Original Article

Role of immunohistochemistry in diagnosing round-cell tumours affecting the oral and maxillofacial regions

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Abstract

Background: One of the most challenging spectra of lesions in the oral and maxillofacial region (OMFR) are round-cell tumours (RCTs). They show a considerable degree of overlap in microscopy and immunophenotypes. The main aim of this study is to analyse the spectrum of RCTs encountered in the oral and maxillofacial regions. We emphasise the role of immunohistochemistry (IHC) which in conjunction with histological, clinical, and imaging findings is necessary for their correct characterisation. The secondary objectives are to discuss differential diagnosis, workflow, and diagnostic algorithm for round-cell lesions affecting the OMFR.

Methods: Formalin-fixed, paraffin-embedded sections of RCTs were retrieved from the archives of the Department of Oral Pathology (January 2018 to March 2020). These cases were analysed by three pathologists independently by evaluating haematoxylin and eosin-stained sections, and immunohistochemical markers employed to characterise these lesions.

Results: Under the spectrum of RCTs, 11 cases (0.53%) were diagnosed with a predominance of non-Hodgkin lymphoma (55%) followed by Ewing sarcoma (18%). The remaining were Langerhans cell histiocytosis (9%), neuroendocrine carcinoma (9%), and sinonasal undifferentiated carcinoma (9%). Except for one case, in all cases, the final diagnosis was established with the use of adjunctive IHC.

Conclusion: RCTs can pose a diagnostic challenge for inexperienced oral pathologists. Thorough knowledge of the differentials of RCT occurring in oral and maxillofacial is helpful. An algorithm-based diagnostic approach incorporating the clinical, imaging, and histomorphological findings and immunohistochemical evaluation can help in minimizing diagnostic confusion and errors.

Keywords: Diagnostic approach, immunohistochemistry, round-cell tumours, oral and maxillofacial region, workflow

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INTRODUCTION

Round-cell tumours (RCTs), although rare, encompass a wide spectrum of highly aggressive malignant tumours exhibiting similar cytomorphology and varying origins.[1] They exhibit monomorphic small to large round cells with hyperchromatic nuclei, increased nuclear–cytoplasmic ratio,

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and no definitive line of differentiation. Their spectrum affecting the oral and maxillofacial region (OMFR) generally includes rhabdomyosarcoma (RMS), Ewing family of tumours, haematolymphoid malignancies, neuroblastoma, mucosal melanoma, small-cell osteosarcoma, and mesenchymal chondrosarcoma. Additionally, the sinonasal region harbours heterogeneous tumours such as sinonasal undifferentiated carcinoma (SNUC) and neuroendocrine carcinomas (NECs). The differential diagnosis also includes tumours that can rarely manifest in the OMFR, namely, poorly differentiated synovial sarcoma, round-cell liposarcoma, and desmoplastic small round-cell tumour (DSRCT).[2-4] Rarely, Merkel cell carcinoma can affect mucosal sites (4.5%) including the oral cavity, although it primarily involves the skin of the head and neck regions with rare lymph node involvement.[5]

These tumours display various diagnostic challenges including overlapping clinical, imaging, and microscopic features with a lack of specific immunohistochemical markers; and considerable variation within and amongst different subtypes. Furthermore, biopsies are small and perilous in these lesions due to the chances of troublesome haemorrhage. Thus, accurate diagnosis of these lesions remains a challenge. However, conclusive diagnosis is of utmost importance, which substantially decides the treatment and prognostication. The demographics, imaging, and microscopic details are invaluable in shortlisting the differential diagnoses. These are further evaluated and ruled in or excluded by using an appropriate panel of immunohistochemical markers.[3]

The aim of this article is to analyse the spectrum of RCTs affecting the OMFR which were encountered in a referral centre. It emphasises the diagnostic role of adjuvant immunohistochemistry (IHC) informed by the basic histomorphological, clinical, and radiological findings. We also discuss the differential diagnoses and propose a diagnostic algorithm for RCTs affecting the oral and maxillofacial regions [Table 1 and Figure 1].

MATERIAL AND METHODS

The study was conducted in accordance with the Declaration of Helsinki and was reviewed and approved by the Institutional Ethics committee. All patients enrolled completed the informed consent form. Formalin-fixed, paraffin-embedded sections of round-cell malignancies were retrieved from the archives of the department of oral pathology of a tertiary care centre (from January 2018 to March 2020). All round-cell malignancies affecting the oral and maxillofacial regions were included in the study irrespective of age, sex, soft tissue, or bone involvement. Bony tissues were subjected to decalcification before routine processing. These cases were analysed by three pathologists independently on the basis of haematoxylin and eosin (H&E)-stained sections and re-evaluation of immunohistochemical markers. Information about the clinical history and other pertinent details were retrieved from the case files of patients. The final diagnosis was decided on the basis of histomorphology supplemented by an array of immunohistochemical markers (the IHC panel employed in the study is given in Table S-1) and integration of available clinical, imaging, and laboratory findings. Furthermore, the treatment profile and follow-up status were also analysed.

Inclusion criteria

All confirmed cases of round-cell malignancies affecting the OMFR (bone and soft tissues) with available records were included in the study.

Exclusion criteria

Cases with non-availability of patient case records were excluded from the study.

RESULTS

Demographics

11 cases (0.53%) were diagnosed as RCTs in our department out of a total of 2066 biopsies received from January 2018 to March 2020. The spectrum included 7 haematolymphoid malignancies comprising NHL (6 cases) and LCH (1; 9%), 2 ESs (18%) and 2 carcinomas including sinonasal NEC (1 case; 9%) and SNUC (1; 9%) [Table 2]. The median age of the patients was 41 years (range 2–73 years) with male predominance (73%). The youngest patient was diagnosed with ES, and the eldest one had NHL. The mandible was the predominant site of involvement (55%), followed by maxilla (36%), and 1 case (LCH) showed diffuse involvement of both jaws. 10/11 cases were categorised with an appropriate panel of immunohistochemical markers; however, for 1 case, diagnosis was rendered on the basis of H&E-stained sections as insufficient tissue was available for immunophenotyping.

Radiology

Osteolytic permeative lesions involving bone and soft tissue with predominant bone involvement were present in LCH, Burkitt lymphoma (BL), plasmablastic plasma cell myeloma (PCM), and ES. An osteolytic lesion with extensive soft tissue components mimicking chronic osteomyelitis was seen in diffuse large B-cell lymphoma (DLBCL). Plasmablastic lymphoma (PBL) and HGBLNOS showed a non-specific generalised bone loss. SNUC exhibited

Table 1: Differential diagnosis of round-cell lesions of the OMFR

	RMS[1] (ERMS and ARMS)	Ewing's sarcoma/ PNET	NHL	LCH	Round-cell liposarcoma (rare variant of myxoid liposarcoma)[2]	Small-cell osteosarcoma	Mesenchymal chondrosarcoma
Age (yrs)	ERMS#: 8–12 yrs ARMS: 10-25 yrs	<30 yrs	Variable in different subtypes	Wide age range peak 3-5 yrs Mostly <15 yrs	Adults, rarely children (mean 60 yrs)	Children and adolescents (jaws-10–20 years younger than peripheral counterparts)	Children and young adults
Gender	M=F	M >F	M >F	M >F	M >F	M=F	M=F
Site	Palate and Paranasal sinus >nasal cavity[1]	2–10% cases in H & N [skull, 1–2% in jaws (mandible>maxilla) and sinonasal tract] and extraskeletal tumours affecting soft tissues	Lymph node >GIT >Waldayer ring >oral cavity	Single site or multiple sites within same organ or widely disseminated multiple system involvement. 60–80% in H & N (jaw bones in 10–20% cases, cervical lymph nodes, paranasal sinus and oral mucosa) and in 25% cases it is a part of multisystem disease.	Lower limbs (thigh) are most common site. IRarely seen intraorally (tongue is the most common)	Metaphysis of long bones>jaws (6% cases; mandible commoner)	Craniofacial bones (jaws), ribs, ilium and soft tissues
Imaging studies	Depends on size of tumour	B>>S Ill-defined osteolytic permeative (onion skin appearance in long bones seldom in jaw bones) accompanying soft tissue mass in 90% cases	S>>B Soft tissue mass with non-specific radiological destruction Periosteal reaction+/–	Sharply punched out and rarely ill-defined radiolucencies Scooped out appearance in mandible or extensive alveolar involvement leading to teeth 'floating in air' appearance.	Depends on size of tumour	B>>S Usually mixed, lytic and blastic pattern, permeative lesion (sunburn-like) with soft tissue component	B>>S Destructive lesion (eccentric with stippled calcifications) with soft tissue extension
Pattern	Sheets, alveolar	Sheets, nests, lobular, trabecular, cord-like, rosettes	Usually diffuse pattern, but can also invade bone in the form of nests, cords or sheets subtypes common in the OMFR: DLBCL >MALT lymphoma >Extranodal NK/T-cell lymphoma >MCL >FL >PTCLNOS BL: rare in adults, common in children	Diffuse infiltration of pale staining histiocytic cells, admixture of variable eosinophils, other inflammatory cells and occasional MGCs.	Hypercellular or round-cell pattern begin as perivascular distribution (delicate vascular plexiform pattern-chicken wire or crow's feet is less prominent than myxoid)	Sheets with usually scanty osteoid occasionally mixed with cartilage. 3 histological patterns- ES-like, lymphoma-like and small spindle cell.	Biphasic appearance of round cells and cartilaginous differentiation, with HPC-like pattern (spindled morphology)
Morphology/ cytology	Round, strap, spindled, rhabdomyoblasts with eosinophilic cytoplasm and fibrillar material around nucleus	Uniform small to medium round cells well delineated nuclear outlines, fine chromatin and ill-defined cellular borders, 75% cases have glycogen granules in cytoplasm	Round cells (small to intermediate to large) with hyperchromatic nuclei and scanty cytoplasm	Cells have indistinct cytoplasmic borders and rounded or indented/ grooved/vesicular nuclei (coffee bean appearance) with minimal atypia.	Small, round or spindled cells with sparse eosinophilic and granular cytoplasm and large nuclei, scattered lipoblasts, variable background vacuoles, intracytoplasmic vacuoles, fragments of myxoid stroma with neoplastic cells	Small to intermediate uniform round/spindle cells with scanty cytoplasm	Round cells with ovoid and hyperchromatic nuclei, scanty cytoplasm, occasionally spindled morphology
Mitosis	Variable	Common	Variable	Very brisk but no atypical mitosis	Frequent	Variable as per grade	Common
Necrosis	Limited	Frequent	Variable	Limited	Frequent	Variable as per grade	Infrequent
PanCK	S (up to 10%, P & D)	R (<30% cases) (+ in adamantinoma-like variant)	-	-	-	R	-/O (<5% cases)

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Table 1: Contd...

	RMS[1] (ERMS and ARMS)	Ewing's sarcoma/ PNET	NHL	LCH	Round-cell liposarcoma (rare variant of myxoid liposarcoma)[2]	Small-cell osteosarcoma	Mesenchymal chondrosarcoma
EMA/MUC1	R (<1%)	R (<20% cases) (+ in adamantinoma-like variant)	S	-	S[4]	-[5]	-
p40[6]	-	- (+ in adamantinoma-like variant)	-	-	NR	NR	NR
Synaptophysin/ Chromogranin	S/R (F)	+ (65%)	-	-	NR	-	-
S 100	R (<10%)	S (up to 30% cases show F)	-	+	+	S	- (+ only in cartilaginous areas)
CD56	+	R (10% cases show F)	In some subtypes	S	-	-8	Variable
NSE	R (<8%)	+	-	-	NR	-8	+
CD99/MIC2	R (10-25%) cytoplasmic	+ (membranous)	+ (50% cases)[9]	-	-	+ (cytoplasmic)	+ (membranous)
NKX2-2[10]	-	+[11]	-	NR	NR	NR	+
FLI-1[12]	R (F)	+ (75% cases)	Variable[12]	NR	NR	NR	-
LCA	R (<1%)	-[13]	+[14]	Variable	-	-	-
CD117	R (<15%)	S (35% cases)	R	Variable	+	NR	S
Vimentin	+	+	+	+	+	+	+
Desmin	+[15]	R	-	-	-	O	- (occasionally focal expression)
Newer/other lesion specific markers	Myogenin, MyoD1	PAS, NKX2-210	Pan B-cell and T-cell markers, TdT, CD15, CD30, ALK etc., depending upon subtype	CD68, CD1a, CD207 (langerin), peanut agglutinin lectin (PAL), PDL−1 (CD274)	NY-ESO-1* MDM2/CDK4 +in liposarcomas except myxoid, pleomorphic variants	SATB2, osteocalcin[16], osteonectin[16]	NKX2-210 SOX-9
Molecular alterations	ARMS: Fusion between PAX3 FOXO1A PAX7-FOXO1A PAX3-NCOA1 PAX3-AFX only ERMS: None	Gene fusion EWSR1-FLI1 (85% cases) EWSR1-ERG (10% cases) EWSR1- other members of ETS family (3% cases) Rarely FUS-ERG FUS-FEV	Ft (14; 18) IGH-BCL2 translocation seen in FL. t (8;14) c-Myc-IGH translocations seen in most of the BL and 8-16% of DLBCL. c-Myc with BCL-2 and/or BCL-6 in high grade B-cell lymphomas	IGH and/or T-cell receptor gene rearrangements- (in 30% cases) BRAFV600 mutations or less commonly MAP2K1 or ARAF mutations	FUS-DDIT3 also called TLS-CHOP fusion and EWSR1-DDIT3 fusion	-	HEY1-NCOA2 fusion

	Oral mucosal melanoma	NEC	SNUC	NUT carcinoma	Olfactory neuroblastoma	Poorly differentiated synovial sarcoma	DSRCT
Age (yrs)	Adults	Middle to older age adults	50–60 yrs	Variable age (range 0.1–82 years with median age 21.9 yrs)	2–90 years with peak in 5th–6th decades	Mean age: 3rd to 4th decade	Children and young adults
Gender	M=F	M >F	M >F	F >M	M >F	M >F	M >>F
Site	Palate and gingiva	Ethmoid sinus >nasal cavity >maxillary sinus and sphenoidal sinus	Large masses affect multiple sites, mostly involve nasal cavity and ethmoid sinus	UADT, in H and N (65% cases in nasal cavity and paranasal sinuses), generally in mildine	Cribriform plate, superior turbinate and superior half of nasal septum	4–9% cases in H& N. Sinonasal tract and skull are rare sites. Intraorally, tongue and cheek are reported sites	Abdomen, retroperitoneum, or pelvis are most common sites. Rarely involves extra abdominal sites, H&N involvement is exceedingly rare (isolated cases are reported in salivary glands)

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Table 1: Contd...

	Oral mucosal melanoma	NEC	SNUC	NUT carcinoma	Olfactory neuroblastoma	Poorly differentiated synovial sarcoma	DSRCT
Imaging studies	Central destructive mass	-	Marked destruction and spread	Extensive local invasion into neighbouring structures	Dumbbell shaped cribriform plate mass	-	-
Pattern	Protean: solid and organoid pattern mostly and sometimes fascicular, nests or single cells	Syncytial, islands, ribbons, sheets, sometimes pseudorosettes	Sheets and nests, rare pseudorosettes	Sheets, solid nests	Lobular, pseudorosettes and true rosettes	Monophasic in poorly differentiated type- may be round cell, spindle or epithelioid variant	Angulated nests of small round cells within an abundant desmoplastic stroma
Morphology/ cytology	Junctional activity seen. ; large, polygonal, usually epithelioid cells, sometimes plasmacytoid or spindle cells are seen. Pleomorphism, intranuclear inclusions, prominent nucleoli present.	Round cells, with high N/C ratio, nuclear molding, nuclei crushed, inconspicuous nucleoli	Medium cells, pleomorphism, nucleoli	Medium cells, monotonous, high N/C ratio, abrupt keratinisation or squamous eddies	Salt-and-pepper chromatin, small nucleoli (grade-dependent)	Small round cells	Small round cells cells with minimal cytoplasm
Mitosis	High	High	High	High	Variable	Common	Frequent
Necrosis	Variable	Prominent	Prominent	High	Scanty	Variable	Frequent
PanCK	-	+ (D&P)	+	+	R (F and W)	F (90% cases) 3	+ (85% cases)
EMA/MUC1	R	+	+ (50%)	S (30%)	R (F only)	+Patchy to focal (95-100%) 9	+
p406	-	-	-	+	-	-	-7
Synaptophysin/ Chromogranin	-5	+	R(<15%)	R (<15%)	>90% (can be weak)	-	R (15% cases)
S100	+	R	R(<15% +	R (F and W)	+ (sustentacular only)	R (30% cases)	O (<5% cases)
CD56	R	+	R (<5%)	-	+ (membranous)	+	S
NSE	-5	+	+(50%)	+	+	NR	+
CD99/MIC2	R	+ in high grade	S (<10%)	-	-	F+	R (<20% cases) (cytoplasmic
NKX2-210	R	-10	-	-	+	R (10%)	R (10%)
FLI-112	Variable12	R (F)	NR	R (F)	R	S	-/variable
LCA	-	-	O	-	-	-	-
CD117	Variable	+	+	-	-	-/O	-
Vimentin	+	S	-	S	R	+	+
Desmin	-	-	-	-	-	-	+ (dot-like nuclear reactivity in 80% cases)
Newer/other lesion specific markers	HMB-45, Melan-A, MITF, SOX10, tyrosinase	TTF-1 in some cases	-	NUT IHC	NFP Calretinin	TLE-117 NY-ESO-118	WT119 (to c-terminal antibody)

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Table 1: Contd...

	Oral mucosal melanoma	NEC	SNUC	NUT carcinoma	Olfactory neuroblastoma	Poorly differentiated synovial sarcoma	DSRCT
Molecular alterations	Gene mutations in KIT (10–30% cases)**, RAS (10-20% cases) and BRAF (<10% cases)	-	SOX-2 amplification in 1/3rd of tumours	NUTM1 gene rearrangements. Fusion of NUTM1 with BRD4 (70% cases), BRD3 (6% cases) or WHSC1L1 (also called NSD3)	Numerous chromosomal deletions, aberrations, and gains with no consistent pattern	Fusion SS18 (also called SYT) with SSX1, SSX2 or SSX4 resulting from t (X; 18) translocation	EWSR1-WT1 rearrangement resulting from t (11; 22) (p13; q12) translocation

Abbreviations: + almost always positive, −; negative, S=sometimes positive, R=rarely positive, F=focal, W=weak, P&D=punctuate and dot, NR=not reported, NK=not known, O=occasionally positive, LD=limited data. RMS=rhabdomyosarcoma, PNET=primitive neuroectodermal tumour, NHL=non-Hodgkin's lymphoma, LCH=Langerhans cell histiocytosis, SNUC=sinonasal undifferentiated carcinoma, NEC=neuroendocrine carcinoma, SS=synovial sarcoma, DSRCT=desmoplastic small round-cell tumour, UADT=upper aerodigestive tract, H & N=head and neck, MGC=multinucleated giant cells, GIT=gastrointestinal tract, M=male, F=female, DLBCL=diffuse large B-cell lymphoma, MALT=mucosa associated lymphoid tissue, MCL=mantle cell lymphoma, FL=follicular lymphoma, PTCLNOS=peripheral T-cell lymphoma not otherwise specified, NLP=nodular lymphocytic predominant type, NS=nodular sclerosis, LP=lymphocytic predominant, LD=lymphocytic depletion type, MC=mixed cellularity type, R-S cells=Reed Sternberg cells, HPC=haemangiopericytoma, PanCK=pancytokeratin, EMA=epithelial membrane antigen, MUC1=Mucin 1, cell surface associated , NSE=neuron-specific enolase, NKX2-2=NK2 homeobox 2, FLI-1=friend leukaemia virus integration-1, LCA=leucocyte common antigen, CD117=c-kit, ALK=anaplastic lymphoma kinase, PDL-1=Programmed death-ligand 1, NY-ESO-1=New York oesophageal squamous cell carcinoma-1MDM2=mouse double minute 2 homolog, CDK4=Cyclin dependent kinase 4, SATB2=Special AT-rich sequence-binding protein 2, HMB-45=human melanoma black-45, MITF=microphthalmia-associated transcription factor, SOX-10=r SRY-related HMG-box 10, TTF-1=thyroid transcription factor 1, TLE1=transducin-like enhancer of split 1, WT1=Wilms tumour 1. 1; Embryonal RMS (ERMS) is common in head and neck, alveolar RMS (ARMS) in extremities followed by H&N. #; children and young adults (embryonal type) and adults (alveolar type). 2; round-cell liposarcoma as per WHO 2013 is called as high-grade myxoid liposarcoma. 3; EMA may be a more sensitive marker than keratins for monophasic and poorly differentiated synovial sarcomas, and most cases show patchy or streaky reactivity in melanocytic tumours. 4; EMA is also expressed by plasma cells so positive in plasmacytomas. 5; Small cell (neuroendocrine) variant common in nasal cavity and paranasal sinuses, NSE in approximately 50%, and neuroendocrine markers (CD56, CD57, and synaptophysin) in up to 13%, chromogranin, however, is consistently absent. 6; p40 is squamous differentiation marker. 7; case report by Zhenjian Cai *et al.* (2020) DSRCT of parotid gland+ve for p40. 8; EMA, CD56, NSE has been reported in rosette-forming osteosarcomas in case reports. 9; 71-93% of lymphoblastic lymphomas and leukaemia express CD99. 10; NKX2-2 is a transcription factor has been demonstrated to be expressed in 93% of Ewing sarcomas/primitive neuroectodermal tumours (more sensitive and specific than FLI-1 based on limited literature) and was not expressed in most of the other small RCTs, with exception of olfactory neuroblastomas and a minor subset of synovial sarcomas, mesenchymal chondrosarcomas, and malignant melanomas. Also observed positivity in gastrointestinal and pancreatic neuroendocrine tumours (NET) but not in lung NET in literature. 11; combination of CD99 and NKX2-2 is highly specific for ES. 12; FLI somewhat more specific for ES that CD99 but specificity is limited by its expression in lymphoblastic leukaemia/lymphomas, NHLs, endothelial derived neoplasms and wide variety of mesenchymal neoplasms, recently seen in melanomas as strong nuclear positivity as well (higher expression in metastatic than primary in studies. 13; few studies in literature mentions LCA positivity in ES. 14; negative in lymphoblastic lymphomas and plasma-cell dyscrasias. 15; RMS is positive for actin, desmin, myogenin, and myoD1. 16; Practical difficulties limit their use in practice. 17; TLE-1 expression also seen rarely in RMS (<20%), in BCOR-CCNB3 Ewings-like sarcomas and occasional in DSRCT but consistently negative in ES. 18; recently reported in 76% of SS cases with strong and diffuse pattern. 19; non-specific cytoplasmic reactivity in RMS, oral mucosal melanomas, not a specific marker also seen in synovial sarcoma, osteosarcoma, and myxoid liposarcoma. *; NY-ESO-1 is positive in myxoid and round-cell liposarcoma, **; skin melanoma shows more BRAF mutations; in contrast, OMM more commonly shows KIT mutations, indicating different pathogenesis.

Table 2: Summary of round-cell malignancies reported in our institute

Cases	Age (Years)	Sex	Clinical Site	Radiology	Other Investigations	Final Diagnosis	Treatment	Follow-Up
1	42	M	Lip	NA		NHL	RT	Died after 2 cycles
2	22	M	Mandible and maxilla (Diffuse)	Generalised bone loss (floating teeth appearance)		LCH (recurrence)		
K/c/o LCH treated 2 years back	Chemotherapy Inj Vinblastine 8 mg	6 cycles completed, PET scan post chemo reveals no active disease						
3	41	M	Mandible	B+S (S>>B) osteolytic permeative	Serum LDH raised (360 units)	DLBCL (NHL)	Chemotherapy (R-CHOP#) followed by RT	Completed 6 cycles of chemotherapy and 3 cycles of RT, swelling subsided
4	08	M	Mandible	B+S (B>>S) osteolytic permeative		BL (NHL)	Chemotherapy (MTX, ICE regimen)	Completed 6 cycles, stable condition
5	65	M	Mandible	B+S (S>>B) Osteolytic	HIV+	PBL (NHL)	Returned to hometown	Lost to follow-up
6	73	M	Mandible	B+S (B>>S) Osteolytic lesion entire ramus and pterygoid muscles	70% of clonal plasma cells in bone marrow anaemia, reversal of albumin to globulin ratio (A/G; 0.8), Inc. serum gamma globulin, M band serum electrophoresis, β2 microglobulin levels >5.5 mg/L, serum globulin (4.4 g/dl), serum phosphate (8.7 mg/dl) and Dec. serum albumin (3.3 g/dl)	PCM (NHL)	VRD* chemotherapy regimen along with zoledronic acid	2 cycles of chemotherapy completed and is stable (after 4 months)
7	63	M	Maxilla	B+S (S >>> B) Osteolytic permeative		HGBLNOS (NHL) k/c/o NHL of cervical lymph node treated in 2016	ICE** chemotherapy regimen	3 cycles completed and decreased size
8	02	F	Mandible	B+S (B>>S) osteolytic permeative		ES H/o excision of SRCT of left flank region 2 years back	VIDE *** chemotherapy regimen followed by surgical hemimandulectomy	6 cycles completed
9	13	F	Mandible	B+S (B>>S) osteolytic permeative	Normal blood profile	Favouring of ES (advised molecular testing but patient could not afford)	Treated as ES VIDE *** chemotherapy regimen followed by surgery	Diminished size of swelling after chemotherapy. Surgery planned after 1 month
10	60	F	Maxilla	NA		NEC	Lost to follow-up	-

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Table 2: Contd...

Cases	Age (Years)	Sex	Clinical Site	Radiology	Other Investigations	Final Diagnosis	Treatment	Follow-Up
11	38	M	Maxilla	Enhancing mass and obliteration of maxillary sinus, resorption of alveolar bone S>>B		SNUC	Surgery followed by postoperative chemotherapy	Stable

Abbreviations: B=bone, S=soft tissue, >> =more than, Inc.=increase, Dec.=decrease, LCA=leucocyte common antigen, PanCK=pancytokeratin, HMB-45=human melanoma black -45, K/c/o=known case of, NHL=non-Hodgkin lymphoma, EMA=epithelial membrane antigen, IP: immunopositive, IN: immunonegative, FP=focal patchy positive, F: few cells positive, DLBCL=diffuse large B-cell lymphoma, HGBL NOS=high-grade B-cell lymphoma not otherwise specified, BL=Burkitt's lymphoma, PBL=plasmablastic lymphoma, PCM=plasma-cell myeloma, ES=Ewing's sarcoma, NEC=neuroendocrine carcinoma, SNUC=sinonasal undifferentiated carcinoma, MTX=methotrexate, #R-CHOP regimen=rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone, *VRD regimen includes Bortezomib 2 mg, lenalidomide 15 mg, dexamethasone 20 mg weekly, Zoledronic, **ICE regimen includes ifosfamide, carboplatin and etoposide, ***VIDE regimen vincristine, ifosfamide, doxorubicin, and etoposide. IP: immunopositive, FP=focally positive, FPP=focal patchy positive, IN: immunonegative

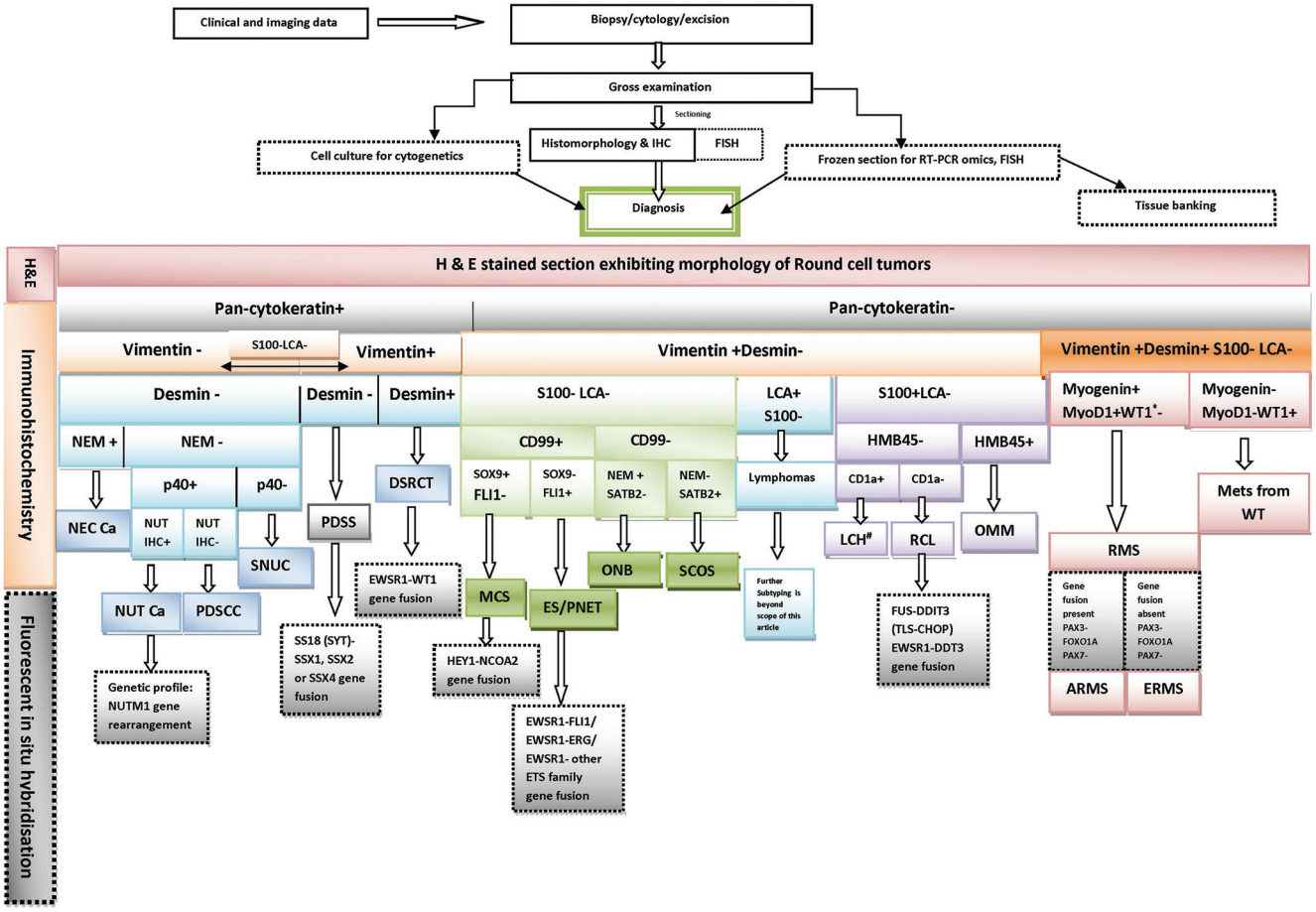

Figure 1: Workflow and IHC diagnostic algorithm for RCTs affecting the OMFR. Abbreviations: H&E; haematoxylin and eosin-stained section, IHC; immunohistochemistry, NEM; neuroendocrine markers, NEC ca; neuroendocrine carcinoma, NUT Ca; NUT carcinoma, NUT; nuclear protein in testis, PDSCC; poorly differentiated squamous cell carcinoma, SNUC; sinonasal undifferentiated carcinoma, PDSS; poorly differentiated synovial sarcoma, DSRCT; desmoplastic small round-cell tumour, MCS; mesenchymal Chondrosarcoma, ES/PNET; Ewing sarcoma/primitive neuroectodermal tumour, ONB; olfactory neuroblastoma, SCOS; small-cell osteosarcoma, OMM; oral mucosal melanoma, LCH; Langerhans cell histiocytosis, RCL; round-cell liposarcoma, RMS, rhabdomyosarcoma; ARMS; alveolar rhabdomyosarcoma, ERMS; embryonal rhabdomyosarcoma, *, positive cytoplasmic but not nuclear staining in RMS; and WT1, Wilms tumor1, Mets; metastasis. *; cytoplasmic staining in RMS and nuclear positive staining in WT. #; LCH otherwise shows classical histomorphology and rarely is confused with other RCTs and can show weak positivity for LCA/CD45, it is mentioned in an algorithm to complete discussion of RCTs of the OMFR. Solid lines/boxes represent an absolute requirement for diagnosis and dotted boxes indicate not an absolute requirement but in the difficult spectrum for study and research purposes

enhancing mass and obliteration of maxillary sinus with resorption of the alveolar bone. Radiographs were not available for case 1 (NHL) and case 10 (NEC).

Histopathology

LCH showed a collection of epithelioid histiocytic cells with abundant pale eosinophilic cytoplasm and grooved nuclei (coffee bean) in a background of abundant eosinophils and chronic inflammatory infiltrate. In our study population, the majority of the cases (6/11) showed a monotonous distribution of atypical lymphoid cells with histologic differentials of poorly differentiated carcinoma and NHL. Large cells with crushing artefacts and a barely discernible morphology were seen in DLBCL. BL showed the characteristic starry-sky appearance due to interspersed tingible body macrophages. A blastic morphology was present in plasmablastic lymphoma (PBL), intermediate cells with a starry-sky appearance in HGBLNOS and atypical plasmacytoid cells with blastic morphology in plasmablastic PCM [Figure 2]. ES showed nests and sheets of small round cells within the dense stroma and crushing artefact in one case. NEC and SNUC exhibited atypical round cells arranged in varying-shaped islands, nests, trabeculae, and cords with perineural, intramuscular, and perivascular invasion [Figure 3].

Immunoprofile

Epithelioid histiocytic cells in LCH showed diffuse CD1a immunopositivity, confirming the recurrence of LCH after 3 years. Cases exhibiting histomorphology favouring NHL were immunostained with the leucocyte common antigen (LCA). DLBCL, BL, PBL, and HGBLNOS were strongly positive, and plasmablastic PCM was negative. They were further categorised into B-cell and T-cell lymphomas by staining with CD20/PAX-5 and CD3. Three NHL cases expressed PAX-5/CD20 immunopositivity and CD3 immunonegativity. These were classified as B-cell NHLs. PAX-5 negative NHL (PBL and PCM) were negative for CD3 as well, thereby excluding the possibility of T-cell NHL.

CD20/PAX-5-positive B-cell NHLs were further subcategorised as BL (case 4) because of young age, immunopositivity for germinal centre markers (CD10 and BCL6) and c-Myc (essential in germinal centre formation),[6] immunonegativity for BCL2, and high Ki-67 index. Immunonegativity for terminal deoxynucleotidyl transferase (TdT) and normal blood profiles excluded lymphoblastic lymphoma, which is the closest differential of BL as both affect similar age groups. Immunonegativity for the germinal centre markers (GCM) in a 41-year-old male with moderate Ki-67 index led to a diagnosis of DLBCL in case 3. Case 7 showing atypical phenotype was diagnosed as HGBLNOS due to a high ki67 index, histomorphology resembling BL (intermediate-sized cells), age, and immunophenotype mimicking DLBCL (old age, GCM−, c-Myc−, BCL2+).

PAX-5-negative B-cell NHL (case 5) exhibited plasmablastic morphology, CD138 immunopositivity, and high Ki67 index with human immunodeficiency virus (HIV) seropositivity, supporting a diagnosis of PBL. Both LCA- and PAX-5-immunonegative NHL (case 6) exhibited diffuse membranous CD138-positive plasmablasts and high Ki67 index along with anaemia and >70% clonal plasma cells in bone marrow aspirate contemplating diagnosis of plasmablastic PCM. Additionally, β2 microglobulin levels >5.5 mg/L (6.08) indicated stage III as per the International Staging System of myeloma [Tables 2 and 3].[7]

Round cells exhibited CD99 immunopositivity in both cases of ES; however, case 8 exhibited diffuse CD99 membranous positivity and case 9 showed CD99 mild positivity in few cells and LCA positivity in a focal area. Thus, the patient was advised molecular testing for confirmation; however, due to economic limitations, the molecular testing could not be done. RCTs of epithelial origin (NEC and SNUC) showed diffuse PanCK immunopositivity. NEC (case 10) additionally showed expression of synaptophysin, NSE, and immunonegativity for p40. Case 11 was negative for neuroendocrine markers in addition to p40 and other lineage-specific markers excluding other RCTs and was diagnosed as SNUC [Figure 3]. Table 1 and Figure 1 summarise the differentiating features and algorithm for RCTs affecting the OMFR.

DISCUSSION

The diagnostic difficulty of RCTs is attributed to their undifferentiated/primitive morphology, making it arduous to characterise them by light microscopy alone.[8] A multidisciplinary approach for accurate diagnosis involves marrying the clinical and imaging findings of the case with a critical interpretation of the histomorphology and the IHC pattern.

Molecular techniques such as flow cytometry, reverse transcriptase polymerase chain reaction, and/or fluorescence *in situ* hybridisation and cytogenetics should be essentially applied in difficult cases presenting with unusual morphology, with typical morphology but unusual age or location, and for distinguishing between sarcoma and its mimics.[4] A proposed algorithm for streamlining

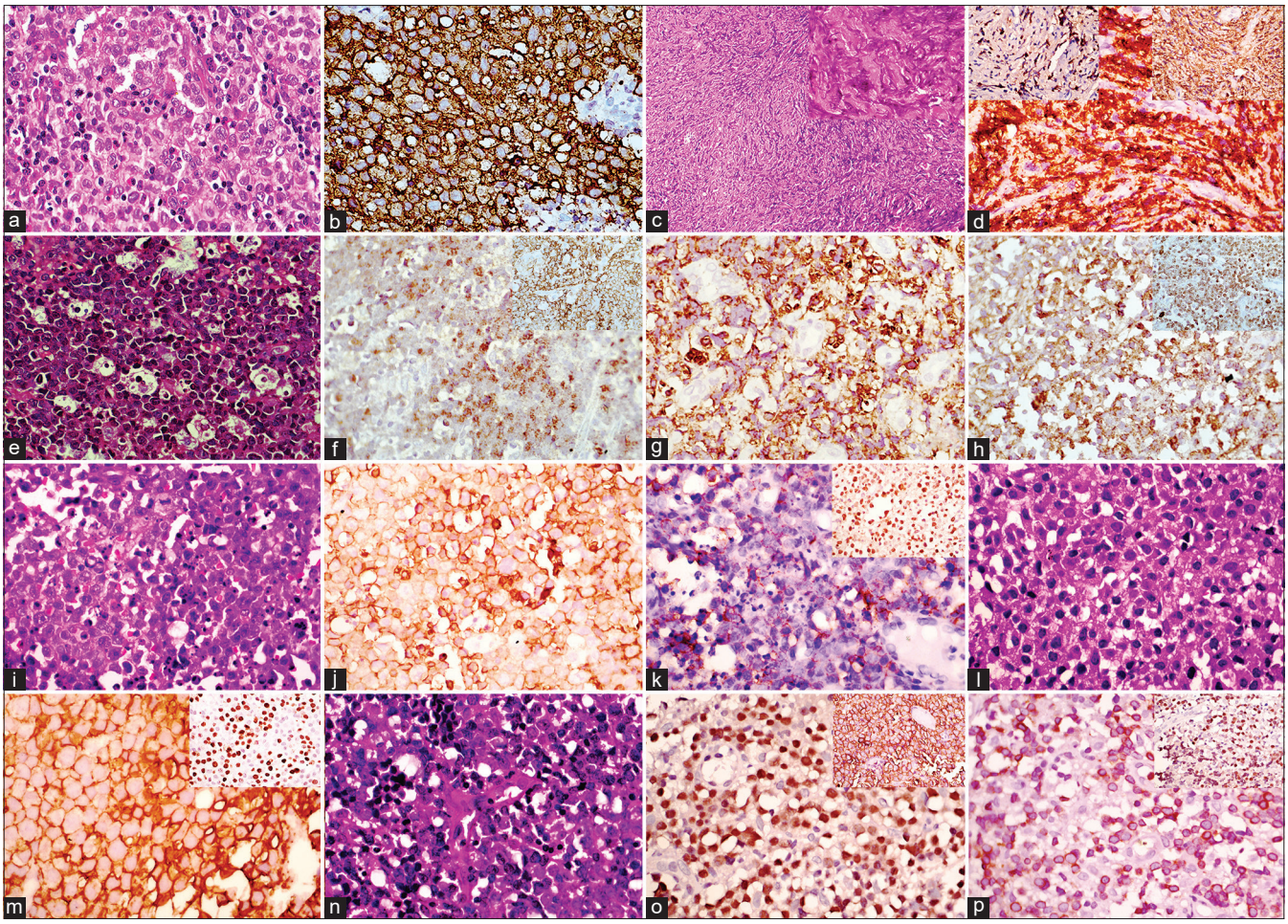

Figure 2: Histopathology and immunohistochemical markers for Haematolymphoid malignancies (cases 2– 7): a: Case of LCH (case 2) showing histiocytes in the background of eosinophils (H&E stain; 40 × magnification) and diffuse CD1a immunopositivity (b). c: Case of DLBCL (case 3) showing monotonous atypical lymphoid cells with crushing artefacts (H&E; 10×, inset showing crushed cells in 40×) and diffuse CD20 immunopositivity [d; inset showing moderate Ki67index (left) and LCA immunopositivity (right)]. e: Case of BL (case 4) showing atypical lymphoid cells with interspersed tingible body macrophages showing a starry-sky appearance (H&E; 40×). Tumour cells show immunopositivity for PAX-5 (f; inset showing LCA positivity), CD10 (g), and c-Myc (h; inset showing a high Ki67 index). i: Case of PBL (case 5) showing atypical lymphoid cells with blastic morphology (H&E; 40×), immunopositivity for LCA (j) and CD138 (k; inset showing a high Ki67 index). l: Case of plasmablastic PCM (case 6) showing atypical cells with plasmacytoid appearance with blastic morphology (H&E; 40×) and diffuse CD138 immunopositivity (m; inset with a high Ki67 index). n: Case of HGBLNOS (case 7) showing monotonous atypical lymphoid cells (H&E; 40×), immunopositivity for PAX5 (o; inset showing LCA immunopositivity) and BCL2 (p, inset showing high Ki67 index)

the diagnostic strategy has been elucidated in [Figure 1 and Table 1].

Whereas clinical and radiological details with histomorphology provide a list of differentials, an array of IHC markers ultimately narrow down the differentials to a final conclusive diagnosis. However, IHC is an adjunct to basic histomorphology and the correct panel of markers is to be decided on the basis of histological differentials rather than random panels.[9] Furthermore, correct interpretation of IHC is the responsibility of the pathologist to avoid diagnostic pitfalls, taking into consideration various antibody-specific characteristics and technical errors.[9,10]

In our study, 11/2066 cases belonged to the spectrum of RCTs and in 10/11 cases, conclusive diagnosis was established with the use of an appropriate panel of IHC markers. Although DLBCL showed barely identifiable cell morphology microscopically, however, IHC markers (LCA++ and CD20+) led to the final diagnosis. Similarly, ES (case 9) showed faint CD99 positivity and focal LCA positivity, creating a diagnostic confusion, but age, radiologically periosteal reaction, and histomorphology of nests of round cells led to the diagnosis of ES. In certain cases, when the haematological profile and IHC markers are negative for other possible entities, molecular studies are needed to rule out undifferentiated sarcomas (CIC and BCOR rearranged sarcomas) [Tables 2 and 3].[11] A study by Louati S *et al.*[12] has shown CD99 positivity in 92.7% of

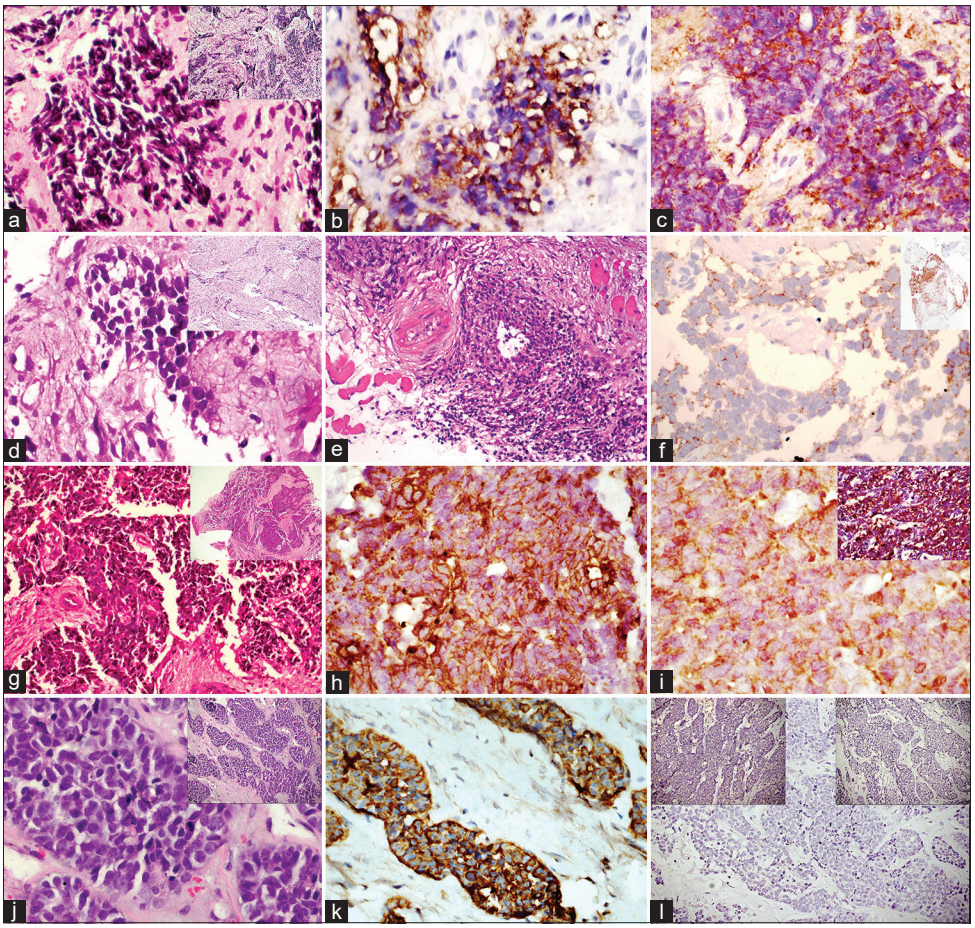

Figure 3: Histopathology and immunohistochemical markers of ES, NEC, and SNUC (cases 8–11): a: case of ES (case 8) showing atypical round cells with crushing artefacts (H&E; 40 × magnification, inset showing pattern of round cells under 10×), membranous immunopositivity for CD99 (b) and NSE (c). d: Another case of ES (case 9) showing atypical round cells with hyperchromatic nuclei (H&E; 40×, inset showing pattern of round cells under 10×), rosette-like pattern of round cells near blood vessels (e; H&E; 20×), mild CD99 immunopositivity (f, inset showing focal LCA immunopositivity). g: Case of NEC (case 10) showing monomorphic round cells arranged in large islands (H&E; 40×, inset showing pattern of round cells under 10×), immunopositivity for PanCK (h) and NSE (I, inset showing synaptophysin). j: Case of SNUC (case 11) showing monomorphic round cells with barely visible cytoplasm and arranged in trabeculae and islands (H&E; 40×, inset showing pattern of round cells under 10×), immunopositivity for PanCK (k) and immunonegativity for chromogranin (l; inset showing negativity for synaptophysin (left) and p40 (right))

the sampled cases of ES. The sensitivity and specificity for IHC was 88% and 58%, respectively. LCA positivity was also observed in rare reported cases of ES.[13]

In our study, NHL formed the most predominant subtype of RCTs followed by ES which was similar to the study by D'cruze L *et al.*,[7] showing NHL (44.2%) being the most common subtype followed by ES (14%); however, a study by Joshi MR *et al.*[14] has found the highest incidence of ES (36%), followed by neuroblastoma (21%) and NHL (15%). However, these studies discussed round-cell malignancies affecting the entire body and were not restricted to the oral and maxillofacial regions. A further small sample size of our study may provide justification for this disparity.

LCA is virtually expressed by all lymphomas, and CD20, PAX-5, and CD3 are important for categorisation into B- or T-cell lymphomas.[8,9] In our study, 4/6 NHL were LCA-positive and 3 displayed positivity for B-cell markers and were thus categorised as B-cell lymphomas. PBL is the most common lymphoma affecting the oral cavity in HIV-positive individuals. Despite being B-cell NHL, PBL showed immunonegativity for PAX5 and immunopositivity for the plasma-cell differentiation marker, CD138. The immunoprofile (PAX5 − and CD138+) is justified as plasmablasts representing the transition stage between the B-cells to plasma cells, thereby losing one or more of B-cell markers and expressing plasma cell-specific markers.[15]

The spectrum of NHL also includes plasma-cell dyscrasias, which originate from bone marrow-homing plasma cells, derived subsequent to antigenic stimulation of B-cells.[16] They lose LCA and one or more B-cell markers and express plasma cell-specific markers (CD138 and

Table 3: Summary of histological differentials and immunoprofile of RCTs described in this study

Case No.	Histologic diagnosis/differentials	CD1a	CD45	CD10	CD20/Pax-5	c-Myc	TdT	BCL6	BCL2	CD138	CD3	NSE	Synaptophysin	Chromogranin	PanCK	CD99	p40	Vimentin	Ki67 (%)	Final diagnosis
	LCH	++																		LCH (recurrence)
	NHL poorly differentiated carcinoma		++		++						-				-			FP	35	DLBCL
	NHL (Burkitt's lymphoma, lymphoblastic lymphoma)		++	+	+	+	-	FP	-						-	-			>95	BL
	NHL		++	FP	-				-	FP	-			-	-				100	PBL
	NHL poorly differentiated carcinoma		-							++					-				90	PCM
	NHL		++	-	+	-			+		F		-		-	-			90	HGBLNOS
	ES NHL (lymphoblastic lymphoma) small cell osteosarcoma Rhabdomyosarcoma		-									+			-	++				ES
	ES lymphoblastic lymphoma rhabdomyosarcoma small-cell osteosarcoma, DSRCT	-	F	-	F		-			-			-	-	-	+		FP	52	ES
	Poorly differentiated carcinoma NEC											+	++		++		-			NEC
	Poorly differentiated carcinoma NEC SNUC											-	-	-	++		-	-	63	SNUC

++=strongly positive, +=mild positivity, FP=focal patchy positive, F: few cells positive. DLBCL=diffuse large B-cell lymphoma, HGBL NOS=high-grade B-cell lymphoma not otherwise specified, BL=Burkitt's lymphoma, PBL=plasmablastic lymphoma, PCM=plasma cell myeloma, ES=Ewing's sarcoma, NEC=neuroendocrine carcinoma, SNUC=sinonasal undifferentiated carcinoma

MUM1/IRF4),[9,17] consistent with our case (plasmablastic PCM; LCA − and CD138+). Our case fell into stage III of plasmablastic PCM presenting with a blastic morphology, high Ki67 index, anaemia and bone marrow showing >70% clonal plasma cells.[7] However, there is microscopic and immunophenotype overlap between PBL and plasmablastic PCM. Diagnostic clues are mucosal involvement with stronger HIV and Epstein–Barr virus (EBV) association in PBL than plasmablastic PCM. The latter presents with osteolytic lesions in rare HIV and EBV positivity. Sometimes atypical presentation (PBL with bony involvement, EBV and HIV negative) may create a diagnostic dilemma requiring exclusion of PCM by a systemic workup.[18]

High-grade B-cell NHL (HGBL) showing an intermediate morphology between DLBCL and BL is classified into two categories namely double hit (DHL) and triple hit lymphomas (THL) exhibiting gene translocations (c-Myc with BCL6 and/or BCL2) and HGBLNOS (without translocations).[19] Thus, case 7 was diagnosed as HGBLNOS because of the intermediate morphology between BL and DLBCL and c-Myc immunonegativity, thus excluding DHL and THL.

RCTs of the sinonasal tract microscopically exhibiting neuroendocrine features with immunopositivity for cytokeratin and at least one neuroendocrine marker (NEM) favour the diagnosis of NEC[3] (case 10; Pan CK+, synaptophysin+, NSE+). Another epithelial-derived RCT of the sinonasal tract (SNUC) is usually a diagnosis of exclusion with a lack of glandular, squamous, and neuroendocrine differentiation. It shows immunopositivity for epithelial markers, immunonegativity for NEM (focal or weak positivity sometimes) without a neuroendocrine morphology, and consistent negativity for p40, as seen in case 11 (Pan CK+, p40−, and NEM−) of the present study [Tables 2 and 3].[3]

CONCLUSIONS

Considerable confusion prevails among oral pathologists with regards to the diagnostic approach to the infrequently encountered lesions displaying round cells in the OMFR. Our diagnostic algorithm is an attempt towards simplifying the diagnostic strategy and is expected to be practically useful for the oral pathologist encountering such rare entities.

In our study, immunohistochemistry was indispensable in the accurate categorisation of RCTs, emphasizing its crucial role in correct interpretation. Furthermore, an improved understanding of genetics has established molecular techniques as an invaluable tool for deciding the final diagnosis of questionable cases and new undifferentiated sarcomas such as CIC and BCOR rearranged sarcomas.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Ethical approval

This study was approved by the Ethics Committee of the All India Institute of Medical Sciences, Delhi (approval numbers IEC-720/04.10.2019 and RP-31/2019).

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Table S-1: Immune cell antigens detected by monoclonal antibodies used in the present study for diagnosing RCTs

Origin/lineage	Immunohistochemical markers
Epithelial	Pancytokeratin- PanCK, epithelial membrane antigen- EMA, CK7
Neural crest	S100
Mesenchymal	Vimentin
melanocytic lesions	S100, HMB45
Ewing's group of tumours	CD99 (also called MIC2), NSE
Neuroendocrine	Synaptophysin, chromogranin, NSE, CD56
Muscle	SMA, desmin, myogenin
Osteosarcoma	SATB2
Proliferation marker	Ki67
Lymphoid neoplasms	CD45/LCA (leucocyte common antigen), B-cell markers (CD20, PAX5), BCL-2, c-Myc, CD15, CD30 Germinal centre markers; GCM (CD10, BCL6) lymphoblastic lymphoma marker: TdT (terminal deoxynucleotidyl transferase) T-cell markers –CD3 NK cell marker: CD56 Plasma-cell differentiation marker: EMA, CD138 CD1a, langerin: LCH