EUROPEAN OPHTALMIC PATHOLOGY SOCIETY
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Case Number: 895-21**,** 922-21
Material submitted: 1 histology H&E slide
Title of Case Presentation:

ENTK lymphoma, nasal type of the eyelid and orbit in a 47 yo female.

CLINICAL HISTORY:

A 47 yo female noticed a progressive swelling and redness of her left superior eyelid, initially unsuccessfully treated as a chalazion. The persistence of swelling prompted further investigations with an MRI demonstrating an orbital infiltrate predominating in the superolateral orbit with involvement of the lachrymal gland leading to a differential diagnosis of a pseudotumor.

Ophthalmological examination revealed no decrease in VA. There was a limitation of left eye elevation and abduction. Slit lamp examination showed a chemosis. There was no intraocular inflammation and fundus were normal bilaterally. Orbital biopsies were performed and initially sent to a private laboratory that established an initial diagnosis of vasculitis. The material was then sent to our laboratory. Systemic investigations for sarcoidosis, Lyme disease, Weggener granulomatosis, Rhumatoid arthritis, COVID, IgG4 were all negative.

Higher doses of steroids only briefly amended the symptomatology that progressively worsened with proptosis, increased upper and lower eyelid swelling, redness and painful eye movements. MRI of the orbit demonstrated an increase in the diffuse orbital infiltrate that involved all the ocular muscles, and partially compressed the optic nerve sheath. As the clinical situation worsened despite 4 weeks of high dose corticosteroids, a conjunctival biopsy was further performed.

OCULAR PATHOLOGY:

Macroscopy:

Initial eyelid, orbital and conjunctival biopsies:

2 skin fragments measuring 0.7 x 0.1 x 0.1 cm and 1.3 x 0.2 x 0.2 cm, 6 fibro-adipose fragments measuring 0.7 x 0.2 x 0.2 cm, 0.9 x 0.2 x 0.1 cm, 0.5 x 0.3 x 0.1 cm, 0.6 x 0.1 x 0.2 cm, 0.4 x 0.1 x 0.1 cm, 0.5 x 0.1 x 0.3 mm and 2 small fragments with a maximum diameter of 0.2 cm.

Secondary conjunctival biopsies:

2 small conjunctival fragments measuring 5.5 x 1.7 x 1.6 mm and 6 x 2 x 1 mm.

Microscopy:

Initial biopsies:

In all the fragments, there is a lymphocytic infiltrate with a striking perivascular predominance. A noticeable infiltration of the adipose tissue and areas of liponecrosis can also be observed. Lymphocytes are circumferentially surrounding adipocytes. There is a majority of small lymphocytes, but in some areas, cells with larger, irregular hyperchromatic nuclei with prominent nucleoli can be can be identified within the adipose tissue. There are no eosinophils, no neutrophils, no granulomas, no necrotizing vasculitis. In the skin fragment, no epidermotropism can be seen.

Secondary conjunctival biopsies:

In the oedematous stroma, there is a diffuse lymphoid infiltrate containing intermediate to large atypical cells with irregular nuclei with a perivascular tropism. The proportion of these cells is much higher than in the orbital biopsies. Small lymphocytes, histiocytes and plasmocytes are also present. Necrosis is absent.

Immunohistochemistry and in situ hybridization:

Initial biopsies:

There was a majority of T CD3+ cells over B CD20+. However, in some areas, notably in the adipose tissue and around the wall of some vessels, local accumulation of B cells could be seen. Among T cells, there was a predominance of TCD8 + over T CD4+. CD2 was preserved while CD5 and CD7 were lost. Cytotoxic markers (Granzyme, TIA1, perforin) were mostly expressed by small lymphocytes. Larger CD56+, CD57+ cells could be observed as well as numerous CD68+ macrophages. The CD56+ cells were also expressing cytotoxic markers. There was no expression of LMP-1 and EBNA2. EBV could be identified by in situ hybridization both in T CD3+ and more rarely in CD20+ cells. Rare CD30+ could also be observed.

Secondary conjunctival biopsies:

The large cells expressed CD56, CD3, CD2, and cytotoxic markers, but not CD5 and CD7. There was a partial expression of CD8 and CD4. Smaller B CD20+ could be found around the vessels. The proliferation index was around 70% in the areas of large atypical cells. In situ hybridization clearly demonstrated EBV within T cells. There was no expression of LMP1.

Clonality Analyis:

Multiplex PCR did not show any monoclonal rearrangement for IGH, IGK, IGΛ, TRG and TRB genes.

NGS:

A probably damaging *TET2*^{Y1902N} mutation as well as a probably damaging mutation in exon 9 of *BCOR* (c.4071+1G>A) were identified.

Diagnosis:
Extranodal Natural Killer/T (ENTK) Cell Lymphoma, nasal type of the right orbit and eyelid.

DISCUSSION:

Extranodal Natural killer/T cell lymphoma, nasal type are angiodestructive aggressive lymphomas. These lymphomas, associated with EBV virus, predominate in Asia and in South America, while they are rare in Western countries [1-4].The median age at presentation is 52 yo with a male predominance (2:1)[1]. These lymphomas usually occur in the nasopharyngeal area (>70%) and extranasal location most commonly include skin (10-26%), spleen and kidney, testes, GI tract, uterus, lungs and salivary glands. Primary localization within the orbit with primary orbital or ocular symptoms as illustrated in our case seems to be rare with only 34 previously reported cases to date[5-20].

Eyelid swelling was the commonest presentation (27/34, 79,4%)[5,7-10,13,15,17,18,20,21], followed by proptosis (32.3%)[6,7,9,13], ptosis (29.4%)[13], decreased visual acuity (20.5%)[6,7,14,17,19], diplopia (17.6%)[13,16], ocular pain (11,8%)[13,15], and epiphora (5.9%)[13]. Decreased visual acuity was found in patents with uveitis (anterior uveitis[6,17] , vitritis[12,14]) or papilloedema[6], but also in patients with decreased ocular motility[5,9,19] probably reflecting the extent of orbital extension by the lymphoma possibly altering optic nerve function.

From a histopathlogical perspective, the tissues are infiltrated by medium to large cells that can be admixed with small cells as seen in our situation. The cells have usually scant cytoplasm, irregular and folded nuclei with heterogeneous chromatin. Angiotropism is characteristic, but not essential for diagnosis. It can be associated with vessels fibrinoid necrosis that was not observed in our situation. The atypical cells usually express a NK phenotype with CD56, CD2, cytoplasmic CD3 (not membranous CD3), as well as cytotoxic markers (granzyme, perforin, TIA-1) usually without T cell receptor[22]. CD4, CD8, CD7 and rarely CD30 can also be found. The cells of origin is believed of NK lineage, but TCR rearrangements have occasionally been found, suggesting also a γδ–T cell origin[23].

The diagnosis of ENTK lymphoma of the orbit was not easy in our situation due to difficulties to precisely determine if EBV was localized in B cells or in T cells: there were indeed areas of accumulation of B cells with presence of EBV within B cells leading to the suspicion of a lymphomatoid granulomatosis. Numerous T cells containing EBV could only be demonstrated in an additional conjunctival biopsy. Our case also illustrate the difficulties to reinterpret and challenge an initial diagnosis established elsewhere.

EBV DNA and oncoproteins (EBV Nuclear Factor, EBNA1, as well Latent Membrane Protein 1, LMP-1) have been detected in ENTK lymphoma [24]. While EBV is ubiquitous, the higher incidence of ENTK lymphoma in Asia and in some areas of Central and South America is not clear. Associations with genetic predisposition (HLA A29)[25], environmental factors (pesticides, herbicides) as well as specific EBV strains with mutations in LMP-1 TCD8 epitope have been identified [23]. In chronic active EBV infections (CAEBV), EBV has been found in B cells in Western countries, while in NK/T cells in Eastern Asia[26]. While CD21 has been linked to the entry of EBV within B cells, the ligand of EBV in NK/T cells is not known and it has been hypothesized that EBV might infect a CD21 expressing progenitor cell that might subsequently differentiate into NK or T cells[25]. EBV viral copy number in blood has been correlated with disease severity and is an independent prognostic factor for survival[27]. LMP-1 is believed to be have an oncogenic drive in ENTK lymphoma through apoptosis inhibition, cycle cell progression, migration and invasion[28]. LMP-1 notably increases MYC, survivin and PD-L1 expression through NF-κB, MAPK, JAK/STAT and PI3k/mTOR signalling in EBV positive ENTKL cell lines[29-32].

The genomic landscape of ENTK lymphoma revealed frequent inactivating mutations (~50%) in *DEAD-BOX-3X (DDX3X)*[33], a RNA helicase. *DDX3X*, located on X chromosome, is a tumor suppressor gene and in vitro experiment with *DDX3X* mutants revealed increased MAP kinase and NF-κB signalling[33]. Mutations in *BCOR,* as identified in our case, have been previously identified in 13%-32% of the cases [34-36]. The presence of inactivating mutations in *BCOR* as well as loss of the corresponding allele suggest that *BCOR* might be a tumor suppressor gene in ENTK lymphoma[35]. BCOR is a co-repressor of BCL6, the master regulator of germinal center. BCL6 and BCOR are thought to cooperate to silence target genes, notably NOTCH transcription and in vitro experiments with NOTCH inhibitors induced growth arrest in ENTK cell lines[37]. Oncogenic *STAT3* mutations, usually occurring in a mutually exclusive way with *DDX3X* and *BCOR* mutations, have been reported in 23-26% of the cases and associated with an increased activity of the JAK/STAT pathway[34].

The outcome of patients with primary orbital ENTK lymphoma has not been favourable with a median overall survival of 5.5 months (SEM 1.6)[5-7,9-12,15,18,19]. As ENTK lymphoma express P-glycoprotein involved in multidrug resistance[38](notably resistance to anthracyclines included in CHOP containing doxorubicin), the worst survival (3 months) has been observed when CHOP regimen has been applied[5]. Extranasal localization is associated with a worse outcome[1] and in a Korean study, the 5 year OS was 13,3% in primary orbital ENTK lymphoma versus 26.4% in ENTK secondarily involving the orbit[13]. The optimal treatment approach combines non anthracycline based chemotherapy and radiotherapy that have proven to be superior to chemotherapy alone (complete response rate 78% in combined approach versus 49% with chemotherapy only, 2 year OS 90% versus 49%)[39]. In our situation, the patient achieved complete remission with an induction using a modified SMILE protocol (methotrexate, leucovorin, ifosfamide, mesna, dexamethasone, etoposide, pegaspargase), followed by radiotherapy (39.6 Gy in both orbits) and cisplatin chemotherapy. Complete remission with a 2 year OS has also previously been observed in 28 yo female with a primary orbital ENTK lymphoma treated with the SMILE protocol and radiotherapy[16].

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