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ORIGINAL PAPER

Primary Diffuse Large B-Cell Lymphoma of the Oral Cavity: Germinal Center Classification

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Abstract Primary lymphomas of the oral cavity are rare and the most frequent type is diffuse large B-cell lymphoma (DLBCL). Recently, several reports have highlighted the value of classifying DLBCL into prognostically important subgroups, namely germinal center B-cell like (GCB) and non-germinal center B-cell like (non-GCB) lymphomas based on gene expression profiles and by immunohistochemical expression of CD10, BCL6 and MUM-1. GCB lymphomas tend to exhibit a better prognosis than non-GCB lymphomas. Studies validating this classification have been done for DLBCL of the breast, CNS, testes and GI tract. Therefore we undertook this study to examine if primary oral DLBCLs reflect this trend. We identified 13 cases (age range 38–91 years) from our archives dating from 2003–09. IHC was performed using antibodies against germinal center markers (CD10, BCL6), activated B-cell markers (MUM1, BCL2) and Ki-67 (proliferation marker). Cases were sub-classified as GCB subgroup if CD10 and/or BCL6 were positive and MUM-1, was negative and as non-GCB subgroup if CD10 was negative and MUM-1 was positive. Immunoreactivity was noted in 2/13 cases for CD10, in 12/13 for BCL6, in 8/13 for MUM-1, and in 6/13 for BCL2. Therefore, 8/13 (58%) were sub-classified as non-GCB DLBCLs and 5/13 (42%) as GCB subgroup. All tumors showed frequent labeling with Ki-67 (range 40–95%). Four of the 8 patients with non-GCB subgroup succumbed to their disease, with the mean survival rate of 16 months. Two patients in this group are alive, one with no evidence of disease and another with disease. No information was available for the other 3 patients in this group. Four of the 5 patients in the GCB subgroup were alive with no evidence of disease and one patient succumbed to complications of therapy and recurrent disease after 18 months. In conclusion, our analysis shows that primary oral DLBCL predominantly belongs to the non-GCB subgroup, which tends to exhibit a poorer prognosis. These findings could allow pathologists to provide a more accurate insight into the potential aggressive behavior and poorer prognosis of these lymphomas.

Keywords Oral large B cell lymphoma · Germinal center · Immunohistochemistry · CD10 · BCL6 · MUM-1

Both authors I. Bhattacharyya and H.K. Chehal have equal contribution in the study.

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most frequently diagnosed type of non-Hodgkin lymphoma (NHL) and is the fifth most frequent cancer, accounting for 30–40% of all cases reported [1]. It is frequently reported in the mediastinum, gastrointestinal tract, bone marrow, central nervous system, breast and testes. [2] Most cases are in patients in the seventh decade of life [1, 2]. NHLs of the oral cavity are rare and account for only 3–5% of the lymphomas

reported [3–5]. They can be primary or secondary to extension from Waldeyer's ring [3, 5]. The most frequent type of primary NHL lymphoma of the oral cavity is DLBCL [3, 5], however, the incidence of primary DLBCL in the oral cavity has not been previously specified in the literature. These lesions are symptomatic and typically present as a rapidly enlarging mass [3, 5]. Outside of the Waldeyer's ring, in the oral cavity, the hard palate and the maxillary vestibule appears to be frequently involved [3, 5].

DLBCL is characterized by a diffuse proliferation of large neoplastic B cells with nuclear size equal to or exceeding normal macrophage nuclei, or more than twice the size of a normal lymphocyte [1, 6]. DLBCL is a heterogenous neoplasm with variable clinical, morphologic, immunophenotypic, cytogenetic, and genetic features [1, 2, 6]. This is reflected in their marked biological heterogeneity and highly variable clinical course [2]. In the past, DLBCL was subclassified based on cytomorphologic features into centroblastic, immunoblastic and anaplastic. Centroblasts are medium to large in size with oval to round nuclei and fine vesicular chromatin patterns. They have two to four nucleoli opposed toward the nuclear membrane, and rarely may have multilobated nuclei, which can predominate in extranodal disease. The tumor can be monomorphic (>90% centroblasts) or polymorphic with admixed immunoblasts (centroblasts $< 90\%$) [1, 2, 6]. Immunoblasts display a uniform cytology and almost all cells exhibit a prominent central nucleoli with distinct rims of basophilic cytoplasm. In the anaplastic variant, the tumor cells are variably large cells with bizarre pleomorphic nuclei. They may mimic Reed Sternberg cells or undifferentiated carcinoma, in which case, immunohistochemistry is helpful. Increased number of intermixed T cells and/or histiocytes can be seen in all variants. In rare cases, the tumor cells may comprise spindle-shaped or signet ring cell forms with a myxoid stroma. The differentiation between morphologic variants is subjective and not reproducible [1]. Moreover, the treatment and importantly, survival rates showed no significant differences between these morphologic variants [7–9].

Most DLBCL show somatic mutations in variable regions of their immunoglobulin heavy chain genes, and are thought to arise from B cells that have passed through the germinal center (GC)—either GC or post-GC B-cells [10–12] Various techniques have been used to sub classify DLBCL including oligonucleotide array [12], cDNA microarray [10, 13], and immunohistochemical expression [14, 15]. Recently, by using the cDNA microarray techniques DLBCL has been subclassified into three molecularly distinct forms, based on their gene expression profiles: germinal center B-cell like (GCB), non-germinal center (non-GCB) or activated B-cell like (ABC), and type 3 gene expression profiles [10, 13]. Patients in the GCB group have demonstrated better outcomes than those with non-GCB and type 3 groups [10, 13]. Subsequently, studies have shown that immunohistochemistry (IHC), which is more practical and widely utilized, is as consistent and accurate in the sub-classification of DLBCLs into these prognostically important subgroups using antibodies against CD10, BCL6 (both markers of germinal center origin) and MUM1 [9, 14, 15]. This sub-classification has not been attempted before for primary DLBCL of the oral cavity. The aim of this study was to apply this approach to sub-classify DLBCLs of the oral cavity into the two prognostically significant subtypes (GCB and non-GCB) using IHC. We believe that such classification will enable pathologists to provide insight into the potential behavior and prognosis of these lymphomas.

Materials and Methods

We searched for cases of primary DLBCL of the oral cavity from the University of Florida Oral and Maxillofacial Pathology Biopsy Service for the period of 2003–2009. The criteria for selection of cases as primary DLBCL included absence of concomitant nodal/extranodal disease at any other site or within a 6 month period of diagnosis of oral lesion. All cases were reviewed and the diagnosis was confirmed according to the published criteria in the 2008 WHO Classification of Hematopoietic tumors [1]. Immunohistochemistry was performed according to standard protocol with antibodies to CD3, CD5, CD20, CD10, Bcl-6, Bcl-2, MUM1 and Ki-67 [9] (see Table 1) applied in all cases. Briefly, multiple unstained sections (5 μm) were then cut from each tissue block and mounted on plus slides. Following deparaffinization, heat-induced antigen retrieval was performed. Rabbit antimouse amplifying kit was used to enhance staining for the above antibodies utilizing an endogenous biotin blocking kit to decrease background staining. Following antigen retrieval and primary antibody incubation, the reaction was completed in a Ventana ES automated stainer (Ventana, Tucson, AZ) using a diaminobenzidine immunoperoxidase detection kit. Negative and positive controls in each run were adequate. All immunohistochemical studies were evaluated independently by 2 pathologists (IB and SA) and immunoreactivity for each antibody was recorded in addition to semiquantitative visual estimation of the percentage of tumor cells nuclei labeling with Ki-67. Disagreements were resolved by joint review on a multihead scope. For antibody against CD10, Bcl-6 and MUM1, cases were considered positive if 30% or more of the tumor cells were stained with an antibody. A uniform cutoff of 30% was used similar to what has been used in other studies [16] (see Table 2).

We classified all the cases into two subgroups: GCB and non-GCB utilizing the flow chart in Fig. 1 [9]. Cases

Table 1 Antibodies used in the study

Antibody	Manufacturer	Source	Clone	Type	Dilution	Characterization
CD20	DAKO, Carpenteria, CA	Mouse monoclonal	L26	IgG29	1:200	Expressed by B-cell lineage
CD3	Ventana Medical Systems, Tucson, AZ	Rabbit polyclonal	PS1	Polyclonal	Prediluted from manufacturer	Expressed by T-cell lineage
CD5	Ventana Medical Systems, Tucson, AZ	Rabbit monoclonal	SP19	IgG	Prediluted from manufacturer	Expressed by T cell lineage
CD10	Ventana Medical Systems, Tucson, AZ	Mouse monoclonal	56C6	IgG1	Prediluted from manufacturer	Expressed by normal GC B-cells
Bcl-2	Ventana Medical Systems, Tucson, AZ	Mouse monoclonal	124	IgG1/K	Prediluted from manufacturer	Expressed in normal lymphoid tissue but is absent in reactive GC B-cells
bcl-6	Cell Marque, Rocklin, CA	Mouse monoclonal	GI191E/A8	IgG1	Prediluted from manufacturer	Expressed in germinal center B cells and a subset of CD4+ T cells. It is expressed in the majority of DLBCL's ranging from 57 to 100%
Ki 67	DAKO, Carpenteria, CA	Mouse monoclonal	MIB1	IgG kappa	1:30	Cell cycle marker used to assess proliferative potential of tumors
MUM1	ID Labs, Inc. London, ON, Canada	Mouse monoclonal	MUM 2p	IgG1	1:20	Expression denotes the final step of intra-GC B-cell

Visualization for all antibodies done by UltraView DAB kit Ventana Medical Systems, Tucson, AZ

Table 2 : Summary of results of immunohistochemical staining

Case	CD 10	CD 20	CD 3	CD 5	Ki 67 (%)	Bcl-2	Bcl-6	MUM1	Sub classification	Outcome
1	−	+	−	−	40–50	−	+	+	Non- GCB	DOD
2	−	+	−	−	80–95	+	−	+	Non- GCB	DOD
3	−	+	−	ND	>75	+	+	+	Non- GCB	DOD
4	−	+	+	−	60–70	−	+	+	Non- GCB	NIA
5	+	+	−	−	40–50	−	+	−	GCB	Alive, NOD
6	−	+	−	−	90	+	+	−	GCB	Alive, NOD
7	−	+	−	ND	50–70	−	+	+	Non- GCB	NIA
8	+	+	−	−	>80	+	+	−	GCB	DOD
9	−	+	−	−	>80	−	+	−	GCB	Alive, NOD
10	−	+	−	−	80	−	+	+	Non-GCB	NIA
11	−	+	−	−	40–50	+	+	+	Non-GCB	DOD
12	−	+	−	−	>80	+	+	−	GCB	Alive, NOD
13	−	+	−	−	40–60	+	+	+	Non-GCB	Alive, with disease

ND not done, *NIA* no information available, *DOD* died of disease, *NOD* no evidence of disease

were subgrouped as GCB if CD10 and/or BCL6 were positive and MUM-1 negative [17]. If CD10 and Bcl-6 were deemed negative, or BCL6 and MUM-1 were positive, the cases were assigned to the non-GCB subgroup [17].

Results

We identified 13 cases of primary DLBCL of the oral cavity. There were six females and seven males ranging in age from 38 to 91 years of age (Table 3). The symptoms in

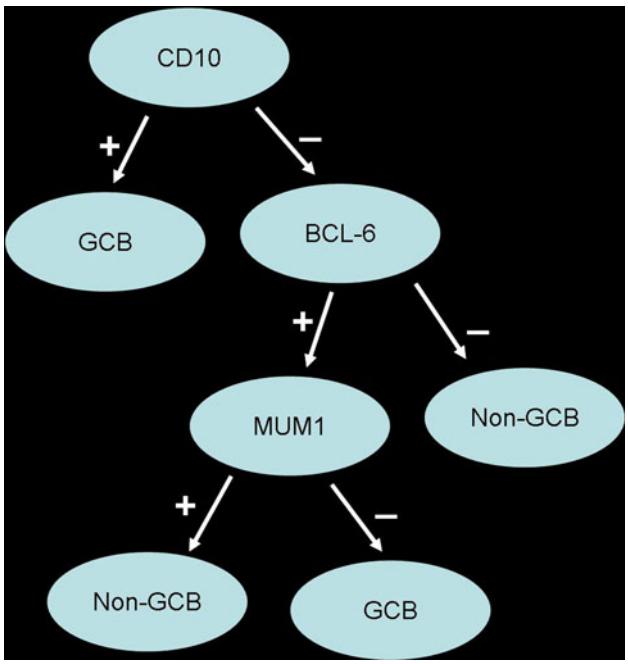

Fig. 1 Flow chart for GCB classification. Adapted from [9]

the cases presented here ranged from none to generalized pain and numbness of chin and lower lip. Varied working diagnoses were provided by the clinicians and included fibroma, pyogenic granuloma, odontogenic cyst, giant cell lesion, myxoma, pleomorphic adenoma, metastatic carcinoma, squamous cell carcinoma and lymphoma. None of these patients had HIV or evidence of immunosuppression. Many of the cases in this study arose in intrabony locations with five in the maxilla around the alveolus or sockets of extracted teeth and case in the right body of the mandible (Fig. 2). The maxillary vestibule was a common site for the occurrence of soft tissue tumors. Seven cases presented as soft tissue swellings with six in the maxilla and one in the mandible. The vestibule was a common site for the occurrence of this lesion as seen in the clinical image from case 11 (Fig. 3). One patient had a recurrence in the vestibule (Fig. 3b).

All cases were initially diagnosed as DLBCL and all demonstrated a diffuse infiltration of medium to large neoplastic B cells (Fig. 4a) that had large nuclei with nuclear size equal to or exceeding normal macrophage nuclei, or more than twice the size of a normal lymphocyte. (Figs 4b, d). Variable proportions of centroblasts and immunoblasts were observed (Fig. 4d) and tumor cells with multilobated nuclei were occasionally seen. There were variable numbers of intermixed histiocytes and/or T cells. These infiltrates demonstrated a diffuse destructive growth pattern and areas of perivascular infiltrate (Fig 4c). In all cases, the tumor cells demonstrated strong immunoreactivity with anti-CD20 antibody (Fig. 5), and were negative for CD3 and CD 5 (cyclinD1 and CD138 in three cases). Immunoreactivity for Bcl-6 was seen in (12/13) cases, for CD10 in 2/13cases, 8/13 for MUM1 and 6/13 for Bcl-2 (Fig. 6). As a result eight cases (58%) were classified in the non-GCB subgroup and five cases (42%) in the GCB subgroup (Fig. 6). All the tumors showed frequent labeling with Ki-67 (40–90%) (Fig. 7; Table 2). Limited clinical data was available for all the cases including status of staging bone marrow biopsy and imaging studies.

Reported treatment included: combination chemotherapy alone (3), combination chemotherapy and radiotherapy (5), treatment declined (2) and unknown treatment status (3). Four of the eight patients with non-GCB subgroup succumbed to their disease, with the mean survival of 16 months. One patient was alive with no evidence of disease and one patient was alive with disease. No information was available for 3 patients in the non-GCB group. In the GCB subgroup four of the five patients were alive with no evidence of disease and one patient succumbed to complications arising from therapy and recurrent disease 18 months after diagnosis.

Discussion

DLBCL is the most common type of NHL in the US and Western world and accounts for 30–40% of all cases [2]. Up to 40% of the cases present with only extranodal involvement, and isolated bone marrow involvement is extremely rare. They are associated with the presence of large cells that express a mature B-cell immunophenotype and have a high proliferative index [1]. Apart from being related to AIDS in a few cases, the etiology of the primary DLBCLs of the oral cavity is unknown [3, 4]. Oral manifestations are seen in 3–5% of the cases [3–5] DLBCL rarely manifests as a primary malignancy in the head and neck region (<1%) [5]. The presence of DLBCL in the head and neck may be associated with undiagnosed HIV infection since they account for 2% of oral neoplasms in patients with AIDS [18]. DLBCL has been reported in the oral cavity in the buccal mucosa, hard palate and gingiva [3, 5]. Many of the cases in our study arose in intrabony locations and the maxillary vestibule was a common site for the occurrence of soft tissue tumors.

The most common presenting symptoms of extranodal NHL in the head and neck region include local swelling, pain and discomfort in the region of involvement [19]. Clinical signs included unilateral enlargement of face, localized swelling, destruction of hard and soft tissue, cavitation and ulceration and/or sessile soft tissue mass formation. This diverse presentation in our study and varied working diagnoses provided, are consistent with the

Table 3 Summaryof clinical findings

Case	Age (in years)	Sex	Race	Site	Duration	Clinical presentation	Working diagnosis	Diagnosis	Treatment & Prognosis
1	91	F	C	Left crest of maxillary alveolus	>1 month	Ulcerated, soft tissue mass	SCCa	Non-GCB	Declined DOD
2	76	M	C	Socket of the extracted mand left 2nd molar	>3 weeks	Pain in extraction socket, numbness in chin & left side of lower lip	PG, Met Ca	Non-GCB	Ch + Xrt DOD
3	80	M	C	Facial aspect of right maxillary tuberosity	2.5 weeks	Swelling	None	Non-GCB	Declined DOD
4	77	F	C	Sockets of extracted max left 2nd & 3rd molars	Unknown	Soft tissue mass	GC lesion, Myxoma	Non-GCB	NIA
5	38	M	H	Maxillary vestibule, buccal to tooth # 9	1.5 month	Painful swelling	Fibroma	GCB	Ch + Xrt NOD
6	51	F	Unk	Right maxillary buccal vestibule	>6 months	Large sessile soft tissue mass	SCCa, Lymphoma, Minor salivary gland tumor	GCB	Ch NOD
7	60	M	C	Right mandibular buccal vestibule	2.5 months	Swelling and numbness in area	Neoplasia	Non-GCB	NIA
8[a]	56	M	C	Maxilla, buccal to edentulous space in area of # 6	>2 weeks	Generalized pain in oral cavity	"Not just necrotic tissue"	GCB	Ch + Xrt DOD
9[a]	45	F	C	Right body of mandible	3 months	Enlargement of right side of face	Myxoma, Pleomorphic Adenoma, a	GCB	Ch NOD
10	54	M	H	Maxilla, area of right first molar	Unknown	Soft tissue swelling over bony exostosis	None	Non-GCB	NIA
11[b]	72	M	C	Maxillary alveolus, left central incisor to right first premolar	Unknown	Swelling with obliteration of vestibule with bone/soft tissue destruction	Odontogenic cyst/ Infection	Non-GCB	DOD
12[b]	89	F	C	Right maxillary vestibule	1 week	Swelling with discomfort in denture wearing, small ulceration on surface	None	GCB	Ch NOD
13	74	F	C	Right maxillary tuberosity	>1 month	Swelling with mass sensation	Lymphoma	Non-GCB	Ch + Xrt Alive with disease

C caucasian, *H* hispanic, *NIA* no information available, *NOD* alive with no evidence of disease, *DOD* died of disease, *Ch* chemotherapy, *Xrt* radiation therapy

[a] radiographs (Fig. 6)

[b] clinical images (Fig. 7)

heterogenous clinical presentation of DLBCLs reported in the literature [3].

In spite of the hematologic and biochemical profiles of affected patients being often normal, patients may have a decreased number of peripheral blood lymphocytes or decreased serum albumin levels and elevated LDH, interleukin-6 (IL-6), IL-10, and IL- 2 receptor levels which have been shown to correlate with a poor prognosis [20].

Most DLBCL have been shown to undergo somatic mutations in the variable regions of their immunoglobulin heavy chain genes, and hence, are thought to be derived from B cells that have passed through the germinal center (GC)-representing either GC or post-GC B-cells [13]. With the help of cDNA microarray techniques, the gene expression profiles of DLBCL have been evaluated and the two distinct molecular forms, the GC-like (GCB) and the activated B-cell like (non-GCB) DLBCL were identified [10]. GCB lesions express genes normally expressed by germinal center B cells and the non-GCB DLBCL express genes normally induced during in vitro activation of peripheral blood B cells [10].

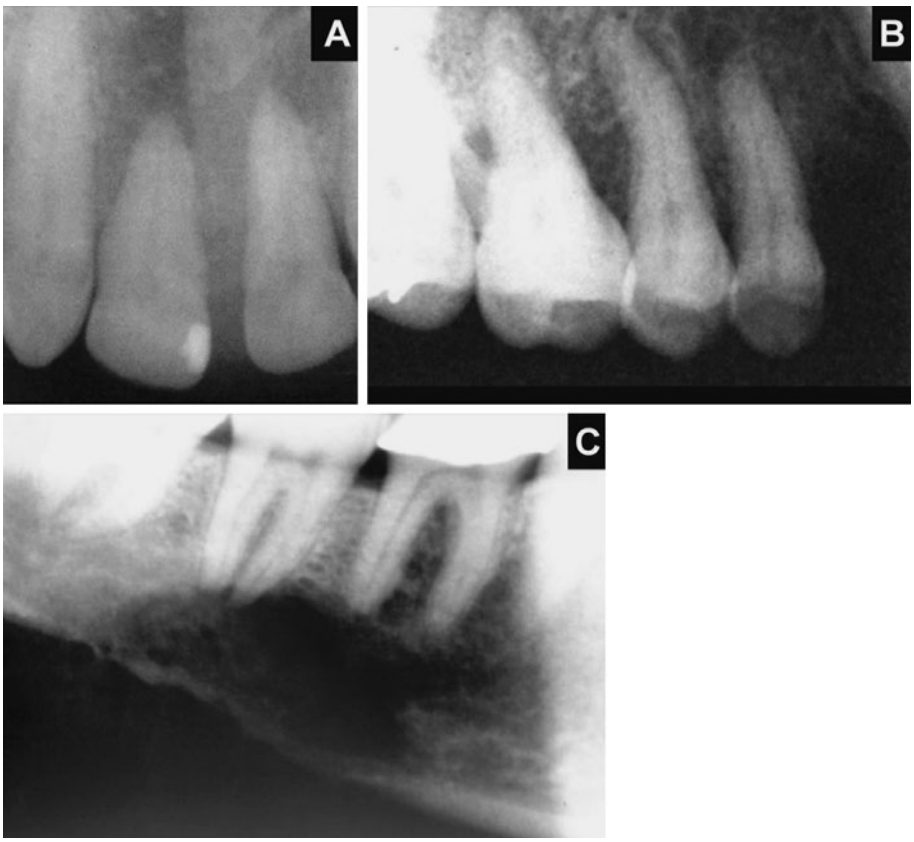

Fig. 2 **a**, **b**. Periapical radiographs demonstrating irregular radiolucencies with indistinct margins seen in a 56-year-old male with swelling in area of right maxillary canine to molars (Case #8 Table 3). **c** Section from panoramic radiograph demonstrating lesion arising within the mandible in the right body of the mandible (Case #9 Table 3)

DLBCLs express CD 45 (leukocyte common antigen) and pan-B-cell antigens (CD19, CD20 and CD79). In some cases, expression of one or more of these antigens may be lacking [20]. CD20 is expressed on B-cells from the mature precursor B cell (hematogone) until the pre plasma cell stage of differentiation [21]. It is a highly specific marker for B-cells lineage and most DLBCL show homogenously bright staining for CD20 [22]. In terminally differentiated DLBCLs with immunoblastic or plasmablastic morphology, CD20 expression can be lost [22, 23]. CD10 is a membrane metalloproteinase that is detected in early lymphoid progenitors. CD10 expression is restricted to germinal centers of secondary follicles [24]. Majority of follicular lymphomas are CD10 positive while other small B-cell lymphomas are CD10 negative [17]. It is expressed in 20–40% of DLBCLs. The staining is membranous and usually homogenous throughout the tumor [8, 25].

CD3 is a T cell marker that is associated with the T-cell receptor and transmits the activation signal to the cytoplasm. It is highly restricted in its expression to T lymphoid cells and is an excellent marker since it is retained following neoplastic transformation [26].

Ki67 is a cell cycle marker used as a proliferation marker. Ki67 defines growth fraction of actively cycling cells. In DLBCL, the expression varies from 30 to 100% but is usually high [27]. Most studies show that a high proliferative index is an adverse prognostic factor. Tumors with a Ki 67 expression of >80% are termed as "highly proliferative" or aggressive tumors [8].

Bcl-2 protein functions as an anti-apoptotic protein protecting cells from programmed cell death [28]. In DLBCL, Bcl-2 protein expression is found in 30–60% of cases with ill understood prognostic value [28]. However, some studies suggest that Bcl-2 expression is associated with a significantly worse overall survival rate [29].

Bcl-6 is selectively expressed by GC B cells in normal lymphoid tissues and a subset of CD4+YT cells [9, 30, 31]. Their expression in DLBCLs has been found in a majority of the cases ranging from 57 to 100% [32]. Bcl-6 is reported in about 40% of DLBCL but the biological significance of this expression is not clear [8, 10, 31, 33].

MUM 1 is a lymphoid-specific member of the interferon regulatory factor family of transcription factors [9]. The staining is nuclear and may be associated with cytoplasmic positivity. In DLBCLs MUM1 is expressed in 50–75% of cases and is seen both with and without Bcl-6 expression [34]. Hans et al. report that expression of MUM1 in at least 30% of tumor cells in their series of DLBCL and were associated with a significantly worse overall survival rate

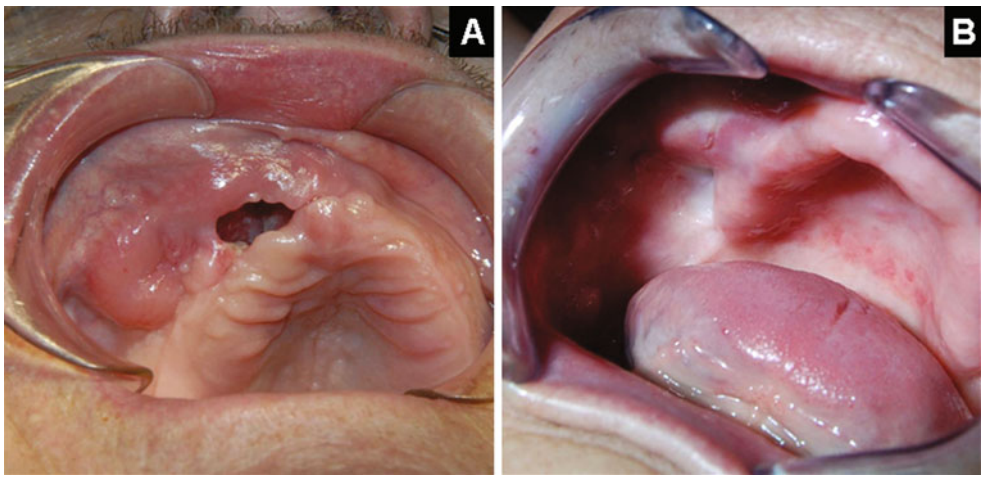

Fig. 3 **a** 72-year-old male with swelling and obliteration of the anterior maxillary vestibule presented with bone and soft tissue destruction (Case #11 Table 3). **b** 89 year old female with swelling exhibiting a small ulceration on the surface in the right maxillary vestibule presented with discomfort in denture wearing. (Case #12 Table 3)

[9]. MUM1 is considered to be a marker of the non-GCB phenotype especially when used in conjunction with CD10 and Bcl-6 [8–10, 35, 36].

In normal B-cell differentiation, the B cells go through the pre-germinal center, GC and post GC stages. Pre GC B cells are virgin B cells. Cells comprising the GC consist of small blast cells, centroblasts, centrocytes and occasionally plasma cells. The B cells that leave the GC and enter the post GC phase, differentiate either towards the memory cells or plasma cells [8]. Normal GC B cells express CD10 and Bcl-6 [17]. Bcl-6 may be lost by late GC B cells, which in turn acquire MUM-1 expression [34]. MUM-1, and CD138 are expressed by post GC B cells while MUM-1 may be acquired late in the GC reaction, CD138 expression is usually restricted to cells exhibiting plasmablastic differentiation and to plasma cells [8].

It has now been accepted that the distinct gene expression profiling subtypes, the GCB and non-GCB of DLBCL can be predicted using a panel of antibodies by immunohistochemistry that include CD10, Bcl-6 and MUM1 [9, 35, 37] If both CD10 and Bcl-6 are positive or even if CD10 alone is positive the tumor can be assigned in the GCB subgroup. If CD10 is negative and Bcl-6 is positive, the expression of MUM-1 determines the subgroup. If MUM-1 is negative the tumor represents a GCB subgroup whereas if MUM-1 is positive, a non-GCB designation is given. These antibodies recognize molecules whose mRNA expression is highly associated with the GCB and non-GCB subtypes in cDNA microarray studies [10, 13]. Cytogenetic abnormalities in DLBCL point to genes that are responsible for the early steps of lymphomagenesis [10]. Majority of the genes identified effect either the apoptotic pathway and/or the cellular proliferation pathways [38]. Two of the most common translocations found in DLBCL are *t*(14;18) and *t*(3;14). *t*(14;18) juxtapose Bcl-2 which encodes an antiapoptotic protein on 18q21(apoptic pathway) [39, 40]. Whereas *t*(3;14) juxtapose Bcl-6 which encodes a proliferative transcription factor on 3q27 (cellular proliferation pathway), with the immunoglobulin heavy chain on 14q32, leading to upregulation [41]. Chromosomal gains such as 8q and 2p are correlated with upregulation of c-myc, a proliferative protein and rel, which plays a role in preventing apoptosis [42, 43]. Chromosomal loss of 17p correlates with the deletion of tumor suppressor gene encoding for the p53 protein [42, 44]. Gene expression profiling techniques are very powerful and probably predictive of prognosis but are not easily available in clinical settings.

In the early 1990s, based on five clinical parameters the International Prognostic Index IPI) was developed [45]. This has developed into the most successful tool in predicting survival in lymphoma patients to date. The parameters included are age, stage of disease, extranodal site, performance status and serum lactate dehydrogenase (LDH) level [45]. Patients with a low risk IPI score had an approximate 5-year survival rate of 73% compared to 26% for high risk group [45]. Efforts to treat poor risk DLBCL (based on IPI) has produced inconsistent results and this has been attributed to the biologic heterogenicity of this entity [38, 46]. However IPI remains a very valuable tool but recent studies analyzing biologic differences, which are not taken into account by the IPI, significantly modify the clinical outcome [10, 12]. Multiple studies have shown that GCB subgroup lymphomas tend to exhibit statistically significant better prognosis when compared to the non-GCB group [8, 20, 35, 37, 38]. Distinctly poorer prognosis

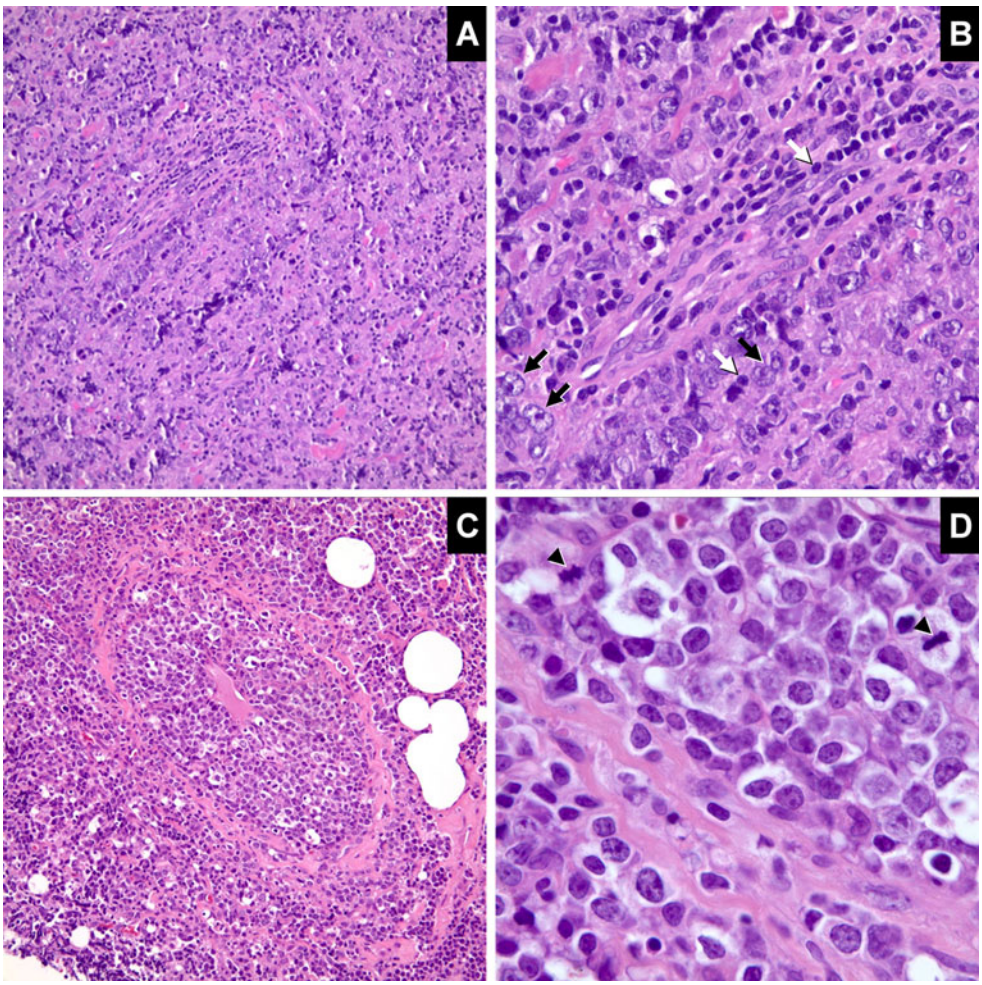

Fig. 4 Composite photomicrograph (hematoxylin and eosin stain) of primary Diffuse large B cell lymphomas in the oral cavity (**a** and **b**, **c** and **d**). **a** Diffuse infiltrate of abnormal lymphoid cells (original magnification ×200). **b** Higher magnification showing that the nuclear size of the abnormal lymphocytes (*black arrows*) is more than two times the size of normal small lymphocytes (*white arrows*). Neoplastic cells demonstrating an immunoblastic morphology with prominent central nucleoli are noted in lower left corner (original magnification ×500). **c** Both diffuse and angiocentric abnormal lymphocytic infiltrates of large cells (original magnification ×200). **d** Higher magnification showing that typical appearance of centroblastic variant (arrow heads highlight mitotic figures). The nuclear size of the abnormal lymphocytes is more than twice the size of a normal lymphocyte (original magnification ×1000)

has been reported in non-GCB DLBCLs in many diverse locations such as the central nervous system, gastrointestinal tract, breast, testes, among others [7, 13, 32, 35–37, 47] Our results appear to indicate a similar trend. A significant proportion (>50%) of the patients with non-GCB DLBCL succumbed to their disease, though limited information was available on the treatment these patients underwent. Importantly, the GCB group patients fared better and four of the five patients are alive and disease free as of submission of the manuscript.

Current treatment of DLBCL usually begins with multiagent chemotherapy, typically CHOP (cyclophosphamide, hydroxydoxorubicin, oncovin and prednisone) [48]. Inspite of the initial response to therapy, more than half the patients succumb to the disease [48]. Early stage disease care involves either chemotherapy alone or a combination of chemotherapy and radiotherapy. The chemotherapy usually involves 3 cycles of CHOP [49].

Patients are considered for bone marrow transplant if remission is not maintained. The role of surgery is severely limited in treatment of DLBCL. Other drugs used in multiagent chemotherapy for advanced stage disease usually involve various combinations of methotrexate, bleomycin, doxorubicin, vincristin, dexamethasone and leukcovorin, etoposide, mechlorethamine, procarbazine, cytarabine [50, 51].

In a recent study, Natkunam et al. [52], demonstrated that the Han's classification cannot predict outcome in a

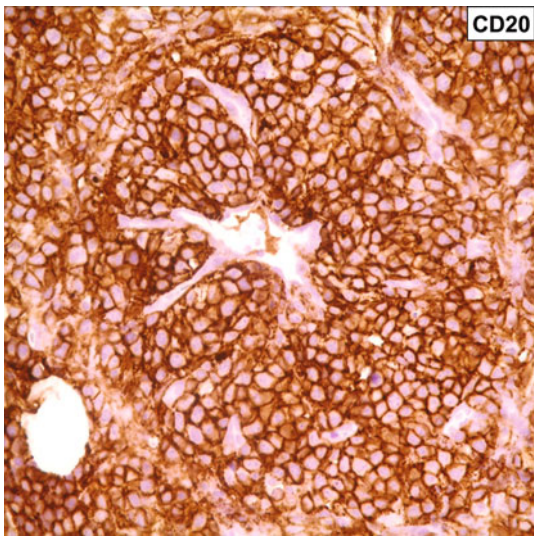

Fig. 5 Immunohistochemical study for CD20. All case demonstrated positive reactivity with this antibody. (Original magnification ×400)

large cohort study of DLBCL patients treated with rituximab. Significant differences in protein expression have been found for DLBCL and have yielded conflicting results [52, 53]. Since many antibodies are not available for many germinal center specific genes, validation of these results in independent groups of patients are necessary. However, treatment for both GCB and non-GCB subgroups is the same. Therefore newer studies should be directed toward developing better predictors of survival in DLBCL patients treated with rituximab.

Conclusions

Similar studies analyzing GCB and non-GCB subgroups of DLBCL have been conducted in tumors of the breast [36], CNS[47] and testes [35] and have revealed that non-GCB tumors exhibit a high proliferative rate and poor prognosis compared to GCB lesions. Our study of 13 primary DLBCLs shows that non-GCB lesions predominate and may exhibit a poorer prognosis with half of the patients succumbing to their disease while majority of the GCB group continue to be disease free. Pathologists and clinicians should be aware of this trend and may consider adding GCB classification to their reports when referring patients with oral DLBCL for further treatment and evaluation. The relatively small number of cases and short follow up periods (less than 5 years) are a limitation of the current study and additional investigation is warranted. However, stratification of DLBCL by immunohistochemitry is possible and is a relatively inexpensive, readily available and effective method of delineating the subtypes of DLBCL.

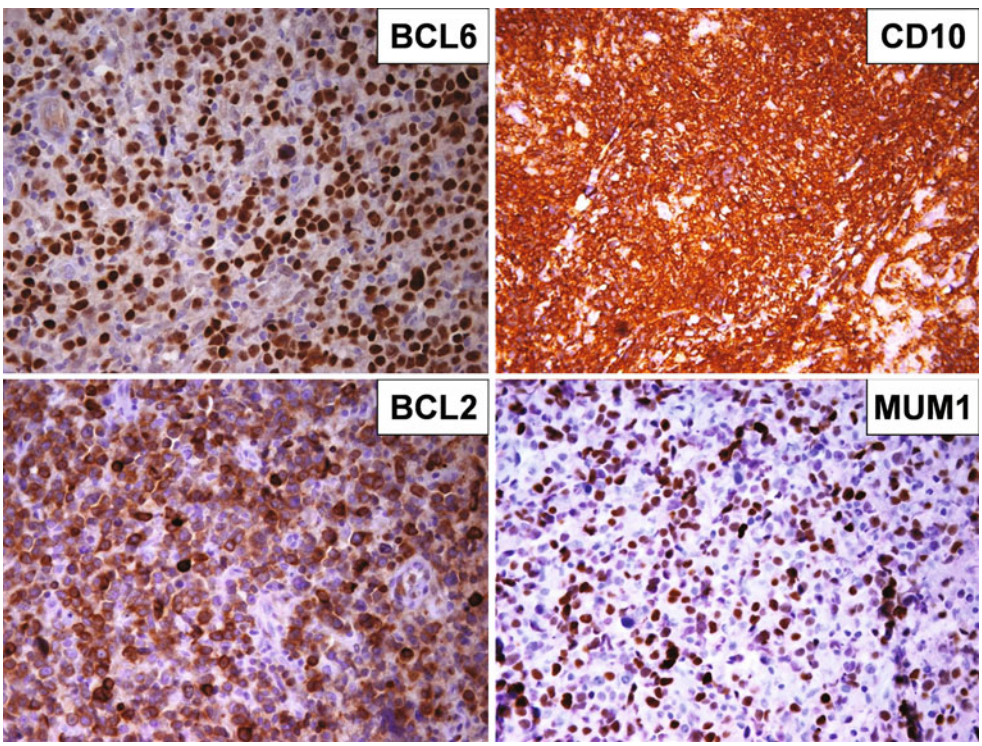

Fig. 6 Immunohistochemical panel (magnification ×40) demonstrating positive reactions

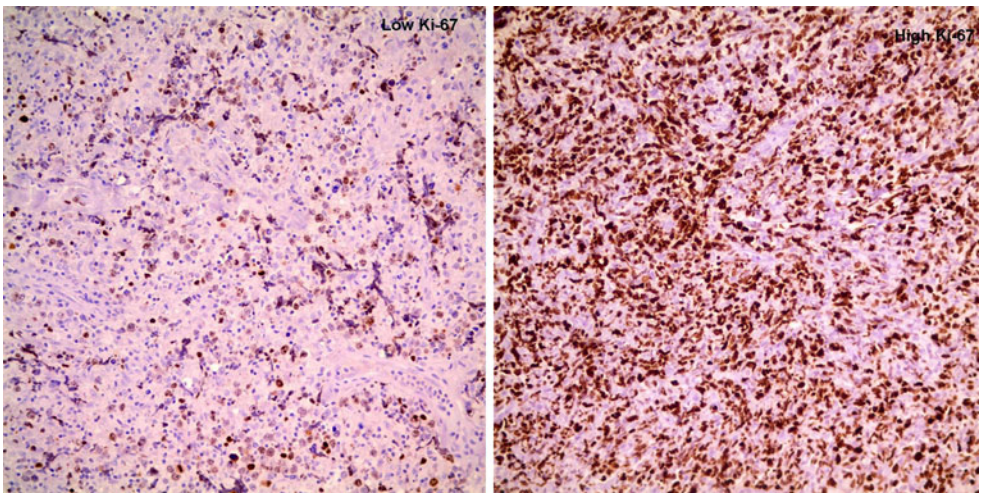

Fig. 7 Immunohistochemical study for Ki-67 (magnification ×20). All tumors showed frequent labeling with Ki-67 (ranging from low 40% to high 95%). **a** low percentage of positivity; **b** high percentage of staining

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