EPITHELIAL EXPRESSION IN HIGH GRADE SARCOMAS

RESEARCH PROJECT IN PARTIAL FULFILLMENT OF THE DEGREE OF MASTER OF PATHOLOGY

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TABLE OF CONTENTS

DECLARATION AND ACKNOWLEDGEMENT

This research report is wholly prepared by me, Dr Siti Aishah Binti Mahamad Dom, based on research work led by my supervisor Associate Professor Dr Nazarina Binti Abdul Rahman.

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This study was approved by the Medical Ethics Committee of the University Malaya Medical Centre.

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

High grade sarcomas are a heterogeneous group of sarcomas often lacking defining cytomorphological or immunohistochemical evidence of specific lineage differentiation. Many of these tumours previously known as Malignant fibrous histiocytoma (MFH) are currently redefined in the WHO 2013 classification of tumours of soft tissue and bone as Undifferentiated or Unclassified high grade sarcomas or Undifferentiated pleomorphic sarcomas (UPS). In some series expression of epithelial markers in high grade sarcomas were recorded.

A retrospective study of a cohort of high grade sarcomas which include UPS cases as well as other more specific high grade sarcomas eg Synovial sarcoma and Epithelioid sarcoma and high grade but lineage specific sarcomas eg high grade Malignant peripheral nerve sheath tumour (MPNST) seen at the University Malaya Medical Centre over an eleven-year period was undertaken to study the frequency and extent of epithelial expression of Cytokeratin and Epithelial membrane antigen (EMA). The clinicopathological features of these cases were also evaluated for any correlation. Immunohistochemical staining for epithelial expressions of cytokeratin - MNF116 and AE1/AE3 and EMA were evaluated in 100 resected specimens of high grade sarcoma cases.

Epithelial expression was seen in 32 (32%) of the 100 cases evaluated either as single marker or multiple marker expression. Cytokeratin was expressed in 12 cases (10 MNF116 and 2 AE1/E3 – positive cases) and EMA was expressed in 26 cases in variable extents of focal, intermediate or diffuse patterns. The figures include the overlap of cases expressing 1 or more than 1 marker. There was a significant association between specific epithelial markers and histological type of tumour and patient gender. There was no significant association of these epithelial markers with the following parameters: patient age and tumour size, location and origin with reference to primary, recurrent and metastatic tumours. Our study highlights the evidence of epithelial differentiation in high grade sarcomas and the diagnostic, therapeutic and prognostic implications.

Key words: high grade sarcoma, epithelial differentiation, cytokeratin, EMA

INTRODUCTION

High grade sarcomas can be diagnostically challenging as they raise several possibilities including focally differentiated or dedifferentiated pleomorphic sarcomas or other non- mesenchymal high grade tumours particularly sarcomatoid carcinomas, anaplastic lymphomas and melanomas amongst others.

Intratumor heterogeneity is known to be present in these tumours often lacking clearly defining features or specific lines of differentiation. The current 2013 WHO classification of tumours of soft tissue redefines entities previously termed Malignant fibrous histiocytoma (MFH) as Undifferentiated or Unclassified sarcomas or Undifferentiated pleomorphic sarcomas (UPS) (Fletcher CDM et al, 2013) (1). According to the French Federation of Cancer Centers Sarcoma Group (FNCLCC) grading system, three independent prognostic factors - degree of differentiation, mitotic activity and necrosis are used for defining the grade. In addition, certain histological types and subtypes of tumour differentiation are considered as high grade. The importance of grading is to indicate the probability of distant metastasis and overall survival.

Broadly, high grade sarcomas are also divided into pleomorphic, spindle cell, round cell and epithelioid subsets. Undifferentiated pleomorphic sarcomas (UPS) are often microscopically patternless, with frequent bizarre multinucleated giant cells. They show no reproducible immunophenotype nor any pattern of protein expression that would allow more specific subclassification. The spindle cell type show fascicular architecture with pale eosinophilic cytoplasm and tapering nuclei. Round cells consist of

relatively rounded to ovoid cells with high nuclear cytoplasmic ratio. The epithelioid morphology closely resembles metastatic carcinoma or melanoma, but generally lack nesting and the appropriate reticulin scaffold, displaying pale eosinophilic cytoplasm and vesicular nuclei.

The cytological appearance and the pattern of growth of tumour cells are helpful morphologic features, but these features are often not sufficient to arrive at the specific diagnosis. In these cases immunohistochemistry and cytogenetic evaluation are essential for diagnosis in identifying the line of differentiation. This determination again is dependent on the availability and extent of ancillary studies including immunohistochemical and molecular techniques, undertaken at any laboratory facility or institution. Epithelial marker expression has been reported in cases of certain specific subtypes of high grade sarcoma eg. Synovial sarcoma, Epithelioid sarcoma and Ewing sarcoma and UPS. This generates diagnostic dilemma because it raises the possibility of a sarcomatoid carcinoma in a patient population in which carcinoma is the most common malignancy prompting further investigation also for exclusion of an alternative primary tumour. The problem is further compounded by the fact that some sarcomatoid carcinomas may be negative for epithelial markers whereas some sarcomas express for such markers (Coindre JM et al, 2003) (2) with obvious implications for treatment and ultimate prognosis (Sha L et al, 2016) (3).

The prognostic significance of the epithelial differentiation is not well studied. To the best of our knowledge most of the related studies have been performed in Western populations and there are no available reports involving the Asian population.

This current study evaluates the expression of certain epithelial markers - Cytokeratin and EMA, in high grade sarcoma cases in a Malaysian patient population at University Malaya Medical Centre and analyzed its association with the following parameters - patient age and gender and tumour size, origin (primary, recurrent or metastatic) and location (extremities, head and neck, trunk, intra-abdominal and others).

MATERIALS AND METHODS

Patients and samples

Archived histopathology reports and slides of high grade sarcoma cases diagnosed in the Department of Pathology, University Malaya Medical Centre between January 2005 and December 2016, including referral and in house material, were retrieved and reviewed.

The selection criteria included confirmed histopathology cases based on histomorphology and availability of immunohistochemical studies of epithelial markers: Cytokeratin - MNF116 and AE1/AE3 and EMA. Resected specimens and cases with specific soft tissue differentiation exhibiting high histological type and grade according to FNCLCC grading system was considered as inclusion criteria. Cases involving biopsy specimens, missing slides, unavailable tissue slides or inadequate or unavailable patient clinical records were excluded. In cases without or with faded haematoxylin and eosin (H&E) slides, 5-μm sections were cut from the paraffin blocks and stained with H&E following standard methodology.

The staining pattern for cytokeratin (MNF116 and AE1/AE3) and EMA were evaluated from available immunohistochemical slides and histopathology reports. The pattern of immunohistochemical staining was graded as positive or negative. The intensity of positivity was assessed as : weak (1+), moderate (2+) and strong (3+), while the extent of positivity was graded as : diffuse, >75% of tumour cells staining (3+); intermediate, 25% to 75% staining (2+); focal, 1% to < 25% (1+); and negative, < 1% staining (0) as proposed by Jun Iwata et al, 2000 (20).

Information regarding patient demographics, tumour characteristics and immunohistochemistry results were extracted from histopathology reports and patient medical records and reviewed.

Immunohistochemistry

Immunohistochemistry for Cytokeratin AE1/AE3 (1:100, Dako North America, Carpinteria, CA,USA), MNF116 (Monoclonal Mouse Anti:Human Cytokeratin, 1:1000, DakoCytomatin, Glostrup, Denmark) and EMA (Monoclonal Mouse Anti-Human Epithelial Membrane Antigen, 1: 200 DakoCytomation , Glostrup, Denmark) as reported had been performed on formalin-fixed, paraffin-embedded tissue sections using steam heat-induced epitope retrieval and the Dako Envision detection system. All the cells labelled by these antibodies displayed cytoplasmic staining pattern for MNF116 and AE1/AE3 and cytoplasmic and membrane staining for EMA.

Normal or tumour tissue known to express the test antigens (i.e. Cytokeratins and EMA) were used as positive controls and included routinely in the immunohistochemical assay.

Statistical analysis

Data was entered and analyzed by SPSS version 23.0. Categorical data had been expressed by frequency and percentage while numerical data as mean and standard deviation.

Expression of Cytokeratin and EMA in high grade soft tissue sarcomas were analyzed by Chi-squared test. Differences with p-value <0.05 were considered significant.

RESULTS

100 cases were entered into the study on the basis of adequate documentation and suitability of available material as stipulated for evaluation of the cytomorphological features and immunohistochemistry.

Patient demographics

The 100 cases were diagnosed mainly in older adults (mean age: 49.53± 19.174, range: 4– 88 years).

52 (52%) patients were male and 48 (48%) female with 41 (41%) Malay, 43 (43%) Chinese and 16 (16%) Indian.

Clinicopathological features of tumour

There were 77 (77%) primary, 17 (17%) recurrent and 6 (6%) metastatic tumours.

Tumour size variation was categorized as less than 5 cm numbering 29 (29%), 5 - 10 cm numbering 29 (29%) and more than 10 cm numbering 40 (40%); no size was recorded in 2 (2%) cases.

Topographic evaluation revealed 73 (73%) from the extremity sites: 53 (53%) from the lower limbs (pelvis – 7, thigh – 29, knee – 8, leg – 8 and foot – 1) and 20 from the upper limbs (shoulder – 5, arm – 4, elbow – 2, forearm – 6, hand – 3). 4 (4%) were located in the trunk (chest wall – 2 and abdominal wall – 2), 6 (6%) in the head and neck (head – 1, nasal – 3 and neck – 2) and 7 (7%) intra - abdominal (stomach – 1, colon – 2, intra-peritoneum – 1, cervix – 2 and uterine adnexa– 1). Other sites – 9 (9%) included 5 from breast and 4 from lung. No location of the tumour was recorded in 1 (1%) case.

Refer Tables 1.0 Patient demographics and 2.0 Clinicopathological features of tumour

The diagnostic spread of cases included 78 (78%) of high grade sarcomas (UPS) with no specific differentiation diagnosed with slight variations in terminology – 18 (18%) undifferentiated pleomorphic sarcomas, 26 (26%) high grade sarcomas, 21 (21%) pleomorphic sarcomas and 13 (13%) malignant fibrous histiocytomas. The remaining 22 (22%) of cases showed specific differentiation subtypes – 11 (11%) synovial sarcomas, 4 (4%) epithelioid sarcomas, 3 (3%) pleomorphic leiomyosarcomas, 3 (3%) high grade malignant peripheral nerve sheath tumours (MPNST) and 1 (1%) uterine sarcoma.

Refer Table 3.0 Histological type.

Table 1.0 Patient demographics

Characteristic	**No of cases (n=100)**
Age (year) Mean Range	49.53 ±19.174 (4-88)
Gender Male Female	 52 (52%) 48 (48%)
Ethnicity Malay Chinese Indian	 41 (41%) 43 (43%) 16 (16%)

Table 2.0 Clinicopathological features of tumour

Characteristic	No of cases (n=100)
Origin	
Primary	77 (77%)
Recurrent	17 (17%)
Metastatic	6 (6%)
Size	
< 5cm	29 (29%)
5-10 cm	29 (29%)
>10 cm	40 (40%)
No size recorded	2 (2%)
Topography	
Extremities	**73 (73%)**
Lower limb	**53**
Pelvis	7
Thigh	29
Knee	8
Leg	8
Foot	1
Upper limb	**20**
Shoulder	5
Arm	4
Elbow	2
Forearm	6
Hand	3
Head and neck	**6 (6%)**
Head	1
Neck	2
Nasal	3
Trunk	**4 (4%)**
Chest wall	2
Abdominal wall	2
Intra-abdominal	**7 (7%)**
Stomach	1
Colon	2
Intra-peritoneum	1
Cervix	2
Uterine adnexa	1
Others	**9 (9%)**
Breast	5
Lung	4
No location recorded	**1 (1%)**

Table 3.0 Histological type

Characteristic	No of cases (n=100)
No specific differentiation	**78 (78%)**
Undifferentiated pleomorphic sarcoma	18 (18%)
High grade sarcoma	26 (26%)
Pleomorphic sarcoma	21 (21%)
Malignant fibrous histiocytoma	13 (13%)
Specific differentiation	**22 (22%)**
Synovial sarcoma	11 (11%)
Epithelioid sarcoma	4 (4%)
Pleomorphic leiomyosarcoma	3 (3%)
High grade MPNST	3 (3%)
Uterine sarcoma	1 (1%)

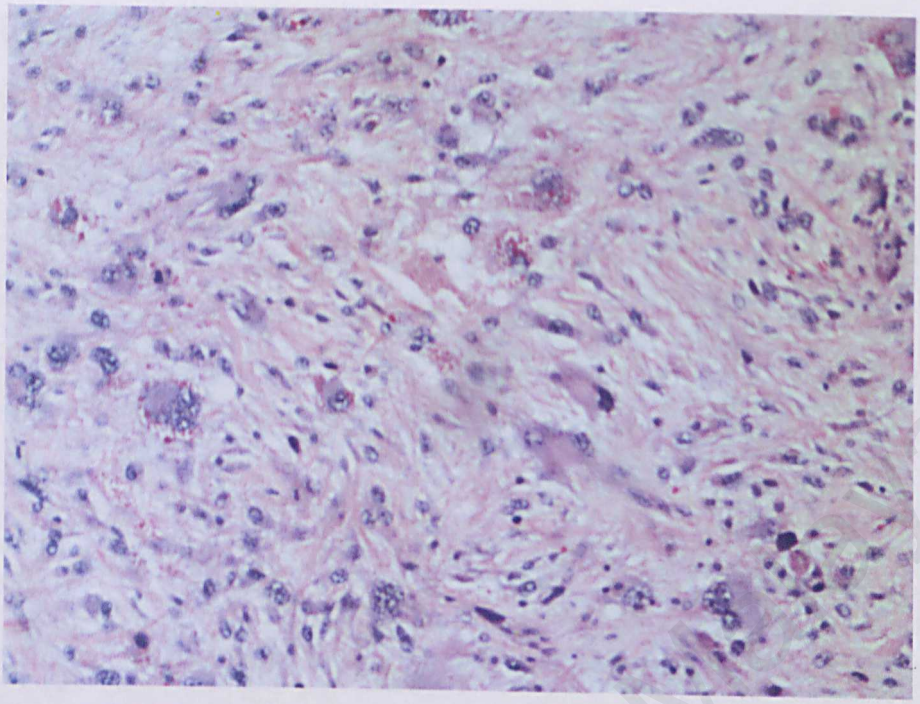


FIG 1: Undifferentiated pleomorphic sarcoma (UPS), H&E x10. Patternless sheets of pleomorphic cells with bizarre multinucleated tumour giant cells.

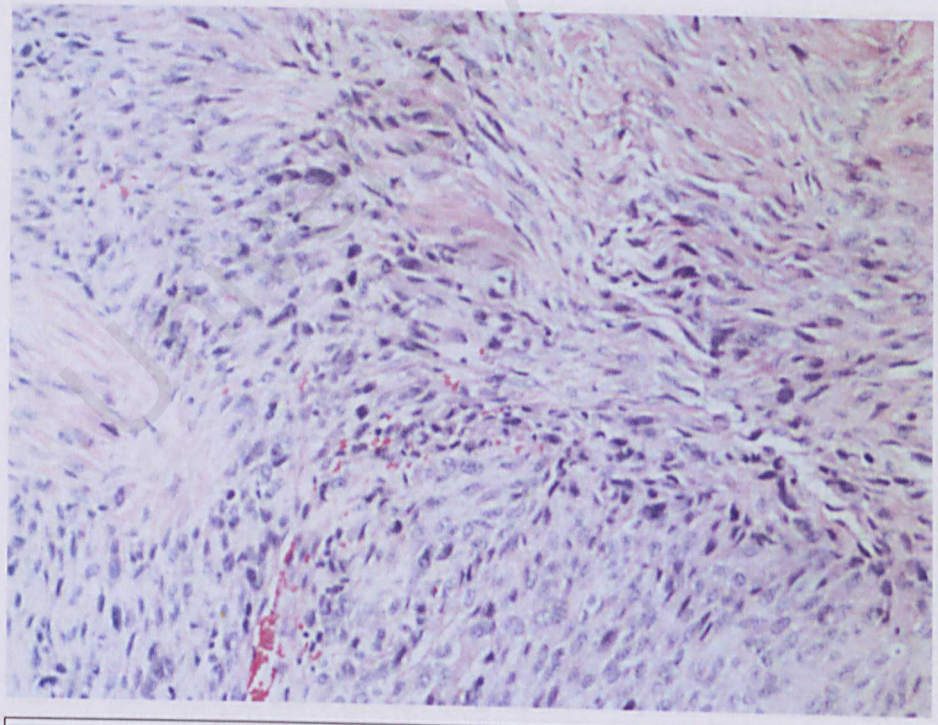


FIG 2: Malignant fibrous histiocytoma (MFH), H&E x10.
Diffuse sheets, storiform and fascicular patterns of spindle cells with indistinct eosinophilic cytoplasm.

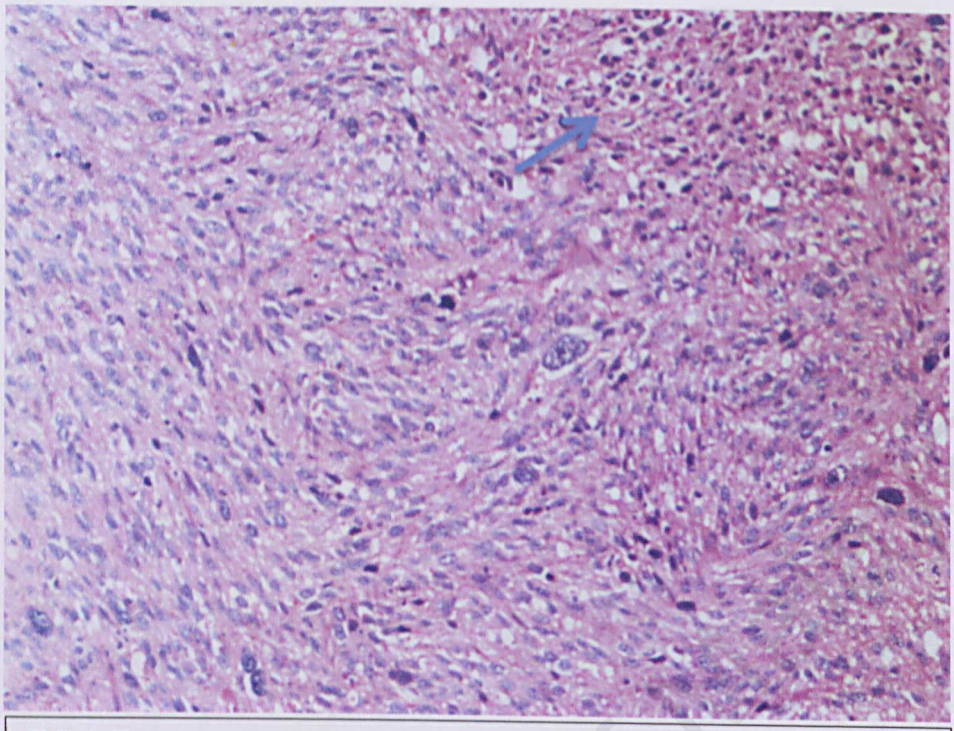


FIG.3: Pleomorphic leiomyosarcoma, (H&E x 10).
Fascicles of spindle cells with indistinct eosinophilic cytoplasm and poorly differentiated pleomorphic cells and necrosis (arrowed).

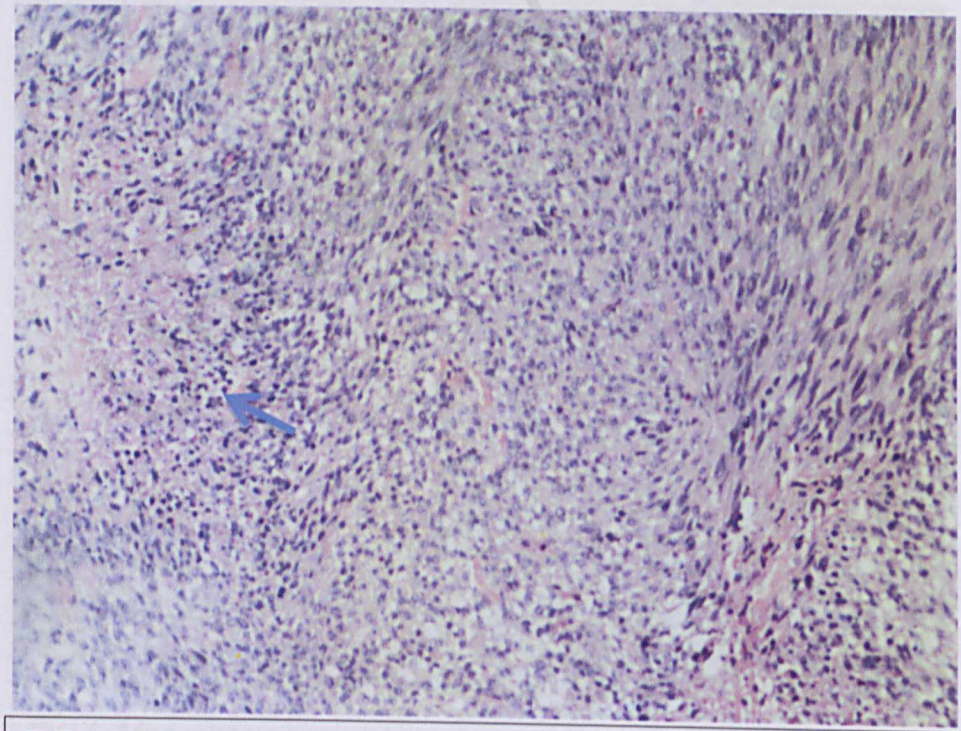


FIG.4: Malignant peripheral nerve sheath tumour (MPNST), (H&Ex10).
Fascicles of spindles cells palisading around the geographical necrosis (arrowed).

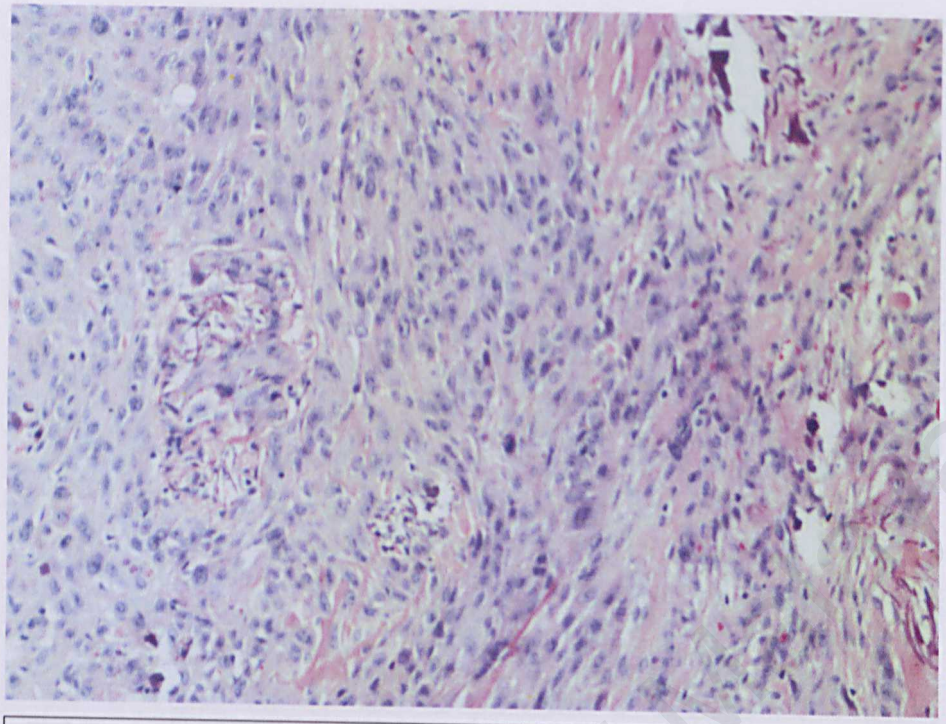


FIG.5: Epithelioid sarcoma, (H&E x10).
Epithelioid and plump spindled tumour cells.

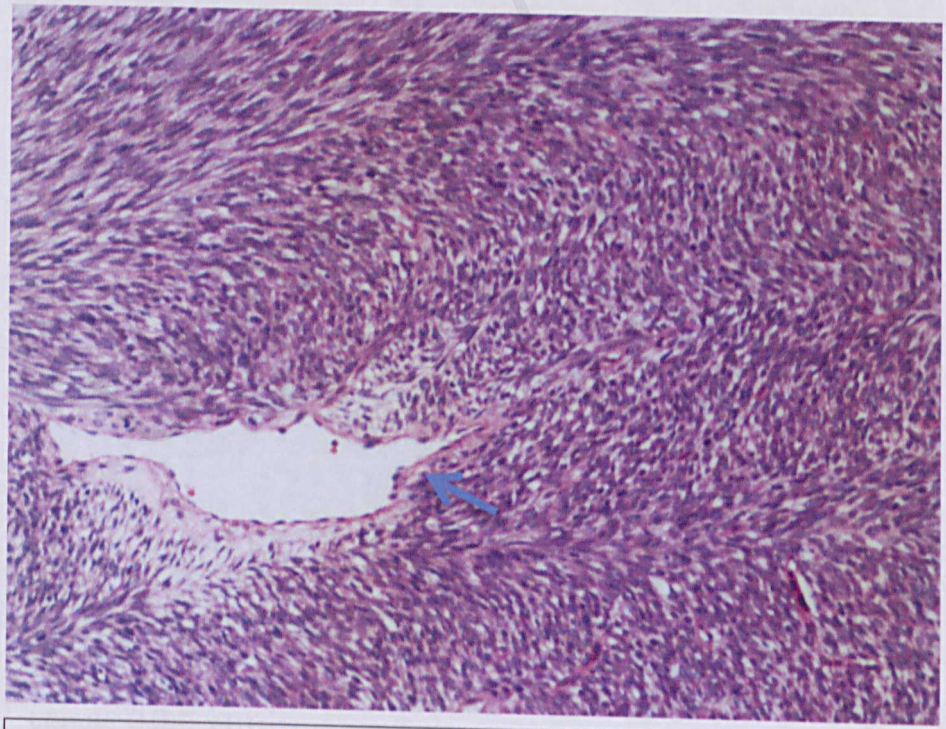


FIG.6: Monophasic synovial sarcoma, (H&E x10).
Fairly uniform spindle cells in dense cellular sheets and fascicles with staghorn vascular pattern (arrowed).

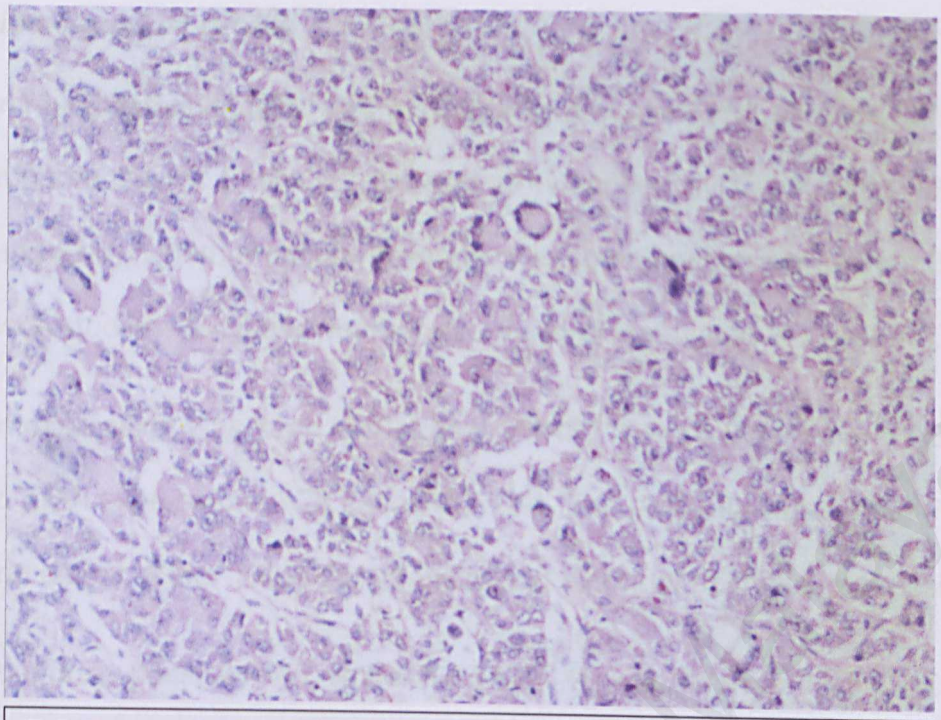


FIG.7: High grade uterine sarcoma , (H&E x 10).
Diffuse sheets of large bizarre pleomorphic cells.

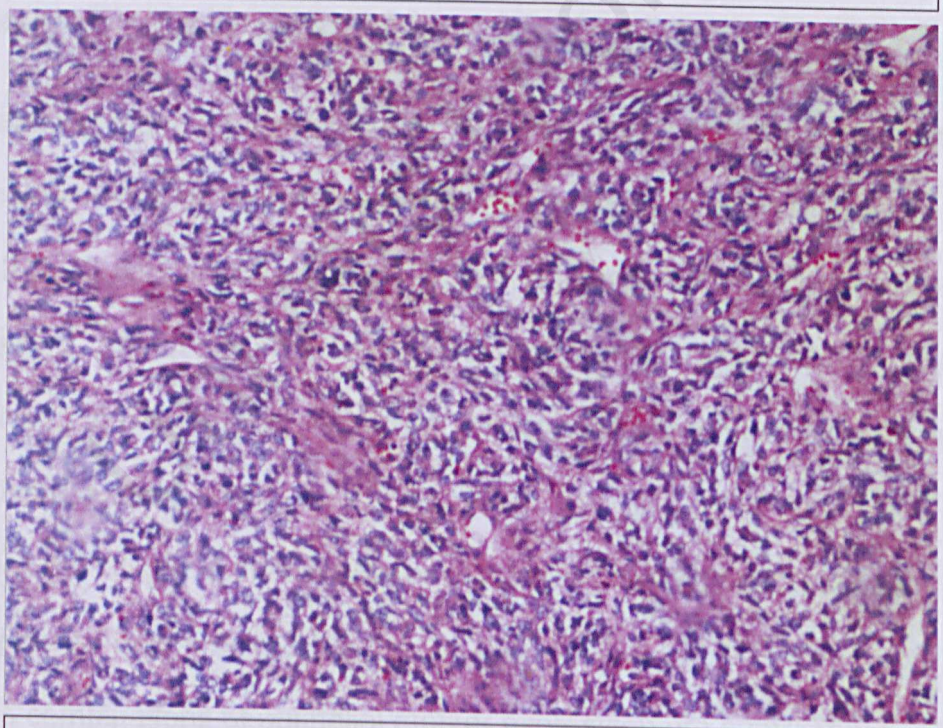


FIG.8: High grade breast sarcoma, preceded by previous phylloides tumour (H&E x10).
Patternless pattern of spindled tumour cells separated by vascular channels.

Immunohistochemical profile

Vimentin immunohistochemistry was available in 83 cases and all showed strong and diffuse immunoreactivity. Epithelial expression evidenced by Cytokeratin MNF116 and AE1/AE3 and EMA, whether occurring singly or in combination was seen in 32 (32%) of the 100 cases studied. Epithelial marker expression is tabulated in Table 4.0.

Cytokeratin expression was seen in 12 cases (MNF116 – 10 (14.3%) and AE1/AE3 – 2 (20%)) and EMA expression in 26 cases (37.7%). The positivity was variable in staining intensity and extent. Refer Table 5.0 Intensity and extent of epithelial expression.

Strong and diffuse positivity of Cytokeratin MNF116 was seen in 1 case of epithelioid sarcoma and strong and diffuse positivity of EMA was seen in 5 cases : 1 epithelioid sarcoma and 4 synovial sarcomas.

MNF116 staining alone was available in 29 cases and 2 (6.9%) were positive in high grade sarcoma with specific subtypes: synovial sarcoma – 1 and epithelioid sarcoma – 1. AE1/AE3 alone was available in only 1 case of pleomorphic sarcoma of the colon and it was negative. EMA staining alone was available in 26 cases and 5 cases (19.2%) were positive: 2 in pleomorphic sarcomas, 3 in specific sarcomas (synovial sarcoma – 2 and epithelioid sarcoma – 1).

Refer Tables 6.0 Single epithelial marker expression and Table 7.0 Intensity and extent of single epithelial expression.

The various combinations of epithelial marker expression are summarized in Table 8.0. 4 cases showed combination of epithelial markers, cytokeratin MNF116 and EMA with variation in the intensity and extent. 2 cases showed combination of both Cytokeratin

MNF116 and AE1/AE3. Cases with combinations of 2 epithelial markers of AE1/AE3 and EMA and 3 epithelial markers of MNF116, AE1/AE3 and EMA were all negative.

Table 4.0 Epithelial marker expression

Grading	MNF116 (n=70)	AE1/AE3 (n=10)	EMA (n=69)
Positive	10 (14.3%)	2 (20%)	26 (37.7%)
Negative	60 (85.7%)	8 (80%)	43 (62.3%)

Table 5.0 Intensity and extent of epithelial expression

Epithelial markers	Intensity			Extent		
	Weak (1+)	Moderate (2+)	Strong (3+)	Focal (1+)	Intermediate (2+)	Diffuse (3+)
MNF116 (n=10)	5 (50%)	4 (40%)	1 (10%)	6 (60%)	3 (30%)	1 (10%)
AE1/AE3 (n=2)	1 (50%)	1 (50%)	-	1 (50%)	-	1 (50%)
EMA (n=26)	14 (53.9%)	5 (19.2%)	7 (26.9%)	16 (61.5%)	4 (15.4%)	6 (23.1%)

Table 6.0 Single epithelial marker expression

Grading	MNF116 (n=29)	AE1/AE3 (n=1)	EMA (n=26)
Positive	2 (6.9%)	0	5 (19.2%)
Negative	27 (93.1%)	1 (100%)	21 (80.8%)

Table 7.0 Intensity and extent of single epithelial expression

	MNF116	EMA
Positive	2	5
Intensity	Moderate : 1 Strong : 1	Weak :1 Moderate: 1 Strong: 3
Extent	Intermediate: 1 Diffuse : 1	Focal : 2 Intermediate: 1 Diffuse: 2
Histological type	Epithelioid sarcoma : 1 Synovial sarcoma : 1	Pleomorphic sarcoma: 2 Epithelioid sarcoma : 1 Synovial sarcoma : 2

Table 8.0 Intensity and extent of multiple epithelial expression

	MNF116 and AE1/AE3	MNF116 and EMA
Positive	2	4
Intensity	MNF116 Weak : 1 Moderate: 1 AE1/AE3 Weak: 1 Moderate: 1	MNF116 Weak: 2 Moderate: 2 EMA Weak: 1 Moderate: 1 Strong: 2
Extent	MNF116 Focal :1 Diffuse: 1 AE1/A3: Focal:1 Diffuse: 1	MNF116 Focal: 2 Intermediate: 2 EMA Focal: 2 Diffuse: 2
Histological type	High grade sarcoma: 1 Pleomorphic sarcoma: 1	Undifferentiated pleomorphic sarcoma: 1 High grade sarcoma: 1 Synovial sarcoma: 1 Epithelioid sarcoma: 1

Note: No epithelial expression in combination of 2 markers AE1/AE3 and EMA and 3 markers MNF116, AE1/AE3 and EMA.

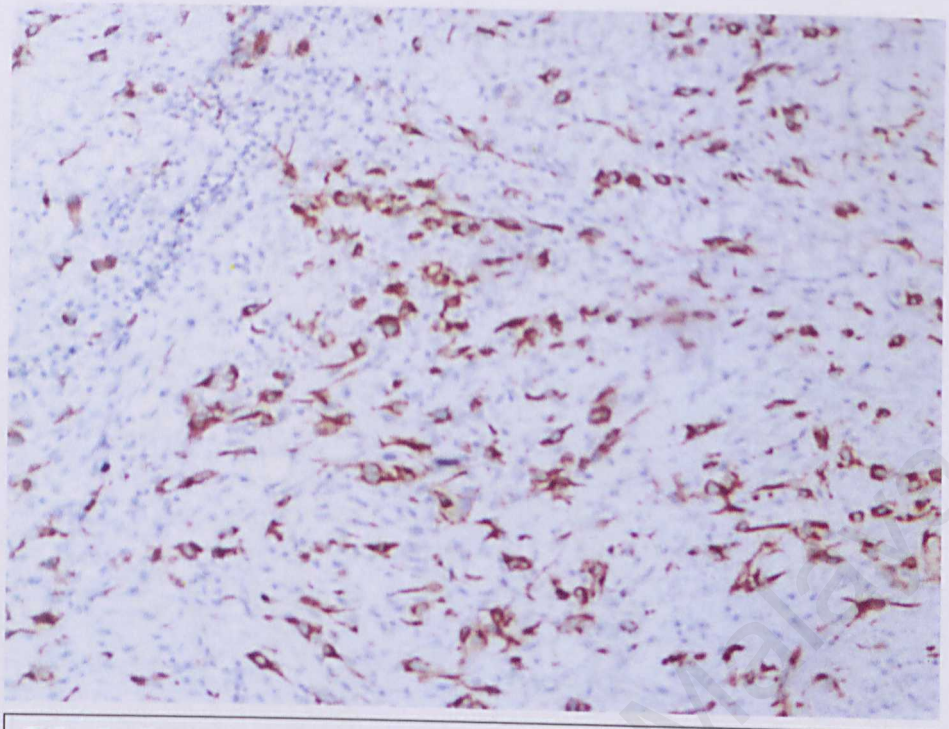


FIG. 9: Strong and intermediate cytoplasmic staining of MNF116

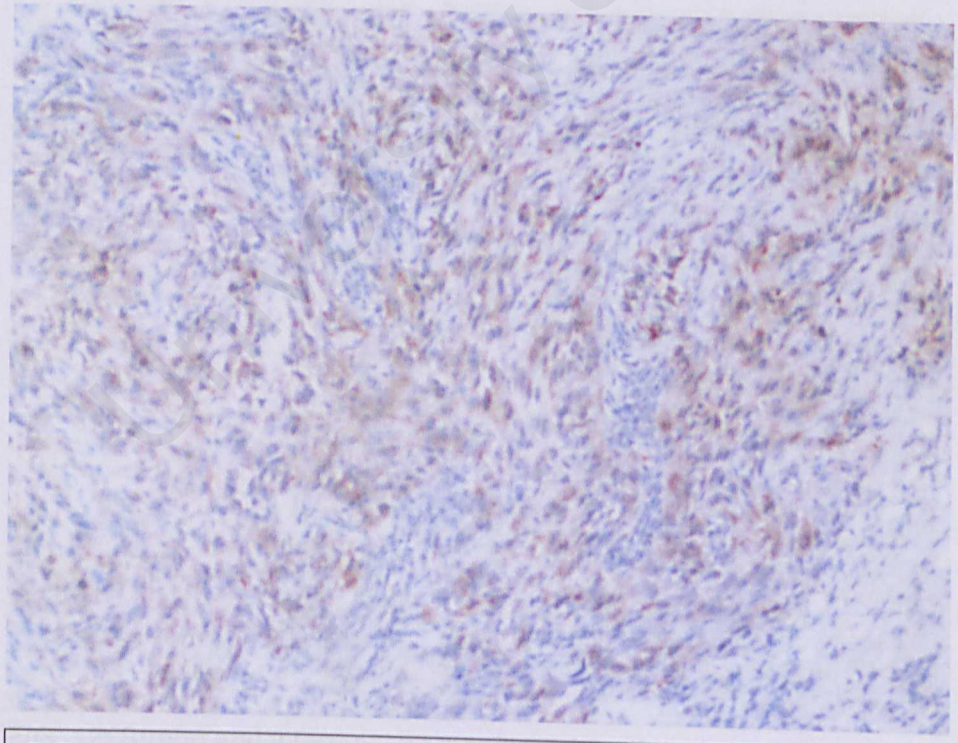


FIG. 10: Moderate and intermediate cytoplasmic staining of MNF116 (x10)

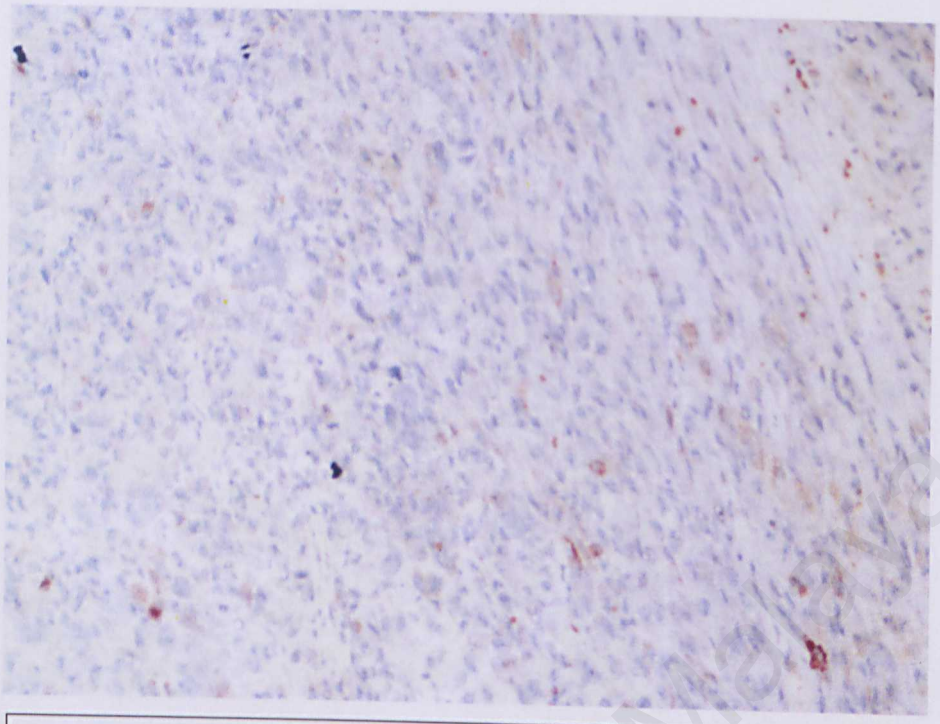


FIG. 11: Weak and focal cytoplasmic staining of MNF116 (x10)

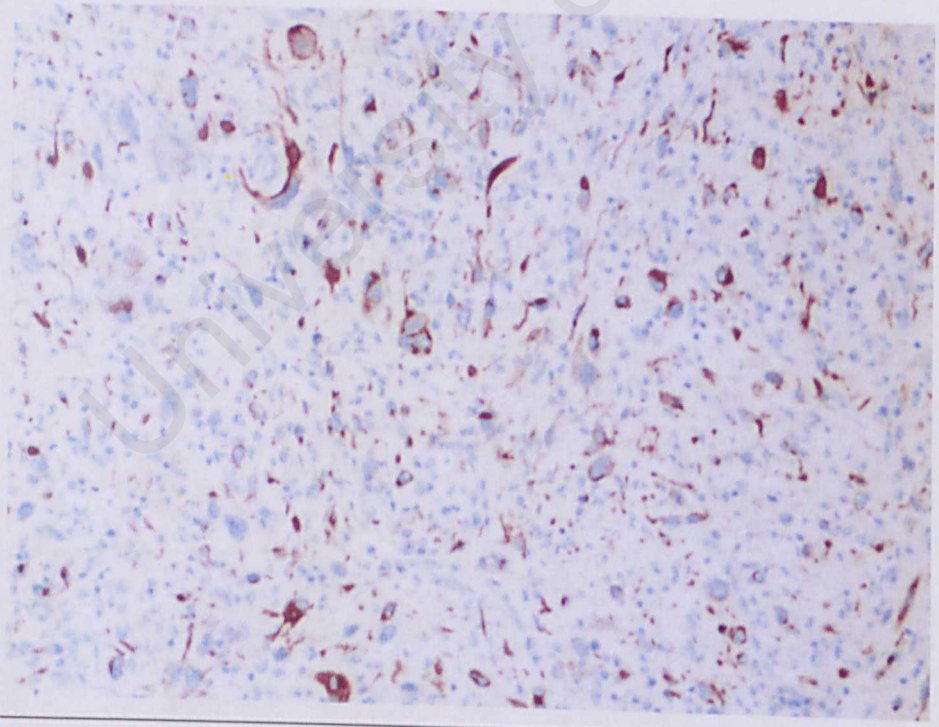


FIG. 12: Strong and intermediate cytoplasmic staining of AE1/AE3 (x10)

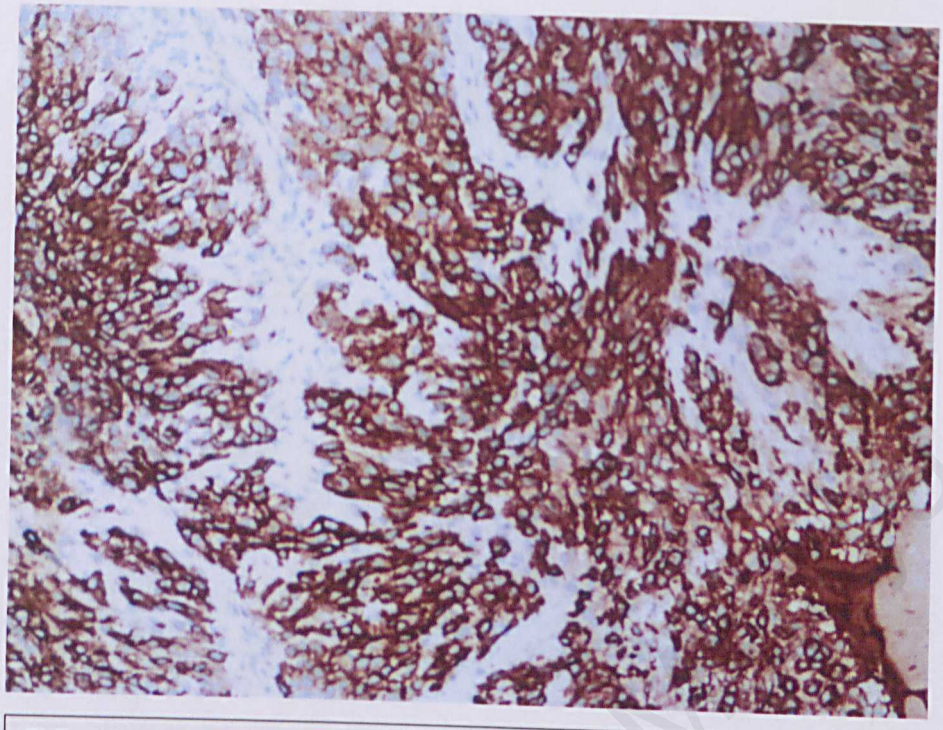


FIG. 13: Strong and diffuse cytoplasmic staining of EMA (x10)

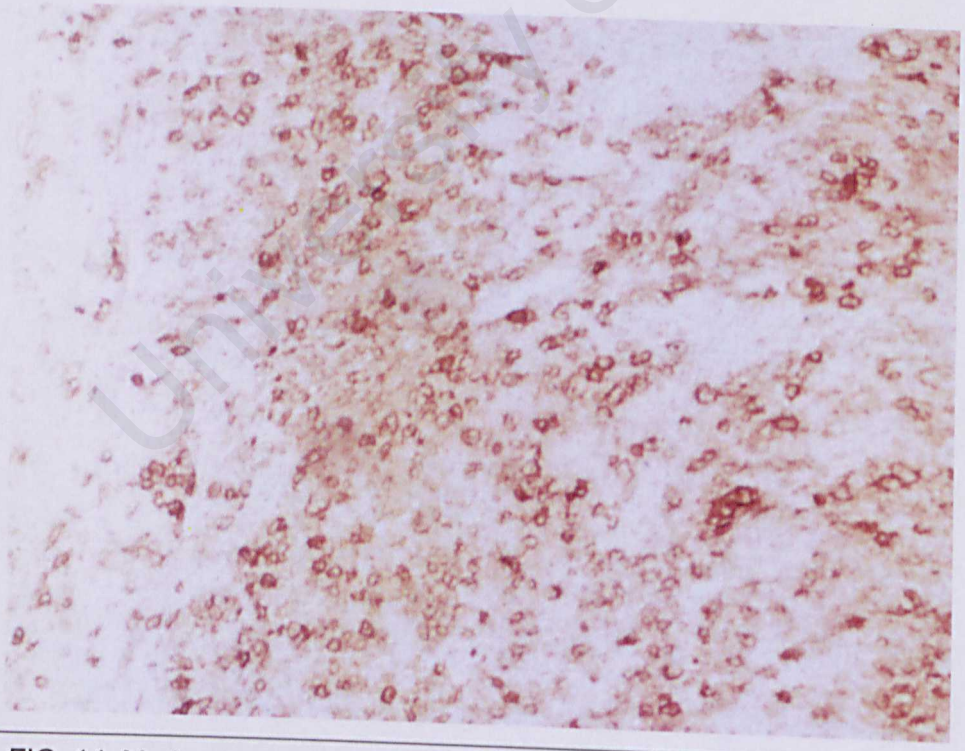


FIG. 14: Moderate and intermediate cytoplasmic staining of EMA (x10)

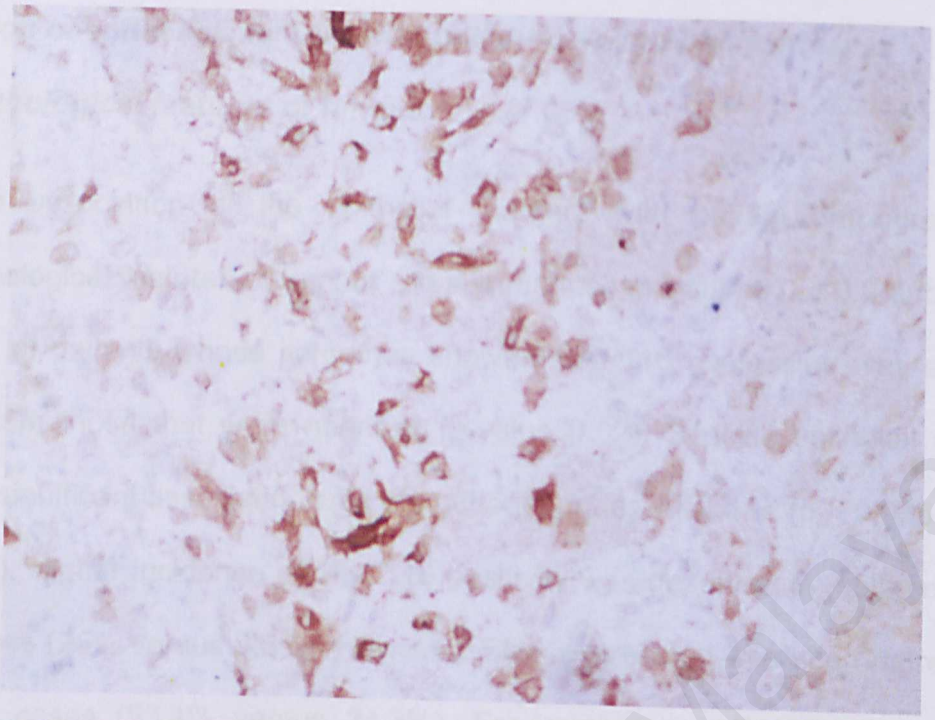


FIG. 15: Strong and focal cytoplasmic staining of EMA (x10)

Association of epithelial markers with patient demographics and clinicopathological features of tumour.

The association of the epithelial markers with patient demographics and clinicopathological features of tumour are summarized in Table 9.0, 10.0 and 11.0. The mean age of patients whose sarcomas showed MNF116 expression was significantly different from those that were negative (p-value 0.02). Specific epithelial expression showed a significant association with the patient gender, MNF116 (p – 0.036) and EMA (p – 0.014). Higher incidence of MNF116 positivity was observed in male compared to female cases (25% versus 3.5%). However, EMA expression was seen more in female than male cases (53.1% versus 24.3%). For cytokeratin AE1/AE3 Chi-square test cannot be performed due to lack of adequate sample numbers.

Epithelial marker expression showed a significant association with tumour histological type: MNF116 (p – 0.022) and EMA (p – 0.012). The histological type was categorized into 2 groups in this study - tumour with no specific differentiation and tumour with specific differentiation. Cytokeratin MNF116 positivity was observed equally in both tumour with specific and no specific differentiation (50%). A slightly higher incidence of EMA positivity was detected in tumour with no specific differentiation (53.8%) including high grade sarcomas – 6, pleomorphic sarcomas – 6 and undifferentiated pleomorphic sarcomas – 2 as compared to tumour with specific differentiation (46.2%): Synovial sarcomas – 8, Epithelioid sarcomas – 2 , pleomorphic LMS – 1 and uterine sarcoma – 1.

Refer Tables 12.0 and 13.0 Tumour histological type and epithelial expression.

Table 9.0 Comparison of clinicopathological features and Cytokeratin MNF116 expression

Characteristic	Total (n=100)	MNF116 expression (n=70)		p- value
		Positive (n=10)	Negative (n=60)	
Age (year) Mean Range	49.53±19.172 (4-88)	36.7±19.368 (16-74)	51.5±17.421 (4-81)	**0.02***
Gender Male Female	52 48	8 (25%) 2 (5.3%)	24 (75%) 36 (94.7%)	**0.036****
Tumour origin Primary Non – primary (recurrent and metastatic)	77 23	10 (17.5%) 0	47 (82.5%) 13 (100%)	0.190***
Tumour size < 5cm 5-10 cm >10 cm No size recorded	29 29 40 2	4 (20%) 4 (21.1%) 2 (6.7%) -	16 (80%) 15 (78.9%) 28 (93.3%) -	0.548***
Tumour location Extremities Non - Extremities (Head and neck, trunk Intra-abdominal and Others) No location recorded	73 16 1	7 (14.3%) 3 (15%) -	42 (85.7%) 17 (85%) -	0.990***

*Independent sample t-test **Pearson Chi-square test ***Fisher Exact Test

Table 10.0 Comparison of clinicopathological features and Cytokeratin AE1/AE3 expression

Characteristic	Total (n=100)	AE1/AE3 expression (n=10)	
		Positive (n= 2)	Negative (n=8)
Age (Year) Mean Range	49.53±19.17 (4-88)	40.37±18.65 (33-60)	46.5±19.09 (11-59)
Gender Male Female	52 48	2 (66.7%) 0	1 (33.3%) 7 (100%)
Tumour origin Primary Non – primary (recurrent and metastatic)	77 23	2 (25%) 0	6 (75%) 2 (100%)
Tumour size < 5cm 5-10 cm >10 cm No size recorded	29 29 40 2	1 (33.3%) 0 1 (20%) -	2 (66.7%) 2 (100%) 4 (80%) -
Tumour location Extremities Non - Extremities (Head and neck, trunk Intra-abdominal and Others) No location recorded	73 16 1	1 (25%) 1 (16.7%) -	3 (75%) 5 (83.3%) -

Note: Chi square cannot performed due to lack of adequate sample numbers (n<)

Table 11.0 Comparison of clinicopathological features and EMA expression

Characteristic	Total (n=100)	EMA expression (n=69)		P value
		Positive (n=26)	Negative (n=43)	
Age (Year)				0.409*
Mean Range	49.53±19.17 (4-88)	47.19±19.46 (11-81)	51.14±18.94 (13-88)	
Gender				**0.014****
Male Female	52 48	9 (24.3%) 17 (53.1%)	28 (75.7%) 15 (46.9%)	
Tumour origin				0.990***
Primary Non – primary (recurrent and metastatic)	77 23	21 (37.5%) 5 (38.5%)	35 (62.5%) 8 (61.5%)	
Tumour size				0.264**
< 5cm 5-10 cm >10 cm No size recorded	29 29 40 2	5 (27.8%) 11 (52.4%) 10 (35.7%) -	13 (72.2%) 10 (47.6%) 18 (64.3%) -	
Tumour location				0.990**
Extremities Non - Extremities (Head and neck, trunk Intra-abdominal and Others) No location recorded	73 16 1	19 (38%) 6 (33.3%) -	31 (62%) 12 (66.7%) -	

*Independent sample t-test **Pearson Chi-square test ***Fisher Exact Test

Table 12.0. Comparison of tumour histological type and epithelial expression

Histological type	No of cases (n=100)	MNF116 expression (n=70)		AE1/A3 expression (n=10)		EMA expression (n=69)	
		Positive (n=10)	Negative (n=60)	Positive (n=2)	Negative (n=8)	Positive (n=26)	Negative (n=43)
No specific differentiation							
Undifferentiated pleomorphic sarcoma	17	2	11	0	1	2	8
High grade sarcoma	25	2	20	1	3	6	9
Pleomorphic sarcoma	23	1	12	1	1	6	10
Malignant fibrous histiocytoma	13	0	8	0	0	0	9
Specific differentiation							
Synovial sarcoma	11	2	5	0	1	8	2
Epithelioid sarcoma	4	3	0	0	0	2	1
Pleomorphic LMS	3	0	2	0	1	1	1
High grade MPNST	3	0	1	0	0	0	3
Uterine sarcoma	1	0	1	0	1	1	0

Table 13.0 Summary of tumour histological type and epithelial expression

Histological type	Total (n=100)	MNF116 expression (n=70)		p-value	EMA expression (n=69)		p-value
		Positive (n=10)	Negative (n=60)		Positive (n=26)	Negative (n=43)	
				0.022* **			**0.012*** **
No specific differentiation	78	5 (8.9%)	51 (91.1%)		14 (28%)	36 (72%)	
Specific differentiation	22	5 (35.7%)	9 (64.3%)		12 (63.2%)	7 (36.8%)	

***Fischer Exact Test

DISCUSSION AND CONCLUSION

High grade sarcomas previously known as malignant fibrous histiocytoma is a diagnosis of exclusion and should be reserved for sarcoma cases without specific lineage (Rita A. et al, 2010) (4). However according to Fletcher et al, 2013 (1) specific histological types in the FNCLCC grading system are also classified into high grade sarcomas such as: pleomorphic liposarcoma, pleomorphic leiomyosarcoma, synovial sarcoma and epithelioid sarcoma.

Immunohistochemistry is crucial in the diagnosis of high grade sarcomas as their morphological features may simulate other neoplasms especially poorly differentiated carcinomas. This is important because accurate diagnosis can influence the outcome and treatment (Sha L et al, 2016) (3). Some specific sarcomas are known to express reactivity to epithelial markers (Dabbs David J, 2010) (21), usually essential for diagnosing poorly-differentiated or sarcomatoid carcinomas. Unexpected positivity may be seen in numerous tumours such as leiomyosarcoma, rhabdomyosarcoma, Primitive neuroectodermal tumour (PNET) and angiosarcoma (Coindre JM et al, 2003, T Hasegawa et al, 2003, Jun Iwata et al, 2009) (2, 6,20)

Cytokeratin AE1/AE3 is a mixture of two different clones of anticytokeratin antibodies that detects high and low molecular weight keratins, namely high molecular weight cytokeratins 1,2,3,4,5,6,10,14.15 and 16 and low molecular weight cytokeratins 7,8 and 19. MNF116 is also another broad spectrum antikeratin with reactivity corresponding to cytokeratin 5,6,8,17 and 19 (Dabbs David J, 2010)(21). Epithelial membrane antigen (EMA) is one of several human milk fat globule proteins (HMFGPs) that are derived from the mammary epithelium, which vary greatly in molecular weight

(51 kD to >1000 kD). They are predominantly glycoproteinaceous and compose part of the plasmalemma of epithelial cells in areas of the cell membrane and hence the EMA reactivity must be cell membrane based.

Among our Cytokeratin positive cases, 10 cases expressed MNF116 (14.3%), 2 cases (20%) expressed AE1/AE3 and 26 cases (37.7%) expressed EMA. This frequency is comparable to Rosenberg et al, 1992. MFH has been reported to express epithelial markers Cytokeratin (25.4%) and EMA (20.6%). Litzky and Brook reported cytokeratin immunoreactivity 35% cases of frozen section tissue samples and 16% of paraffin embedded tissue samples of MFH.

Primary MFH or pleomorphic sarcoma of the breast is rare and diagnostically challenging as the histology displays pleomorphic spindle cells in storiform pattern associated with brisk mitosis, negative for all keratins including CK18 and CK5/6 (Paul HH et al, 2011) (10). This result is comparable to the 5 cases of breast sarcoma which were all negative for cytokeratin and also EMA. 2 cases of this study were previously diagnosed as phylloides tumours. Some entrapment of breast ductal epithelium can be misinterpreted as epithelial component of biphasic phylloides tumour or metaplastic carcinoma. Careful attention to and correlation with clinical and radiological features augmented by immunohistochemistry can avoid misdiagnosis.

Pleomorphic leiomyosarcoma (LMS) is a high grade tumour at least focally exhibiting features of smooth muscle differentiation with immunoreactivity for Actin, Calponin and H-caldesmon (Coindre JM et al, 2003, Louis G et al, 2008) (2,11). It has also been shown to display aberrant expression of the epithelial markers, Cytokeratin and EMA in 10-20% of cases as previously reported in Jun Iwata et al, 2000. These

cases displayed intracytoplasmic diffuse or fibrillary staining as well as dot-like patterns. In our study only 1 of 3 cases showed focal immunoreactivity for EMA.

Synovial and epithelioid sarcomas, soft tissue sarcomas with established epithelial differentiation are generally considered high grade and aggressive tumours. In this study the synovial sarcoma cases all of which were monophasic showed expression of Cytokeratin MNF116 and EMA in 36.4% and 72.7% of cases respectively. Expression of epithelial markers in 90% of synovial sarcomas appears more intense in the epithelial component rather than spindle component (Khin Thway et al, 2014) (18). In comparison, the epitheliod sarcoma cases showed a stronger and more diffuse immunoreactivity for both Cytokeratin MNF116 and EMA.

There are significant differences between expression of Cytokeratin and EMA in relation to patient gender and histological types of high grade sarcomas. A variable frequency of cytokeratin expression in high grade sarcoma cases has been shown. Awareness of this fact particularly in relation to histological type, is of practical importance as this variable expression can be applied in different sarcoma types to aid diagnosis particularly when considering poorly-differentiated carcinoma or sarcomatoid carcinomas.

One of the limitations of this study which includes multiple variables and minimal sample sizes is the dependancy on preexisting epithelial immunohistochemistry records providing only a non-uniform panel of epithelial markers used singly or in combination. Nonetheless this study highlights the importance of including epithelial markers in the diagnostic immunohistochemical assay of high grade sarcomas to verify tumour histogenesis.

REFERENCES

1. Fletcher CDM, Julia AB, Pancras CW, Fredrick M. WHO classification of tumour of soft tissue and bone, 2013

2. Coindre JM. Immunohistochemistry in the diagnosis of soft tissue tumours. Histopathology 2003; 43: 1-16

3. Sha L, Benjamin B, Marieke AG, Arjen HGC, Inge BB, Judith VGMB, Liam AMD. High grade sarcoma diagnosis and prognosis: Biomaker discovery by mass spectrometry imaging. Proteomics 2016; 16: 1802-1813.

4. Rita. A, Paola CR, Lor R, Phillip B, Lynn M. Undifferrentiated High Grade Pleomorphic Sarcomas in Children. Pediatric and Development Pathology 2010; 13: 209 – 217.

5. Martina S, Tiziana P, Bruna W, Vincenzo C, Alfonso C, Fabrizio I, Paola S. Expression profiles in malignant fibrous histiocytomas: Clues for differentiating 'spindle cell' and 'pleomorphic' subtypes. European Journal of Cancer 2008; 44: 298-309

6. Hasegawa T, Hasegawa F, Hirose T, Sano T, Matsuno Y. Expression of smooth muscle markers in so called malignant fibrous histiocytomas. Jurnal Clinical Pathology 2003; 56: 666-671.

7. Rosenberg AE, Connell JX, Dickersin GR, Bhan AK. Expression of epithelial markers in malignant fibrous histiocytoma of musculoskeletal system. Human pathology 1993; March 24 (3):284-293

8. Litzky LA, Brooks JJ. Cytokeratin immunoreactivity in malignant fibrous histiocytoma and spindle cell tumours: comparison between frozen and paraffin embedded tissues. Modern Pathology 1992; Jan; 5 (1): 30-4

9. Robert AE, Cristina RA. The rise and fall of Malignant Fibrous Histiocytoma. Ultrastructural Pathology 2004; 28:283-289

10. Paul HH, Gary B, James VH, Julie CF. Primary malignant fibrous histiocytoma (myxofibrosarcoma/pleomorphic sarcoma not otherwise specified) of breast: clinicopathologic study of 19 cases. Annals of Diagnostic Pathology 2011; 15: 407-413

11. Louis Guillou. Pleomorphic sarcomas: sub classification, myogenic differentiation and prognosis. Diagnostic Histopathology 2008; 14: 527-535.

12. Melike O, Mesut E, Emrah E, Sinan K, Dogan Y. et al. Evaluation of prognostic factors in localized high grade undifferentiated pleomorphic sarcoma. Tumour Biology 2015.

13. John R Goldblum. An approach to pleomorphic sarcomas. Modern Pathology 2014; 27: 539-546.

14. Igor Matushanky, Elizabeth C, Joslyn Mills, Sarra Siddiqi, Todd Hricik and Carlos Cordon-cardo. MFH classification : differentiating and undifferentiated pleomorphic sarcoma in the 21st Century. Expert review Anticancer Therapy. 2009; 9(8):1135-1144.

15. Salvatore R, Judith VM, Herman MK, Roberto T, Cristina N, Lucia Z. et al. Malignant fibrous histiocytoma and fibrosarcoma of bone. Virchows Arch 2012; 461: 561-570.

16. Robert DZ, Marcos M, Carlos Z, Edwin N, Dilara A, Melitina C, Jorge A. Primary mesenteric undifferentiated pleomorphic sarcoma masquerading as a Colon Carcinoma. A case report and literature review. Hindawi Publishing corporation case report in Oncological Medicine 2015.

17. Nicole AC, Pawel K, Rima A, Vikram D, Francis JH, John TM. Prognostic value of myogenic differentiation in undifferentiated pleomorphic sarcomas of soft tissue. Human Pathology 2014; 45:1504-1508.

18. Khin Thway, Cyril Fisher. Synovial sarcoma: defining features and diagnostic evolution. Annals of Diagnostic Pathology 2014; 18: 369-380.

19. Laila Chbani, Louis Guillou, Philippe Terrier, Anne VD, Fleur G, Marie Joe TL, Dominique R, Yves Marie et al. Epithelioid sarcoma; A clinicopathologic and Immunohistochemical analysis of 106 cases. American Journal Pathology 2009; 131: 222-227.

20. Jun Iwata and Christopher D.M Fletcher. Immunohistochemical detection of cytokeratin and epithelial membrane antigen in leiomyosarcoma. Pathology International 2000; 50: 7-14

21. Dabbs, David J. Diagnostic Immunohistochemistry Theranostic and Genomic Applications 3rd edition, 2010: 90-91.

22. Okado K, Hasegawa T, Yokoyama R, Itoi E. Osteosarcoma with cytokeratin expression. Jurnal Clinical Pathology 2003; 56:742-746

23. Nicole AC, Pawel K, Rima AA, Vikra D, Francis JH, John TM, Petur N. Prognostic value of myogenic differentiation in undifferentiated pleomorphic sarcomas of soft tissue. Human Pathology 2014; 45: 1504-1508.

24. Andrea Ferrari, Iyad Sultan, Tseng Tien Huang, Ahmad Shehadeh et al. Soft tissue sarcoma across the spectrum. Pediatric Blood Cancer 2011; 57: 943-949.

25. Roger N, Edward W, David P, Jayesh D, Nugroho P, Beena D, Richard Q. Soft tissue sarcoma in Asia Pacific Region: A systematic review. Asian Pacific Journal of Cancer Prevention 2013; 14: 6821-6832.

APPENDICES

1. RAW DATA
2. SPSS TABLE
3. ETHICS APPROVAL

Extension: **N-negative, F- focal, I-intermediate, D- diffuse**

Expression of Cytokeratin and EMA in high grade sarcoma cases 2005 to 2016

Bil	HPE No	P/R/M	Diagnosis	Age	Gender	Ethnic	Site	Size	EMA		MNF116		Others
1	10/2248	P	UNDIFF PLEOMORPHIC SARCOMA	41	F	M	RIGHT THIGH	10X5.5X5 (M)	P	WI	N	-	Vimentin : P
2	10/3816	R	HIGH GRADE SARCOMA	44	M	C	LEFT SHOULDER	2X1X0.5 (S)	N	-	X	X	Vimentin : P
3	10/4033	P	HGS suggest EPITHELIOID SARCOMA	53	M	C	LEFT THIGH (A) BUTTOCK MASS(B)	6X4X5 2.3X2.2X2 (M)	N	-	P	WF	Vimentin : P
4	10/4391	P	UNDIFF PLEOMORPHIC SARCOMA	74	M	C	LEFT CHEST WALL	12X12.5X3 (L)	N	-	P	WF	Vimentin : P
5	10/6737	R	PLEOMORPHIC SARCOMA	75	F	C	ANTERIOR CHEST WALL	14X6X6 (L)	P	WF	X	X	Vimentin : P
6	10/7795	P	PLEOMORPHIC HIGH GRADE SARCOMA	69	F	C	RIGHT THIGH	27.5X8.5X8.5 (L)	P	WF	N	-	(07/8950)
7	10/6351	P	MFH	80	M	C	LEFT FOREARM	5.5X4X2 (M)	N	-	X	X	Vimentin : P
8	11/721	P	PLEOMORPHIC HIGH GRADE SARCOMA	17	M	C	LEFT ILIOPSOAS	4X3X3 (S)	X	X	N	-	Vimentin : P
9	11/1394	P	HIGH GRADE SPINDLE CELL SARCOMA	51	M	C	LEFT THIGH	5.5X3.5X5 (M)	P	WF	N	-	Vimentin : P
10	11/2591	P	UNDIFF PLEOMORPHIC SARCOMA	53	M	M	LEFT FOREARM	12X12X5.5 (L)	X	X	N	-	Vimentin : P
11	1/2616	P	HIGH GRADE SARCOMA	16	M	M	LEFT HUMERUS	15.5 CM (L)	N	-	X	X	Vimentin : P

12	11/2954	P	PLEOMORPHIC SARCOMA	53	F	I	LEFT BREAST LUMP	9.5X8X6 (M)	P	WF	N	-	Vimentin : P
13	11/4171	P	UNDIFF PLEOMORPHIC SARCOMA	79	M	I	LEFT FOREARM	24X12X6 (L)	N	-	X	X	Vimentin : P
14	11/5311	P	MONOPHASIC SYNOVIAL SARCOMA	32	M	I	RIGHT AXILLARY	4X8X3.5 (M)	P	MI	X	X	Vimentin : P CK7: N
15	12/1118	P	HIGH GRADE SARCOMA	25	F	M	ANTERIOR NECK MASS	8X9X9 M	N	-	X	X	
16	12/2242	M	MET HIGH GRADE SARCOMA	53	M	M	RIGHT ILIAC BONE	11 CM IN DM (L)	P	WF	N	-	Vimentin : P
17	12/6061	P	PLEOMORPHIC SARCOMA	60	M	I	LEFT FEMUR	21X16X5 (L)	N	-	N	-	Vimentin : P
18	12/6331	P	HGS suggestive of MPNST	56	F	M	RIGHT NASAL MASS	11 CM (L)	N	-	N	-	Vimentin : P
19	12/6349	P	MFH	45	M	M	LEFT ANKLE	4.5X3.7X6	N	-	X	X	Vimentin : P
20	13/2876	P	UNDIFF PLEOMORPHIC SARCOMA	57	M	C	LEFT THIGH	23X22X7 (L)	N	-	X	X	Vimentin : P
21	14/7040	P	UNDIFF PLEOMORPHIC SARCOMA	51	M	C	RIGHT THIGH	15X12X7	X	X	N	-	Vimentin : P
22	15/7190	P	UNDIFF PLEOMORPHIC SARCOMA	59	M	M	RIGHT SHOULDER	3.2X3.3X2 (S)	X	X	N	-	Vimentin : P
23	14/885	P	HIGH GRADE SARCOMA	13	F	C	LEFT SUPRAKLAVIKULAR	10.5X11X6 (L)	N	-	N	-	AE1/3:N Vimentin : P
24	14/6649	P	HIGH GRADE SARCOMA	70	M	M	RIGH KNEE	6X3X2 (M)	X	X	N	-	-

25	14/10125	P	UNDIFF PLEOMORPHIC SARCOMA	66	M	C	RIGHT THIGH	17.5X11X7 (L)	X	X	N	-	-
26	15/8833	R	UNDIFF PLEOMORPHIC SARCOMA	59	M	C	LEFT THIGH	12X17 (L)	X	X	N	-	Vimentin : P
27	13/1103	P	HIGH GRADE PLEOMORPHIC SARCOMA	33	M	I	LEFT PELVIS	10X6X5 (L)	N	-	P	W F	AE1/3:W,F Vimentin : P
28	13/3475 (B)	P	HIGH GRADE PLEOMORPHIC SARCOMA	60	M	C	STOMACH	0.7X0.5X0.3 (S)	N	-	P	M D	AE1/3:M,D Vimentin : P
29	10/3500	P	PLEOMORPHIC SARCOMA	50	M	C	RIGHT THIGH	21X16X5 (L)	N	-	N	-	Vimentin : P
30	10/9678	P	UNDIFF PLEOMORPHIC SARCOMA	27	M	M	LEFT POPLITEAL	8.5X8.5X7.5 (M)	X	X	N	-	CK7: N -
31	12/9539	P	MFH	88	M	C	RIGHT LOWER LIMB	33X15X8 (L)	N	-	X	X	Vimentin : P
32	10/5828	P	MFH	72	M	C	ANTERIOR ABDOMINAL WALL	14.5X8X10 (L)	N	-	X	X	Vimentin : P
33	11/5070	R	MFH	71	M	C	LEFT SCAPULA/CLAVICLE	9X8.5X4.5 (M)	X	X	N	-	Vimentin : P
34	11/5073	P	MFH	51	M	M	LEFT FEMUR	13X10X5 CM	N	-	N	-	Vimentin : P
35	10/6515	P	HIGH GRADE SARCOMA	20	F	M	UTERUS (PARACERVICAL)	NO SIZE	N	-	N	-	Vimentin : P
36	11/722	P	HIGH GRADE SARCOMA	59	F	C	SIGMOID COLON N	4X3X2 (S)	P	W F	N	-	CK7/20:N Vimentin : P
37	10/6951	P	UTERINE SARCOMA	54	F	C	UTERUS (POST CERVICAL VAGINAL WALL)	17X12X18 C (L)	P	W F	N	-	Vimentin : P AE1/3:N MYOGENIN: N

38	15/8232	R	HGS Previous Phylloides	53	F	C	ANTERIOR CHEST WALL	3.5-2.5 CM (S)	X	X	N	-	AE1/3:N Vimentin : P
39	11/4234	P	PLEOMORPHIC SARCOMA	32	F	M	LEFT BREAST	3.5X3X2.5 (S)	N	-	N	-	Vimentin : P
40	13/5364	P	HIGH GRADE SARCOMA	51	F	M	LEFT BREAST	17X10X7 (L)	N	-	N	-	AE1/3:N Vimentin : P
41	12/532	P	HIGH GRADE SARCOMA	31	F	I	LEFT BREAST LUMP	4X3X2.5 (S)	X	X	N	-	Vimentin : P
42	10/7292	P	UNDIFF PLEOMORPHIC SARCOMA	31	M	C	RIGHT FOREARM	23X20X15 (L)	N	-	X	X	Vimentin : P
43	15/7362	M	MET PLEOMORPHIC LMS	62	F	C	LEFT ILIAC ACETABULUM	0.6X0.6X0.5 (S)	X	X	N	-	Vimentin : P Actin : F
44	11/103	R	EPITHELIOID SARCOMA	55	F	I	RIGHT WRIST	1 CM (S)	P	S D	X	X	Vimentin : P
45	14/7830	P	HGS SUGG PLEOMORPHIC LMS	59	F	M	RIGHT THIGH	30X13X13 (L)	P	W F	X	X	AE1/3:N Vimentin : P
46	16/3410	P	UNDIFF PLEOMORPHIC SARCOMA	34	F	C	LEFT PELVIS	7.5X10X7.5 (L)	X	X	N	-	Vimentin : P
47	16/7625 ©	P	UNDIFF PLEOMORPHIC SARCOMA	26	M	C	LEFT MAXILLARY	7.5X6.5X8 (M)	P	M F	P	M F	Vimentin : P
48	16/5199	P	HIGH GRADE SARCOMA	54	C	M	LEFT ANTERIOR THIGH	10X6X2.5 (L)	X	X	N	-	--
49	16/5181	P	SYNOVIAL SARCOMA	21	F	I	RIGHT KNEE	1.5X1X0.3 (S)	N	-	N	-	Vimentin : P
50	16/315 (B,C)	R	HIGH GRADE SARCOMA	60	F	C	NASAL SEPTUM	1X0.5X0.3 (S)	N	-	N	-	Vimentin : P

51	16/3229	M	UNDIFF PLEOMORPHIC SARCOMA	44	M	C	LUNG NODULE	1X0.7X0.7 (S)	N	-	X	X	AE1/3:N ---
52	09/1953	R	UNDIFF PLEOMORPHIC SARCOMA	85	F	C	LEFT ELBOW	3.5X2.5 (S)	N	-	X	X	---
53	09/1926	R	UNDIFF PLEOMORPHIC SARCOMA	72	F	C	THIGH	11X8X7 (L)	X	X	N	-	Vimentin : P
54	09/1457	P	PLEOMORPHIC SARCOMA	62	M	M	LEFT CALF	26X16X12 (L)	P	MD	N	-	Vimentin : P
55	09/3883	P	UNDIFF PLEOMORPHIC SARCOMA	21	M	C	PELVIS	13X8X7 (L)	N	-	X	X	-----
56	09/8199	R	HIGH GRADE SARCOMA	71	F	I	LEFT LEG	1.5X1X0.6 (S)	P	WF	N	-	Vimentin : P
57	09/5496	P	UNDIFF PLEOMORPHIC SARCOMA	70	M	C	LEFT THIGH	3 CM (S)	N	-	X	X	Vimentin : P
58	09/5063	P	MFH	63	F	C	RIGHT THIGH	3.8X2X2.5 (S)	N	-	N	-	Vimentin : P
59	09/9712 (B)	P	EPITHELIOID SARCOMA	19	M	M	FOREARM	2.5 CM (S)	X	X	P	SD	Vimentin : P CD99/CD34/ CD31:P
60	09/8801	P	MFH	54	M	M	RIGHT THIGH	6X7X5 (M)	N	-	N	-	Vimentin : P
61	09/6097	R	HIGH GRADE SARCOMA	45	F	M	LEFT CALF	1X1 (S)	X	X	N	-	Vimentin : P
62	09/5894	P	PLEOMORPHIC SARCOMA	73	M	M	RIGHT THIGH	9.5X5X5 (M)	P	MI	N	-	Vimentin : P
63	09/7836	P	PLEOMORPHIC SARCOMA	50	F	I	BREAST	20X15X7 (L)	X	X	N	-	Vimentin : P SMA:F

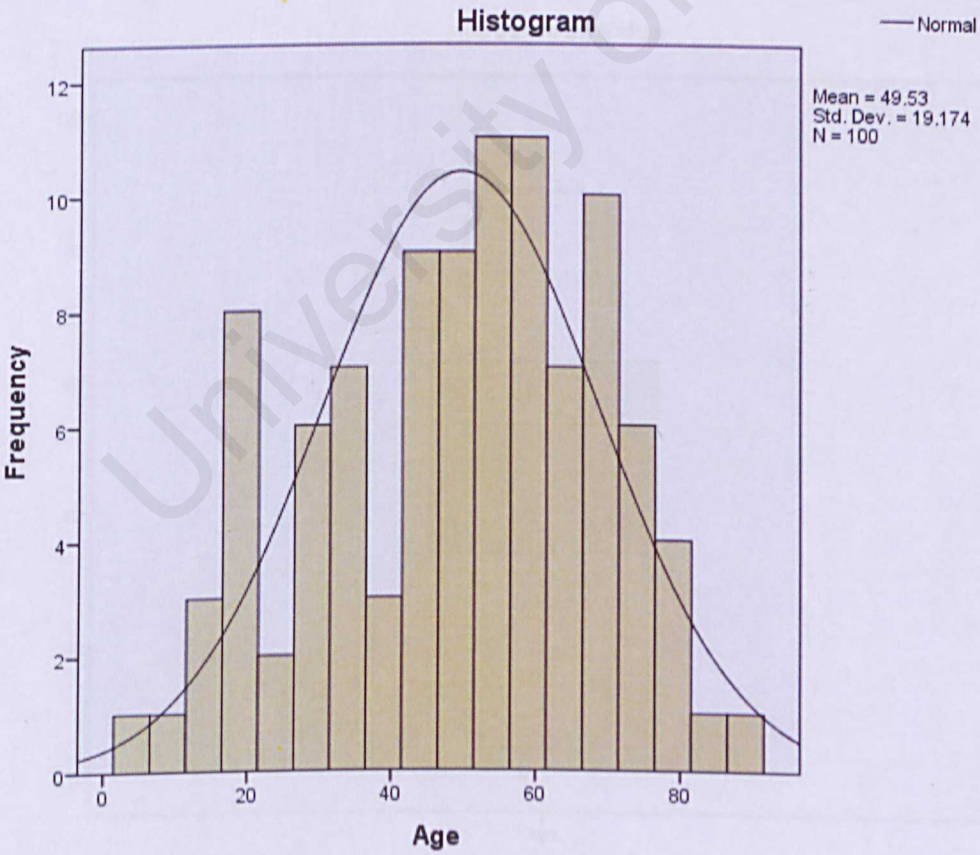
64	08/5794	P	PLEOMORPHIC SARCOMA	60	F	C	LEFT THIGH	6X4X2 (M)	N	-	X	X	Vimentin : P
65	16/8260 (B,C,F)	P	PLEOMORPHIC SARCOMA	38	F	M	ABDOMINAL WALL RECTAL OVARY	4X3X2 2.5X1.6X1.4 5.5X3.8X3.7 (M)	X	X	X	X	AE1/3:N ----
66	08/32	P	SYNOVIAL SARCOMA	46	F	M	xxxxxxx	16X9X9.5 (L)	-	WF	N	-	BCL2: P CD99:F Vimentin : P
67	08/10163 R	R	SYNOVIAL SARCOMA	28	M	I	LEFT LUNG	8.5X8X6.5 (M)	P	SD	x	x	Vimentin : P CD99:P
68	07/8339	P	PLEOMORPHIC SARCOMA	43	F	M	LEFT SHOULDER	8X6X5 (M)	N	-	N	-	BCL2 (F) ----
69	07/56	P	PLEOMORPHIC SARCOMA	34	F	M	RIGHT LEFT FIBULA	7X3X3 (M)	P	SF	X	X	Vimentin : P
70	06/9715	R	SPINDLE CELL SARCOMA	49	F	C	LEFT HIP	NO SIZE	N	-	X	X	Vimentin : P MPNST (062150)
71	05/6439	P	MFH	33	M	M	LEFT AKA	30X13X10 (L)	X	X	N	-	Vimentin : P
72	05/243	P	UNDIFF PLEOMORPHIC SARCOMA	57	F	I	LEFT ADNEXAL	5X4X2 (S)	N	-	N	-	Vimentin : P CK20/CK7:N
73	08/7039	P	BIPHASIC SYNOVIAL SARCOMA	46	F	M	RIGHT KNEE	6.5X5X3.5 (M)	P	SD	P	SF	CK7:P 5/6/CD99:F ----
74	16/6189	P	UNDIFF PLEOMORPHIC SARCOMA	4	F	C	POST NECK MASS	4X1X1.5 (S)	X	X	N	-	Vimentin : P
75	15/5670	M	HIGH GRADE SARCO MA	44	F	I	LUNG	3.5X1X2 (S)	X	X	N	-	Vimentin : P

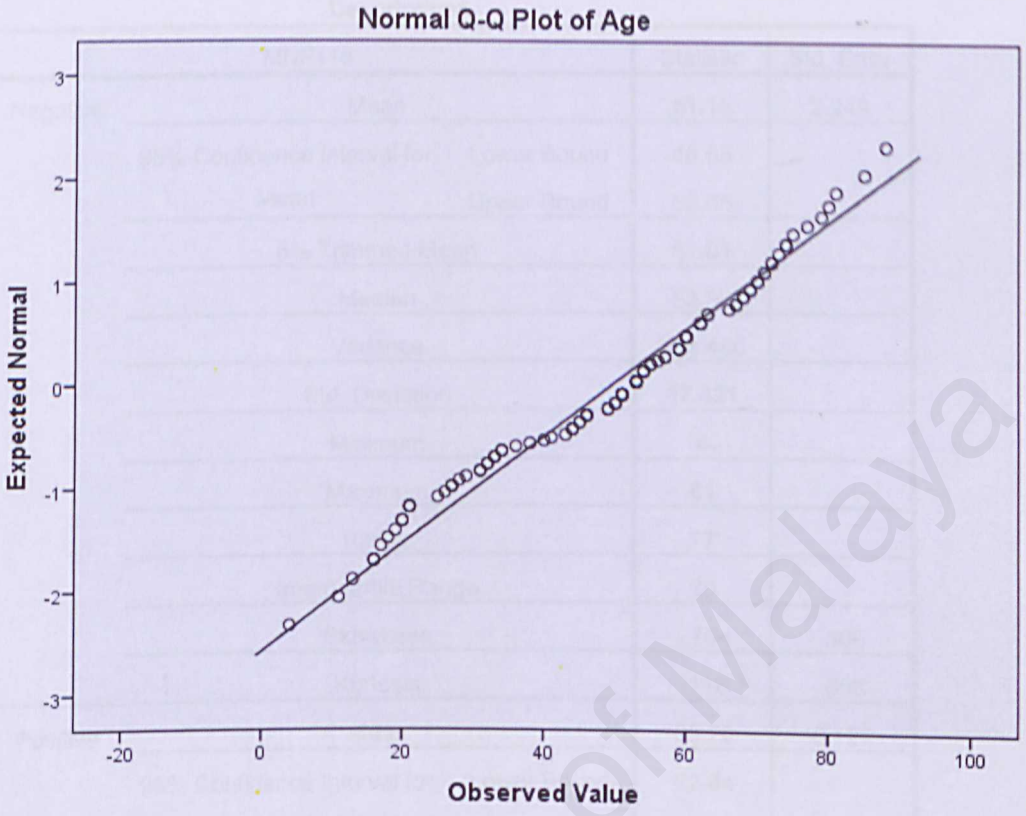
76	15/6568	M	HIGH GRADE SARCOMA	18	F	M	LUNG	1.8X1.5X1.5 (S)	X	X	N	-	Vimentin : P
77	15/6379	P	SPINDLE CELL SARCOMA	68	F	M	LEFT THIGH	14X8X6 (L)	X	X	N	-	Vimentin : P
78	15/620	R	RECURRENT SARCOMA	67	M	C	LEFT CALF	1X1X2 (S)	X	X	N	-	Vimentin : P
79	14/728	M	SARCOMA	62	F	M	HEAD	4X2X2 (S)	X	X	N	-	---
80	14/459	P	HIGH GRADE SPINDLE CELL SARCOMA	68	M	M	RIGHT AXILLARY	6X5X4 (M)	N	-	X	X	SMA: F ---
81	14/4715	P	PLEOMORPHIC SARCOMA	67	M	C	RIGHT THIGH	2X2X2 (S)	N	-	X	X	Vimentin : P
82	13/2648	P	MFH	77	F	M	LEFT THIGH	9X8X6 (M)	X	X	N	-	Vimentin : P
83	13/4231	P	HIGH GRADE SARCOMA	69	M	C	RIGHT ANKLE	4.5X6X8 (M)	X	X	N	-	Vimentin : P
84	13/7123	R	PLEOMORPHIC SARCOMA	49	F	C	LEFT ILIAC	5.5X3.5X4 (S)	N	-	X	X	CD99:P ----
85	13/2420	R	SYNOVIAL SARCOMA	46	M	I	RIGHT ELBOW	1.2X2X1.5 (S)	N	-	X	X	CD99,BCL2: F Vimentin : P
86	12/699	P	LMS	60	M	M	PERITONEUM	10X10X5 (L)	N	-	N	-	SMA:F, Desmin: F ----
87	12/1604	R	MFH	62	M	I	RIGHT FEMUR	4X3.5X3 (S)	N	-	X	X	---
88	12/7506	P	MPNST	27	M	M	RIGHT AXILLA	16X13X10 (L)	N	-	X	X	S100:F Vimentin : P

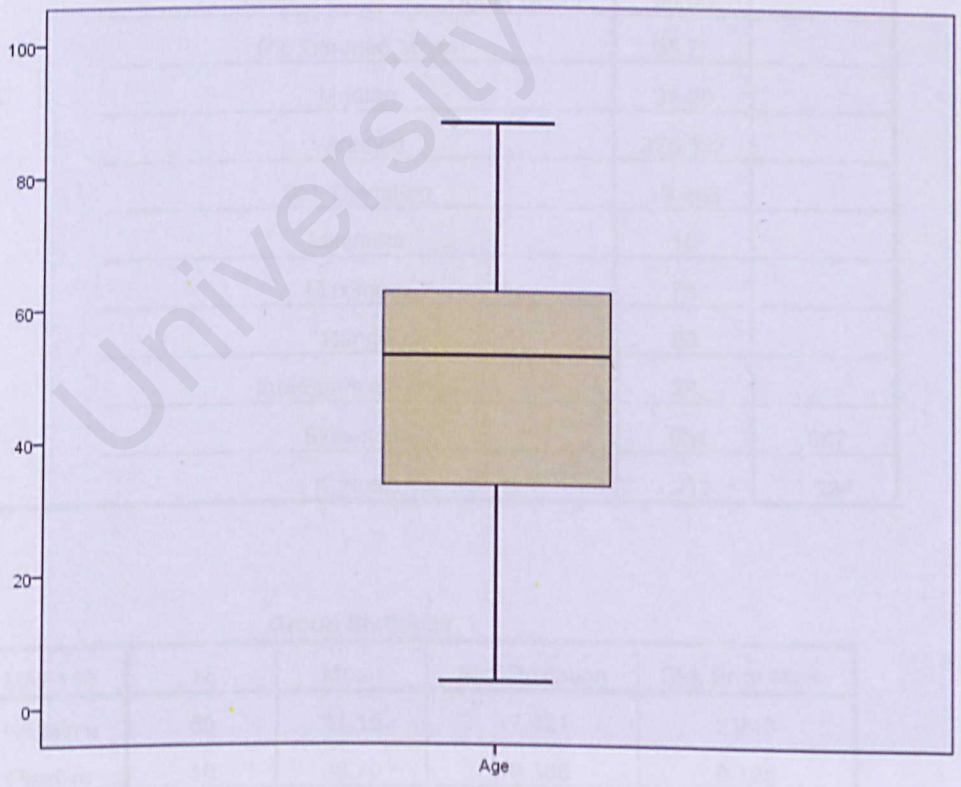
89	13/2010	P	SYNOVIAL SARCOMA	55	F	I	RIGHT AKA	22X9X30 (L)	P	w F	N	-	BCL,CD99:P
90	10/8519	P	SYNOVIAL SARCOMA	21	F	M	LEFT KNEE	8X4X2.5 (M)	P	W F	P	I W	Vimentin : P
91	11/6246	P	SYNOVIAL SARCOMA	20	M	M	LEFT FOOT	16X14X12 (L)	P	W F	N	-	Vimentin : P
92	11/7902	P	SYNOVIAL SARCOMA	11	F	C	LEFT THIGH	9X8X5 (M)	P	W F	X	X	AE1/3:N CK7:P BCL2,CD99: Vimentin : P
93	13/215	P	SYNOVIAL SARCOMA	29	M	M	LEFT THIGH	6X5.5X4 (M)	X	X	P	M I	Vimentin : P
94	10/6398	P	MFH	51	F	M	RIGHT THIGH	18X11X5	X	X	N	-	Vimentin : P
95	10/4038	P	PLEOMORPHIC SPINDLE CELL SARCOMA	74	F	M	RIGHT THIGH	11X6X3.5	X	X	N	-	Vimentin : P
96	14/3903	P	POORLY DIFF SARCOMA	40	F	M	RIGHT KNEE	5.5X5	N	-	N	-	Vimentin : P
97	10/944	P	PLEOMORPHIC SARCOMA	81	F	C	LEFT THIGH	7X4X7	P	M D	N	-	Vimentin : P
98	07/4996 (B)	P	MFH	63	M	M	LEFT SCAPULA	17X12X5.5	N	-	N	-	Vimentin : P
99	14/4082	P	EPITHELIOD SARCOMA	16	M	I	FINGER	1.2 CM	P	S D	P	W I	Vimentin : P
100	07/925 Chong	P	HIGH GRADE SARCOMA	36	F	C	LEFT THUMB	3X2.5X1	P	S D	P	M I	Vimentin : P

Descriptives

			Statistic	Std. Error
Age	*Mean*		*49.53*	1.917
	95% Confidence Interval for Mean	Lower Bound	45.73	
		Upper Bound	53.33	
	5% Trimmed Mean		49.78	
	Median		53.00	
	Variance		367.625	
	Std. Deviation		*19.174*	
	Minimum		4	
	Maximum		88	
	Range		84	
	Interquartile Range		30	
	Skewness		-.315	.241
	Kurtosis		-.683	.478







MNF116 EXPRESSION

Descriptives

	MNF116		Statistic	Std. Error
Age	Negative	Mean	51.15	2.249
		95% Confidence Interval for Mean — Lower Bound	46.65	
		95% Confidence Interval for Mean — Upper Bound	55.65	
		5% Trimmed Mean	51.91	
		Median	53.50	
		Variance	303.486	
		Std. Deviation	17.421	
		Minimum	4	
		Maximum	81	
		Range	77	
		Interquartile Range	20	
		Skewness	-.784	.309
		Kurtosis	.118	.608
	Positive	Mean	36.70	6.125
		95% Confidence Interval for Mean — Lower Bound	22.84	
		95% Confidence Interval for Mean — Upper Bound	50.56	
		5% Trimmed Mean	35.78	
		Median	31.00	
		Variance	375.122	
		Std. Deviation	19.368	
		Minimum	16	
		Maximum	74	
		Range	58	
		Interquartile Range	34	
		Skewness	.934	.687
		Kurtosis	-.213	1.334

Group Statistics

	MNF116	N	Mean	Std. Deviation	Std. Error Mean
Age	Negative	60	51.15	17.421	2.249
	Positive	10	36.70	19.368	6.125

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Age	Equal variances assumed	.297	.588	2.391	68	.020	14.450	6.043	2.392	26.508
	Equal variances not assumed			2.215	11.559	.048	14.450	6.525	.174	28.726

Crosstab

			MNF116		Total
			Negative	Positive	
Gender	Male	Count	24	8	32
		% within Gender	75.0%	25.0%	100.0%
	Female	Count	36	2	38
		% within Gender	94.7%	5.3%	100.0%
Total		Count	60	10	70
		% within Gender	85.7%	14.3%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	5.526[a]	1	.019		
Continuity Correction[b]	4.032	1	.045		
Likelihood Ratio	5.756	1	.016		
Fisher's Exact Test				.036	.021
Linear-by-Linear Association	5.447	1	.020		
N of Valid Cases	70				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.57.

b. Computed only for a 2x2 table

Crosstab

			MNF116		
			Negative	Positive	Total
Location_Gr	Extremities	Count	42	7	49
		% within Location_Gr	85.7%	14.3%	100.0%
	Non-extremities	Count	17	3	20
		% within Location_Gr	85.0%	15.0%	100.0%
Total		Count	59	10	69
		% within Location_Gr	85.5%	14.5%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.006[a]	1	.939		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.006	1	.939		
Fisher's Exact Test				1.000	.603
Linear-by-Linear Association	.006	1	.939		
N of Valid Cases	69				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.90.

b. Computed only for a 2x2 table

Crosstab

			MNF116		
			Negative	Positive	Total
Origin_Gr	Primary	Count	47	10	57
		% within Origin_Gr	82.5%	17.5%	100.0%
	Recurrent and Metastasis	Count	13	0	13
		% within Origin_Gr	100.0%	0.0%	100.0%
Total		Count	60	10	70
		% within Origin_Gr	85.7%	14.3%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.661[a]	1	.103		
Continuity Correction[b]	1.421	1	.233		
Likelihood Ratio	4.474	1	.034		
Fisher's Exact Test				.190	.109
Linear-by-Linear Association	2.623	1	.105		
N of Valid Cases	70				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 1.86.

b. Computed only for a 2x2 table

Crosstab

			MNF116		Total
			Negative	Positive	
Size	Small (<5cm)	Count	16	4	20
		% within Size	80.0%	20.0%	100.0%
	Medium (5-10cm)	Count	15	4	19
		% within Size	78.9%	21.1%	100.0%
	Large (>10cm)	Count	28	2	30
		% within Size	93.3%	6.7%	100.0%
	Total	Count	59	10	69
		% within Size	85.5%	14.5%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	2.936(a)	4	.569	.593		
Likelihood Ratio	3.002	4	.557	.596		
Fisher's Exact Test	3.106			.548		
Linear-by-Linear Association	1.843(b)	1	.175	.179	.101	.025
N of Valid Cases	67					

a 3 cells (33.3%) have expected count less than 5. The minimum expected count is 2.15.

b The standardized statistic is -1.357.

AE1/AE3 EXPRESSION

Descriptives

	AE1_AE3		Statistic	Std. Error
Age	Negative	Mean	40.38	6.595
		95% Confidence Interval for Mean — Lower Bound	24.78	
		95% Confidence Interval for Mean — Upper Bound	55.97	
		5% Trimmed Mean	40.97	
		Median	47.50	
		Variance	347.982	
		Std. Deviation	18.654	
		Minimum	11	
		Maximum	59	
		Range	48	
		Interquartile Range	35	
		Skewness	-.987	.752
		Kurtosis	-.655	1.481
	Positive	Mean	46.50	13.500
		95% Confidence Interval for Mean — Lower Bound	-125.03	
		95% Confidence Interval for Mean — Upper Bound	218.03	
		5% Trimmed Mean	.	
		Median	46.50	
		Variance	364.500	
		Std. Deviation	19.092	
		Minimum	33	
		Maximum	60	
		Range	27	
		Interquartile Range	.	
		Skewness	.	.
		Kurtosis	.	.

Group Statistics

	AE1_AE3	N	Mean	Std. Deviation	Std. Error Mean
Age	Negative	8	40.38	18.654	6.595
	Positive	2	46.50	19.092	13.500

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Age	Equal variances assumed	.031	.866	-.414	8	.690	-6.125	14.791	-40.234	27.984
	Equal variances not assumed			-.408	1.522	.734	-6.125	15.025	-94.724	82.474

Crosstab

			AE1_AE3		
			Negative	Positive	Total
Gender	Male	Count	1	2	3
		% within Gender	33.3%	66.7%	100.0%
	Female	Count	7	0	7
		% within Gender	100.0%	0.0%	100.0%
Total		Count	8	2	10
		% within Gender	80.0%	20.0%	100.0%

Crosstab

			AE1_AE3		
			Negative	Positive	Total
Location_Gr	Extramities	Count	3	1	4
		% within Location_Gr	75.0%	25.0%	100.0%
	Non-extramities	Count	5	1	6
		% within Location_Gr	83.3%	16.7%	100.0%
Total		Count	8	2	10
		% within Location_Gr	80.0%	20.0%	100.0%

Crosstab

			AE1_AE3		
			Negative	Positive	Total
Origin_Gr	Primary	Count	6	2	8
		% within Origin_Gr	75.0%	25.0%	100.0%
	Recurrent and Metastasis	Count	2	0	2
		% within Origin_Gr	100.0%	0.0%	100.0%
Total		Count	8	2	10
		% within Origin_Gr	80.0%	20.0%	100.0%

Crosstab

			AE1_AE3		
			Negative	Positive	Total
Size	Small (<5cm)	Count	2	1	3
		% within Size	66.7%	33.3%	100.0%
	Medium (5-10cm)	Count	2	0	2
		% within Size	100.0%	0.0%	100.0%
	Large (>10cm)	Count	4	1	5
		% within Size	80.0%	20.0%	100.0%
Total		Count	8	2	10
		% within Size	80.0%	20.0%	100.0%

EMA EXPRESSION

Descriptives

	EMA		Statistic	Std. Error
Age	Negative	Mean	**51.14**	2.889
		95% Confidence Interval for Mean — Lower Bound	45.31	
		95% Confidence Interval for Mean — Upper Bound	56.97	
		5% Trimmed Mean	51.21	
		Median	53.00	
		Variance	358.790	
		Std. Deviation	**18.942**	
		Minimum	**13**	
		Maximum	**88**	
		Range	75	
		Interquartile Range	23	
		Skewness	-.221	.361
		Kurtosis	-.506	.709
	Positive	Mean	**47.19**	3.817
		95% Confidence Interval for Mean — Lower Bound	39.33	
		95% Confidence Interval for Mean — Upper Bound	55.05	
		5% Trimmed Mean	47.34	
		Median	52.00	
		Variance	378.802	
		Std. Deviation	**19.463**	
		Minimum	**11**	
		Maximum	**81**	
		Range	70	
		Interquartile Range	29	
		Skewness	-.179	.456
		Kurtosis	-.875	.887

Group Statistics

	EMA	N	Mean	Std. Deviation	Std. Error Mean
Age	**Negative**	**43**	**51.14**	**18.942**	2.889
	Positive	**26**	**47.19**	**19.463**	3.817

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Age	Equal variances assumed	.157	.694	.830	67	.409	3.947	4.754	-5.543	13.437
	Equal variances not assumed			.825	51.735	.413	3.947	4.787	-5.659	13.554

Gender * EMA Crosstabulation

			EMA		Total
			Negative	Positive	
Gender	Male	Count	28	9	37
		% within Gender	75.7%	24.3%	100.0%
	Female	Count	15	17	32
		% within Gender	46.9%	53.1%	100.0%
Total		Count	43	26	69
		% within Gender	62.3%	37.7%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6.061[a]	1	.014		
Continuity Correction[b]	4.897	1	.027		
Likelihood Ratio	6.132	1	.013		
Fisher's Exact Test				.024	.013
Linear-by-Linear Association	5.973	1	.015		
N of Valid Cases	69				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 12.06.

b. Computed only for a 2x2 table

Site_Location * EMA Crosstabulation

			EMA		
			Negative	Positive	Total
Site_Location	Extremities	Count	31	19	50
		% within Site_Location	62.0%	38.0%	100.0%
	Trunk	Count	2	1	3
		% within Site_Location	66.7%	33.3%	100.0%
	Intra-abdomen	Count	2	1	3
		% within Site_Location	66.7%	33.3%	100.0%
	Head and neck	Count	3	1	4
		% within Site_Location	75.0%	25.0%	100.0%
	Others	Count	5	3	8
		% within Site_Location	62.5%	37.5%	100.0%
Total		Count	43	25	68
		% within Site_Location	63.2%	36.8%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.303[a]	4	.990
Likelihood Ratio	.318	4	.989
Linear-by-Linear Association	.068	1	.794
N of Valid Cases	68		

a. 7 cells (70.0%) have expected count less than 5. The minimum expected count is 1.10.

Origin_Gr * EMA Crosstabulation

			EMA		
			Negative	Positive	Total
Origin_Gr	Primary	Count	35	21	56
		% within Origin_Gr	62.5%	37.5%	100.0%
	Recurrent and Metastasis	Count	8	5	13
		% within Origin_Gr	61.5%	38.5%	100.0%
Total		Count	43	26	69
		% within Origin_Gr	62.3%	37.7%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.004[a]	1	.949		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.004	1	.949		
Fisher's Exact Test				1.000	.593
Linear-by-Linear Association	.004	1	.949		
N of Valid Cases	69				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.90.

b. Computed only for a 2x2 table

Size * EMA Crosstabulation

			EMA		Total
			Negative	Positive	
Size	Small (<5cm)	Count	13	5	18
		% within Size	72.2%	27.8%	100.0%
	Medium (5-10cm)	Count	10	11	21
		% within Size	47.6%	52.4%	100.0%
	Large (>10cm)	Count	18	10	28
		% within Size	64.3%	35.7%	100.0%
Total		Count	41	26	67
		% within Size	61.2%	38.8%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	2.664[a]	2	.264
Likelihood Ratio	2.662	2	.264
Linear-by-Linear Association	.117	1	.733
N of Valid Cases	67		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.99.

CYTOKERATIN (MNF116 & AE1/AE3) AND EMA AND TUMOUR HISTOLOGICAL TYPE

AE1_AE3 *Diagnosis_Gr Crosstabulation

			Diagonosis_Gr		
			No-specific differentiation	Specific differentiation	Total
AE1_AE3	Negative	Count	5	3	8
		% within AE1_AE3	62.5%	37.5%	100.0%
	Positive	Count	2	0	2
		% within AE1_AE3	100.0%	0.0%	100.0%
Total		Count	7	3	10
		% within AE1_AE3	70.0%	30.0%	100.0%

EMA * Diagnosis__Gr Crosstabulation

			Diagonosis_Gr		
			No-specific differentiation	Specific differentiation	Total
EMA	Negative	Count	36	7	43
		% within EMA	83.7%	16.3%	100.0%
	Positive	Count	14	12	26
		% within EMA	53.8%	46.2%	100.0%
Total		Count	50	19	69
		% within EMA	72.5%	27.5%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	7.247[a]	1	.007		
Continuity Correction[b]	5.827	1	.016		
Likelihood Ratio	7.119	1	.008		
Fisher's Exact Test				.012	.008
Linear-by-Linear Association	7.142	1	.008		
N of Valid Cases	69				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 7.16.

b. Computed only for a 2x2 table

MNF116 * Diagnosis_Gr Crosstabulation

			Diagonosis_Gr		
			No-specific differentiation	Specific differentiation	Total
MNF116	Negative	Count	51	9	60
		% within MNF116	85.0%	15.0%	100.0%
	Positive	Count	5	5	10
		% within MNF116	50.0%	50.0%	100.0%
Total		Count	56	14	70
		% within MNF116	80.0%	20.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6.563[a]	1	.010		
Continuity Correction[b]	4.557	1	.033		
Likelihood Ratio	5.468	1	.019		
Fisher's Exact Test				.022	.022
Linear-by-Linear Association	6.469	1	.011		
N of Valid Cases	70				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.00.

b. Computed only for a 2x2 table

UNIVERSITY OF MALAYA MEDICAL CENTRE

MEDICAL RESEARCH ETHICS COMMITTEE
(Formerly known as Medical Ethics Committee)
UNIVERSITY OF MALAYA MEDICAL CENTRE
ADDRESS : LEMBAH PANTAI, 59100 KUALA LUMPUR, MALAYSIA
TELEPHONE : 03-79493209/2251 FAXIMILE : 03-79492030

NAME OF ETHICS COMMITTEE/IRB Medical Research Ethics Committee, University Malaya Medical Centre	**MREC ID NO**: 20161031-4469
ADDRESS : LEMBAH PANTAI, 59100 KUALA LUMPUR, MALAYSIA	
PROTOCOL NO(if applicable) :	
TITLE: Expression of cytokeratin and EMA in high grade sarcoma cases	
PRINCIPAL INVESTIGATOR : Master Student Siti Aishah Mahamad Dom	**SPONSOR**

The following item [✓] have been received and reviewed in connection with the above study to conducted by the above investigator.

[✓] Application to Conduct Research Project(form)	Ver.No :	Ver.Date : 01-11-2016
[✓] Study Protocol	Ver.No : 1	Ver.Date : 03-11-2016
[ ] Patient Information Sheet	Ver.No :	Ver.Date :
[ ] Consent Form	Ver.No :	Ver.Date :
[ ] Questionnaire	Ver.No :	Ver.Date :
[✓] Investigator's CV / GCP (Master Student Siti Aishah Mahamad Dom,Prof Nazarina Abdul Rahman,)	Ver.No :	Ver.Date :
[ ] Insurance certificate	Ver.No :	Ver.Date :
[✓] Other documents		

and the decision is [✓]

[ ] Approved (Full Board)
[✓] Approved (Expedited)
[ ] Rejected(reasons specified below or in accompanying letter)

Comments:

Lab-based study on archived material.

The Investigators are required to:

1) *follow instructions, guidelines and requirements of the Medical Research Ethics Committee.*
2) *report any protocol deviations/violations to Medical Research Ethics Committee.*
3) *provide annual and closure report to the Medical Research Ethics Committee.*
4) *comply with International Conference on Harmonization – Guidelines for Good Clinical Practice (ICH-GCP) and Declaration of Helsinki.*
5) *obtain a permission from the Director of UMMC to start research that involves recruitment of UMMC patient.*
6) *ensure that if the research is sponsored, the usage of consumable items and laboratory tests from UMMC services are not charged in the patient's hospital bills but are borne by research grant.*
7) *note that he/she can appeal to the Chairman of Medical Research Ethics Committee for studies that are rejected.*
8) *note that Medical Research Ethics Committee may audit the approved study.*
9) *ensure that the study does not take precedence over the safety of subjects.*

Date of expedited approval : 23-11-2016

This is a computer generated letter. No signature required.