23 (96%)	Weak result	-	-
Amary MFC[10]	Brazil, 2007	132	87/101 (86%)	-	126/131 (96%)	-
Tanas MR[9]	USA, 2010	32	31/32 (96.87%)	-	-	-
Motoi T[4]	Japan, 2010	16	16/16 (100%)	16/16 (100%)	16/16 (100%)	-
Horna H[8]	Germany, 2014	9	8/9 (89%)	-	6/6 (100%)	-

N, Number of cases; FISH, fluorescent in situ hybridization; CISH, chromogenic in situ hybridization; RT-PCR, reverse transcriptase polymerase chain reaction.

sarcoma. This rearrangement was previously reported in 89% to 100% of synovial sarcomas using FISH method.[4-9] However, our positive rate was lower. In fact, break apart FISH probe identified 17 (73.91%) rearranged tumors, but the remaining six (26.09%) showed intact signals. Table 3 summarizes the results of similar studies published on the importance of cytogenetic or molecular diagnosis of synovial sarcoma.

Tumors that did not show SS18-SSX rearrangement were of either monophasic or poorly differentiated type. These types of synovial sarcoma can be difficult to distinguish from other tumors based solely on histomorphology and immunohistochemistry. Four of our negative cases (cases 8, 10, 18, and 20 in table 2) had immunohistochemistry information; however, only one showed typical immunohistochemistry findings of synovial sarcoma (case 20 with positive epithelial markers, CD99, Bcl6, and negative CD34). However, not all rearranged tumors were typical in immunohistochemistry either. For instance, case 4 tumor cells did not express epithelial markers, but they were rearranged for SS18-SSX. We believe that synovial sarcoma, with its wide range of histological variations and considerable immunohistochemical overlapping markers is one of the most difficult sarcomas to diagnose precisely. Moreover, there is no gold standard for diagnosing synovial sarcoma; therefore, the possibility of an incorrect primary diagnosis should always be considered.

Another possible reason for the higher negative FISH rate could be our method of case selection. In some studies, only cases with a previously confirmed SS18-SSX fusion gene by some other methods were selected; therefore, there was a systematic error towards higher positive FISH rates. In contrast, we performed FISH in all cases with a report of synovial sarcoma.

False negative FISH results may be occasionally due to inherent technical difficulties. A neoplasm sometimes harbors a cryptic molecular fusion gene, which is not detectable by FISH method. In such cases, a secondary laboratory technique such as reverse transcriptase polymerase chain reaction might be conducive to find the rearrangement. In addition, our understanding of the molecular alterations of synovial sarcoma is currently incomplete, and there still exist a large number of rare variant translocations linked to this entity.

Two cases showed unexpected FISH signal patterns. One revealed three separate green signals accompanied by three separate red signals suggestive of trisomy. Since no orange/yellow/green fusion signal was detected in this case, we conclude that all three available alleles were rearranged. The other unusual case revealed one orange/yellow/green fusion signal along with a single orange one in almost all tumor cells. This pattern was also present in many other cases, but only in occasional scattered cells. Amary et al. reported this finding in six patients. They associated this result to the fact that the SSX-SS18 transcript on chromosome 18 was more unstable than the SS18-SSX transcript on chromosome X.[10]

We further tried to detect SSX-SS18 translocation by CISH technique; however, the interpretation of CISH slides was impossible mainly due to the weak signals and many lost

dots in most cells. Terry et al. also had this experience with CISH and were not able to obtain acceptable dots. However, Motoi et al. observed that FISH and CISH had similar diagnostic utilities.[4, 11]

Conclusion

We believe that FISH is a practicable method for confirming the diagnosis of synovial sarcomas, particularly in tumors with monophasic and poorly differentiated subtypes with vague immunohistochemical findings. Furthermore, it is logical to perform another molecular method to examine equivocal, unexpected, or discrepant FISH results.

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Conflict of Interest

None declared.

References

1. Goldblum JR, Weiss SW, Folpe A. Enzinger and Weiss's soft tissue tumors. 6th ed. Philadelphia: Saunders; 2014. p. 1052-1070.
2. Goldblum JR, Lamps WL, McKenney JK, Myers JL. Rosai and Ackerman's surgical pathology. 11th ed. Amsterdam: Elsevier; 2018. p. 1876-1879.
3. McPherson R, Pincus MR. Henry's clinical diagnosis and management by laboratory methods. 23rd ed. Amsterdam: Elsevier; 2017. p .1514.
4. Motoi T, Kumagai A, Tsuji K, Imamura T, Fukusato T. Diagnostic utility of dual-color break-apart chromogenic in situ hybridization for the detection of rearranged SS18 in formalin-fixed, paraffin-embedded synovial sarcoma. *Hum Pathol*. 2010;41(10):1397-404. doi: 10.1016/j.humpath.2010.02.009.
5. Tvrdík D, Povy´sil C, Svatosová J, Dundr P. Molecular diagnosis of synovial sarcoma: RT-PCR detection of SYT-SSX1/2 fusion transcripts in paraffin-embedded tissue. *Med Sci Monit.* 2005;11(3):MT1-7.
6. Romeo S, Rossi S, Acosta Marín M, Canal F, Sbaraglia M, Laurino L, et al. Primary synovial sarcoma (SS) of the digestive system: a molecular and clinicopathological study of fifteen cases. *Clin Sarcoma Res.* 2015;5:7. doi: 10.1186/s13569-015-0021-3.
7. Surace C, Panagopoulos J, Palsson E, Rocchi M, Mandahl N, Mertens F. A novel FISH assay for SS18–SSX fusion type in synovial sarcoma. *Lab Invest*. 2004;84:1185-92. doi: 10.1038/labinvest.3700142.
8. Horna H, Allmanritter J, Doglionid C, Marxe A, Müllerb J, Gattenlöhnerf S, et al. Fluorescence in situ analysis of soft tissue tumor associated genetic alterations in formalin-fixed paraffin-embedded tissue. *Pathol Res Pract.* 2014;210:804-11. doi: 10.1016/j.prp.2014.09.009.
9. Tanas MR, Rubin BP, Tubbs RR, Billings SD, Downs-Kelly E, Goldblum JR. Utilization of fluorescence in situ hybridization in the diagnosis of 230 mesenchymal neoplasms. *Arch Pathol Lab Med.* 2010;134:1797-803. doi: 10.1043/2009-0571-OAR.1.
10. Amary MFC, Berisha F, Bernardi FDC, Herbert A, James M, Reis-Filho JS, et al. Detection of SS18-SSX fusion transcripts in formalin-fixed paraffin-embedded neoplasms: analysis of conventional RT-PCR, qRT-PCR and dual color FISH as diagnostic tools for synovial sarcoma. *Mod Pathol.* 2007;20:482-96. doi: 10.1038/modpathol.3800761.
11. Terry J, Barry TS, Horsman DE, Hsu FD, Gown AM, Huntsman DG, et al. Fluorescence in situ hybridization for the detection of t(X;18)(p11.2;q11.2) in a synovial sarcoma tissue microarray using a break apart-style probe. *Diagn Mol Pathol.* 2005;14:77-82. doi: 10.1097/01.pas.0000155021.80213.c9.