

Results: Using a cutoff at 10%, methylation of MGMT was identified in 33% (11/33) cases of glioblastoma and 0% of the non-neoplastic epilepsy brain tissue. The range of percentage of methylation of any CpG island in MGMT promoter is 33-95% with a mean of 65%. By a series dilution of a methylated cancer cell line with an unmethylated normal cell line, pyrosequencing can detect 5% of tumor cells harboring MGMT methylation. The minimal amount of genomic DNA required to be able to successfully detect MGMT methylation by pyrosequencing is at 100 ng (approximately 3,000 cells). In comparison with MSP, pyrosequencing is comparably sensitive with less false-positive cases and also provide quantitative methylation value of each CpG island.

Conclusions: We have studied and validated the quantitative MGMT methylation assay on small biopsy tissue from patients with glioblastoma. We demonstrate that pyrosequencing detection of MGMT methylation has an analytical sensitivity suitable for clinical utility. MGMT methylation assay can provide a useful molecular biomarker for prediction of chemosensitivity in patients with glioblastoma multiforme.

1826 Osteopontin and CD44 Immunorexpression in Primary Central Nervous System Lymphoma, and Comparison with Nodal and Extranodal Diffuse Large B-Cell Lymphoma

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Background: Primary central nervous system lymphoma (PCNSL) is a diffuse large B-cell lymphoma (DLBCL) mostly of non-germinal center-like (non-GCB) type. Osteopontin, recently the most up-regulated gene in PCNSL, contributes to spread of carcinomas and myeloma by binding to CD44 variants (CD44v), especially CD44v6. We studied immuno-expression of osteopontin, and its putative receptor CD44v6 and CD44H in PCNSL.

Design: We studied 49 archival pathology cases, including 20 PCNSL, 12 N-DLBCL, and 17 EN-DLBCL. Immunohistochemical (IHC) staining was performed for osteopontin (OPN), CD44v6, CD44H, CD10, BCL-6, MUM-1, and Ki67, semi-quantitatively scored by % positivity of tumor cells (0%, 1-25%= score 1, 26-50%=2, 51-75%=3, and 76-100%=4) and staining intensity (none=0, weak=1; moderate=2; intense=3), and an overall IHC score (OIS) obtained by multiplying % score with intensity score (0 to 12), and correlated with Ki67 indices, and GCB vs. non-GCB types.

Results: OPN nuclear positivity was variably observed in 20 of 20 (100%) PCNSL cases, 16 of 17 (95%) EN-DLBCL, and 3 of 12 (25%) N-DLBCL. The OIS of OPN in PCNSL (7.0±3.5) and EN- (4.4±4.1) groups was significantly higher than N-DLBCL (0.3±0.6) ($p < 0.001$). The difference in OPN IHC scores between PCNSL and EN-DLBCL group, was not significant ($p=0.053$). Of the 16 of 49 cases positive for CD44v6 (33%), 6 were PCNSL, and 5 each EN- and N-DLBCL; no statistical difference was observed. CD44H was positive in all cases except one PCNSL, but without any significant differences across the three groups. When non-GCB was compared with GCB group, only CD44H expression was significantly different, with higher expression in non-GCB (score 12±1.5) than GCB group (9.5±3.1) ($P=0.015$); the differences were insignificant for OPN and CD44v6. Neither CD44H nor CD44v6 scores correlated with the OPN expression score or Ki67 index.

Conclusions: Osteopontin immunorexpression was highest in PCNSL (PCNSL > EN-DLBCL > N-DLBCL), suggesting its probable role in its pathogenesis. However, its lack of correlation with CD44v6 excludes any major role of the latter in OPN overexpression in PCNSL. Moreover, no association was observed between proliferative index and expression of OPN, CD44H or CD44v6. The significantly higher CD44H expression in non-GCB than GCB group may contribute to the aggressiveness of the non-GCB DLBCL. Further studies with additional CD44 variants may clarify the prognostic/predictive role of osteopontin in PCNSL.

1827 IMP3 Expression in Astrocytic and Oligodendroglial Tumors

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Background: Insulin-like growth factor-II mRNA-binding protein 3 (IMP3), an oncofetal protein, is involved in embryogenesis and is expressed in multiple malignant neoplasms. IMP3 has been shown to have higher expression in high grade tumors and is associated with poor prognosis. With the exception of glioblastoma, IMP3 expression in primary brain tumors of glial origin has not been systematically investigated. In this study, IMP3 expression in a series of astrocytic and oligodendroglial tumors is examined and correlated with histologic type and WHO grade.

Design: Two hundred forty-four (244) cases of neurosurgical biopsy and brain resection specimens from 1998-2008 were retrieved from the pathology archives of our institution. The tumors included: 149 cases of glioblastoma (GBM), (WHO Grade IV); 19 anaplastic astrocytoma (Grade III); 35 anaplastic oligodendroglomas and anaplastic mixed gliomas (Grade III); 14 oligodendroglomas and low grade mixed gliomas (Grade II); and 17 pilocytic astrocytomas (Grade I). Ten cases of gliosis were used as controls. All cases were stained with a monoclonal antibody specific for the IMP3 protein, and evaluated independently by 3 observers. IMP3 expression was divided into 2 categories: positive (moderate or strong cytoplasmic staining with membranous accentuation, >5%) and negative (absent or weak cytoplasmic staining, <5%).

Results: IMP3 was expressed in 93% (138/149) of GBMs, 79% (15/19) of anaplastic astrocytomas, 26% (9/35) of anaplastic oligodendroglomas and anaplastic mixed gliomas, and 18% (3/17) of pilocytic astrocytomas. Grade II oligodendrogloma and low grade mixed gliomas (0/14), and gliosis (0/10) showed no IMP3 expression. This expression was statistically significant ($p < 0.05$) between the high grade tumors and gliosis, among the high grade tumors (Grade III and IV), and additionally between each of the high grade tumors and low grade tumors. Among the low-grade (Grade I and II) tumors and gliosis cases, no statistically significant difference in IMP3 staining was found.

Conclusions: IMP3 expression is positively correlated with WHO grade. IMP3 expression is found in the majority of GBMs (Grade IV). Grade III gliomas have a higher percentage of expression than Grade I and II gliomas. IMP3 is expressed more frequently in astrocytic tumors compared to oligodendroglial tumors of the same grade or even higher grade. IMP3 staining is helpful in distinguishing high grade gliomas from low grade ones and gliosis.

Ophthalmic

1828 Twist, E-Cadherin, and Uveal Melanoma Metastasis

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Background: Metastatic spread is the main cause of death in patients suffering from uveal melanomas, but the molecular basis of tumor dissemination is only partially understood. Increased expression of proteins linked to epithelial mesenchymal transition (EMT) have been previously associated with invasion and metastasis in a number of cancers, but they have not been analyzed in uveal melanoma. We therefore examined expression of the EMT-associated protein Twist in uveal melanoma cell lines and in primary tumors. We also analyzed E-cadherin, which is frequently downregulated by Twist in metastatic carcinomas.

Design: Twist expression was analyzed using Western blots of protein extracts from four uveal melanoma cell lines (OCM1, OMM1, OM431), and by immunohistochemical analysis of a tissue microarray containing representative cores from 80 uveal melanomas. At least three intact cores were required for a tumor to be scored. We also examined E-cadherin levels using immunohistochemistry on the tissue microarray. A semiquantitative (0, 1+, 2+, 3+) scale was utilized for the immunohistochemical evaluations.

Results: Twist and E-cadherin were detected in protein extracts from all three uveal melanoma cell lines examined, while E-cadherin was present only in OCM3. Interestingly, the OCM3 cells expressing E-cadherin appeared to spread more slowly than the other two lines examined. Silencing of Twist expression with shRNA resulted in significant reduced invasion in vitro. On the tissue array, 72 cases had sufficient evaluable cores for analysis, and 27 of these (38%) had high levels (3+) of Twist. In the same 72 tumors, 18 (25%) had very low or absent (0 to 1+) expression of E-cadherin. Interestingly, 11 of the 18 tumors (61%) with low or absent E-cadherin had high levels of Twist, suggesting that Twist might repress E-cadherin expression in uveal melanoma. Statistical analysis of the immunohistochemical staining data in all 72 cases showed a trend towards a negative correlation between Twist and E-cadherin ($p = 0.06$, two-tailed Spearman test). Twist and E-cadherin expression did not appear to correlate with histopathological factors, metastasis, or overall survival, although clinical follow-up was only available for 25 patients with tumors represented on the array.

Conclusions: High levels of Twist protein expression are more common in uveal melanoma with low E-cadherin, suggesting a potential relationship between the two. However, OCM3 cells co-express both proteins, and the overall negative correlation on our tissue microarray was not statistically significant ($p = 0.06$).

1829 Expression of Sonic Hedgehog Signaling Pathway Related Proteins in Retinoblastoma

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Background: Sonic hedgehog (SHH) protein is a member of secreted signaling molecules that is involved in early embryonic development of various organs. Dysregulation of this pathway has been reported in several cancers but not yet assessed in retinoblastoma.

Design: Fifty-four cases of retinoblastoma were investigated immunohistochemically using antibodies against SHH pathway proteins such as SHH, GLI1, GLI2, GLI3, and ABC binding cassette G2 (ABCG2) on tissue microarray blocks. Western blot (WB) analysis was performed to confirm the expression of SHH and GLI proteins in two retinoblastoma cell lines, Y-79 and WERI-Rb-1. Correlation between the expression of SHH signaling proteins and various clinicopathologic parameters was statistically analyzed.

Results: SHH was expressed in most cases of retinoblastoma (52 of 54, 96.3%), 10 cases (18.5%) showing strong expression. GLI1 and GLI2 were also highly expressed, 44 of 54 cases (81.5%) and 49 of 53 cases (92.5%), respectively. GLI3, a transcriptional repressor, was expressed in 23 of 54 cases (42.6%) at low levels. ABCG2 expression was found in 12 of 54 cases (22.2%). High expression levels of these proteins in retinoblastoma cell lines were confirmed by WB. Expression of SHH correlated with advanced T stage ($p=0.026$), optic nerve invasion ($p=0.014$), necrosis ($p=0.036$) and distant metastasis ($p=0.029$). Expression of ABCG2, which represents chemoresistance, positively correlated with that of SHH ($p=0.002$).

Conclusions: SHH related proteins are highly expressed in retinoblastoma tumor cells. SHH expression is closely related to advanced disease and overexpression of chemoresistance protein, ABCG2. These findings strongly suggest that SHH signaling pathway may play a significant role in progression of retinoblastoma.

1830 Squamous Cell Lesions of the Conjunctiva: Evaluation of Current Grading Systems and Patho-Epidemiological Survey of Patients in Blantyre, Malawi

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Background: Conjunctival squamous cell lesions are an uncommon disease with a variable geographic incidence, being more common in countries closer to the equator.

In our experience, squamous cell carcinomas of the conjunctiva are more common in Malawi. To our knowledge, no large study investigating the association of squamous cell lesions in the conjunctiva with clinical, prognostic and pathologic features in Malawian patients has been performed.

Design: We surveyed all cancers diagnosed by histology from 1997 to 2007 from the Department of Histopathology at the University of Malawi College of Medicine (Blantyre, Malawi). We selected and reviewed 160 conjunctival biopsies and applied the published grading system for pre-cancerous versus invasive cancerous epithelial lesions to further understand the patho-epidemiology of conjunctival lesions at presentation. Microscopic criteria of conjunctival lesions were evaluated in both initial biopsies and any subsequent procedures, when possible. We also analyzed the clinical outcome and subsequent follow up for all conjunctival biopsies performed in ophthalmology clinics during 2011.

Results: Squamous cell carcinoma of the conjunctiva was the second most common location for squamous cell carcinoma, after the cervix (19% vs. 44%). Application of the published grading system showed that the majority of lesions biopsied in Malawi were at the invasive stage of cancer, although pre-cancerous lesions adjacent to the site of invasion were also identified.

Conclusions: A modified clinicopathological algorithm for conjunctival squamous cell lesions in African settings is necessary due to late presentations and difficulties with adequate follow up.

1831 No Expression of Proteins Associated with Alzheimer's Disease and Parkinson's Disease in Retina and Lens

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Background: Alzheimer's disease (AD) and Parkinson's disease (PD) are the two most common neurodegenerative disorders and together affect more than 30 million people worldwide. Definite diagnosis of AD and PD still relies on postmortem examination of the brain, but the possibility of earlier pathological diagnosis in living patients using abnormal proteins associated with these diseases is increasingly being explored. It has been previously reported that amyloid plaques were detectable non-invasively in the retina and lenses of a very small group of AD patients, but correlations with cortical pathology were limited. We therefore evaluated expression of proteins associated with AD and PD pathology in postmortem eyes in a larger case-control study.

Design: Eyes enucleated at autopsy from 7 cases of AD, 6 cases of PD or PD with dementia, and 6 age-matched controls were retrieved from the brain resource center and autopsy archives of our hospital. The AD cases included 6 cases of definite AD with a CERAD plaque score C and Braak score V-VI/VI, and one case of probable AD with a CERAD score B and Braak score II/VI. The PD cases included 2 cases of Lewy body disease in a brainstem pattern, 3 cases in a limbic pattern and one case in a neocortical pattern. Immunostains for beta-amyloid and phospho-tau and Congo red stains were performed on the AD and control cases. Immunostains for alpha-synuclein were performed on all cases.

Results: Contrary to previously published results, no amyloid deposits were detected in the retinas or lenses by beta-amyloid immunostains or Congo red stains in AD cases. The phospho-tau immunostains also did not reveal any abnormal tau accumulation in AD eyes. Scattered-to-frequent cells in the ganglion cell layer and inner nuclear layer of retina demonstrated diffuse cytoplasmic alpha-synuclein immunoreactivity in 5 of 7 (71%) AD cases, 4 of 6 (67%) PD cases, and 3 of 6 (50%) control cases. However, no definite Lewy bodies or Lewy neurites were identified. The retinal alpha-synuclein positivity and the frequency of the positive cells did not correlate with the presence or extent of the Lewy body pathology in the brain.

Conclusions: Abnormal protein aggregation characteristic of AD and PD is not present in the retinas or lenses of affected patients. Our data call into question the use of tracing dye or other methods to detect AD or PD pathology in the eyes of presymptomatic patients.

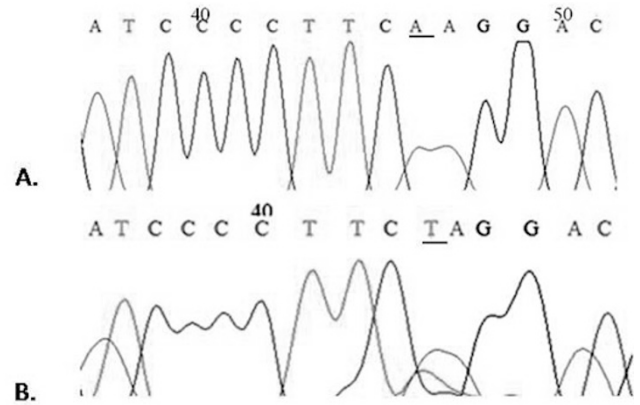
1832 Lymphoid Enhancing Factor-1(lef-1) Gene Mutation in Eyelid Sebaceous Carcinoma

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Background: A higher incidence of Eyelid Sebaceous carcinoma (ESC) is reported from China and India. The molecular mechanisms underlying pathogenesis of ESC are not known. β -catenin signaling pathway has a role in the normal development of sebaceous glands; Lef-1 (Lymphoid enhancing factor-1) gene is a key transcription factor and binding partner of β -catenin molecules within the nucleus. Mutations of Lef-1 have been described in transgenic mice models which lead to spontaneous skin tumors with sebaceous differentiation. The present study was therefore undertaken to determine the role of Lef-1 in the pathogenesis of ESC.

Design: Thirty-six cases of histopathological confirmed ESC diagnosed between 2007-2010 were selected for this study. Fresh tissues were obtained after informed consent and Institute Ethical Committee approval. Lef-1 immunorexpression using monoclonal antibodies against Lef-1 clone (EP2030Y), at a dilution of (1:50) was evaluated on formalin fixed paraffin embedded sections. Mutational analysis of Lef-1 (exon-1, which encodes the β -catenin binding domain) was undertaken by sequencing PCR. The results were correlated with the clinicopathological features.

Results: Loss of immunohistochemical expression of Lef-1 was present in 69% cases (25/36) and Lef-1 mutations in exon-1 were observed in 19% cases (7/36). All 7 samples showed non-sense mutations at codon(27) resulting in amino acid change AAG(Lysine)→TAG (stop).



(figure 1a shows wild sequence chromatogram and figure 1b mutated allele).

All the cases harboring the Lef-1 mutations were also negative for Lef-1 expression. 6/7 ESC patients with Lef-1 mutation had advanced age (> 60 years), 4/7 were females, upperlid was involved in all and 5/7 had tumor size \geq 2cm as well as poor histologic differentiation. There was no significant correlation of Lef-1 mutation with any of the clinicopathological features.

Conclusions: This preliminary study suggests the possible role of Lef-1 mutations in the molecular pathogenesis of Eyelid Sebaceous Carcinoma. However, further studies are needed to elucidate the exact role of Lef-1/ β catenin signalling pathway in its pathogenesis.

1833 Orbital Hamartomatous Mesenchymal Lesions in Adults: An Entity To Be Considered

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Background: A hamartoma is a tumor-like malformation composed by a disorderly proliferation of mature tissue elements normally present in the organ/anatomical site in which it originates. Neuromuscular hamartoma (NMH) is an infrequent pediatric peripheral-nerve tumor characterized by a proliferation of nerve fibers intermingled with mature muscle fibers in the course of the affected nerve, with clinical manifestations resulting from nerve dysfunction. Rhabdomyomatous mesenchymal hamartoma (RMH) is even rarer, composed of striated muscle fibers randomly arranged in the dermis/subcutaneous tissue, associated with other mesenchymal elements of normal appearance.

Design: Clinical history, histological slides, immuno-studies, imaging techniques and outcome of 3 adult male patients without previous ophthalmic history (ages 61, 62 and 80 years), which debuted with unilateral proptosis due to orbital space-occupying lesions, were reviewed. All biopsies showed a similar histology: a hamartomatous mesenchymal lesion. A fourth case with approximate morphology was discarded because the patient had a history of thyroid orbitopathy and bilateral exophthalmos.

Results: All 3 patients presented with unilateral proptosis and an intermittent orbital discomfort (average evolution of 6.6 months, range 4-10 months), without clinical manifestations of nerve dysfunction. Imaging techniques in all 3 cases showed a unilateral, ill-defined, orbital retrobulbar lesion. Biopsies revealed the morphology of a hamartoma: irregular fibers of skeletal muscle disorderly arranged, intimately intertwined with nerve bundles of variable diameter, mature adipose tissue in varying amounts and vessels of malformative aspect. There was neither cellular atypia nor mitosis. The 3 patients received conservative treatment after diagnosis, without evidence of disease progression by an average of 95 months follow-up.

Conclusions: To our knowledge, these hamartomatous orbital lesions in adults without previous pathology have not been described before. All 3 cases show the typical morphology of a hamartoma, and although they remember to a NMH and/or a RMH, do not meet clearly their characteristics. A clinical course associated with mass effect of the lesion without manifestations of nerve dysfunction suggests that they are not associated with a particular nervous branch. We highlight the importance of consider this diagnosis in a patient with an unilateral orbital space-occupying lesion and the relevancy of biopsy in these cases because these lesions may be managed conservatively and evolution is benign.

1834 Primary Signet-Ring/Histiocytoid Carcinoma of the Eyelid

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Background: We present two cases of primary signet-ring/histiocytoid carcinomas of the eyelid. These are rare, aggressive tumors, with approximately thirty reported cases.

Design: Both cases were men, aged 60 and 69-years, who presented with non-painful thickening of the eyelid. Biopsies were submitted for histopathologic review with histochemical and immunohistochemical stains to further classify the neoplasm.

Results: Biopsies showed eyelid skin with a dermal proliferation of histiocytoid cells with eccentrically placed nuclei and eosinophilic cytoplasm. A moderate percentage of these cells had vacuoles in the cytoplasm typical of a signet-ring cell appearance. Tumor cells percolated through the dermis in a patchy pattern without forming a discrete mass. Mucicarmine histochemical stains highlighted the signet-ring cells, and by immunohistochemistry the signet-ring/histiocytoid cells were positive for cytokeratin 7, carcinoembryonic antigen (CEA), epithelial membrane antigen (EMA) gross cystic disease fluid protein-15 (GCDFP-15 / BRST-2), and, e-cadherin. The histological and

immunohistochemical differential diagnosis includes metastatic signet-ring/histiocytoid carcinoma, especially histiocytoid lobular carcinoma from the breast, axillary skin, or other internal organs. Extensive systemic work-ups in both cases were negative for a primary site. Positron emission tomography and computerized tomography scans showed extensive orbital involvement. Orbital exenteration was performed in one case and showed diffuse involvement of upper and lower eyelids, conjunctiva, lacrimal gland, and orbit. Tumor cells focally involved the outer one-third of the sclera, but did not enter the eye.

Conclusions: We emphasize the patchy distribution, and in some small biopsies only a few tumor cells may be seen, giving the tissue the impression of being hypercellular. In these cases, immunostains for cytokeratin are useful.

1835 Accuracy of Frozen Section in the Intraoperative Diagnosis of Ophthalmic Diseases

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Background: Frozen section procedure is often employed during surgery to diagnose obscure lesions, to differentiate between malignant and benign lesions, to determine negative margins in oncological surgeries, and to confirm the presence of lesional tissue. In ophthalmic surgery this procedure is not widely used. This may be attributed to the small quantity of tissue obtained during surgery and lack of proper training in frozen section preparation and diagnosis among ophthalmic pathologists. In this study, we aim to determine the accuracy of intraoperative frozen section diagnosis in ophthalmic pathology at a major tertiary care ophthalmic institute.

Design: Data collection was done by searching the computer data bank for intraoperative and final diagnoses of ophthalmic specimens submitted to the Pathology Department between January 2005 and January 2010. Pathology reports were reviewed and classified as: accurate, inaccurate, and deferred.

Results: Between 2005 and 2010, 1501 ophthalmic specimens were submitted for frozen section diagnosis. Thirty cases (1.9%) were deferred for permanent section diagnosis. Out of the remaining 1471 cases, 1359 (92.4%) frozen section diagnoses were consistent with final diagnosis based on permanent sections, while 112 (7.6%) cases showed discrepancies between frozen section and final diagnosis. By location, highest diagnostic accuracy was achieved for anterior chamber (100%), optic nerve (100%), temporal artery (100%), and eyelid (96.5%) biopsies. Diagnostic accuracy was slightly lower for biopsies of conjunctiva (89.4%), orbit (88.1%), and eyebrow (87.5%). Clinically significant negative discrepancies were highest in the diagnosis of conjunctival/corneal lesions (5.02%). Clinically significant positive discrepancies were highest in orbital biopsies (6.5%). The specificity and sensitivity for frozen section diagnosis are 92.4%; with a positive predictive value of 93.1% and a negative predictive value of 91.6%.

Conclusions: Our data confirm that frozen section diagnosis is a reliable method for ophthalmic surgeries. Diagnostic errors may be reduced by adequate tissue sampling, complete clinical information, and good communication between surgeon and pathologist.

1836 Gender Differences and Estrogen and Progesterone Receptor Expression in Uveal Melanoma

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Background: Older reports have not demonstrated estrogen receptor (ER) expression in uveal melanoma (UVM). A recent study reporting differences in incidence and metastasis-related mortality by gender prompted a re-examination of ER and progesterone receptor (PR) expression by immunohistochemistry with comparison to clinical outcome and chromosome 3 status.

Design: 33 cases of UVM from 2004-09 treated by enucleation were examined for incidence and survival. 23 cases were evaluated for ER and PR by immunohistochemistry. Chromosome 3 status was determined by FISH and SNP array analysis.

Results: There were 19 men (58%) and 14 women (42%). 11 patients were DOD: 8 men (67%) and 3 women (25%); 1 woman was alive with metastasis. 10 men (56%) and 8 women (44%) were alive without metastasis. 3 patients died of other causes. ER was positive in 7 women (11 positive cases) and negative in UVM arising in men (8 of 12 negative cases). PR was negative in 20 of 23 cases and weakly positive or negative in 3. ER did not predict either clinical outcome or chromosome 3 status. Of the ER positive cases, 4 were DOD and 6 were alive without metastasis. Of the ER negative cases, 5 were DOD and 6 alive without metastasis. When compared to chromosome 3 abnormalities, ER was positive in 9/18 monosomy 3 cases and 2/5 disomy cases.

Conclusions: Gender may play a role in UVM. ER expression was present in 48% of cases of uveal melanoma and more likely in women in this small study, but it does not appear to be prognostic. We propose further study, including quantitative evaluation using image analysis.

1837 Association of Chlamydia Psittaci in Cases of Ocular Adnexal MALT Lymphoma

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Background: Extranodal marginal zone B-cell lymphoma of mucosa associated lymphoid tissue type (MALT lymphoma) is the most common type of malignant lymphoma of the ocular adnexa. Recently several studies have indicated that *Chlamydia psittaci* (*C.psittaci*) has been associated to ocular adnexal lymphomas with variable geographic distribution. This observation points toward the opportunity to investigate the prevalence of *C.Psittaci* infection in ocular adnexal MALT lymphoma.

Design: Nine patients with ten cases of biopsy proven ocular adnexal MALT lymphoma from over the past 10 years (from January 1st 2001 to August 31st 2011) were selected

from the archived file. Paraffin embedded, formalin fixed specimens were studied for *C.Psittaci* by immunohistochemistry and molecular analyses.

Immunohistochemistry was performed using anti-genus-specific lipopolysaccharide polyclonal antibody (BD1168, Santa Cruz Biotechnology). The presence of *C.psittaci* was determined by amplification of two separate targets of the *C.psittaci* genome. Nested PCR for the 16S-23S spacer region was performed using touchdown PCR followed by realtime amplification with visualization of melt curves. The second target used for identification was the *omp 1* gene of *C. psittaci*. A real time PCR with primers and FRET probes were used for amplification.

Results: In our study four of ten biopsy cases show immunoreactivity to anti-genus-specific lipopolysaccharide polyclonal antibody indicating that Chlamydial species are present in the lesional tissue. Furthermore, none of the specimens tested showed amplification for *C.psittaci* by molecular studies. The positive control used was successfully amplified and confirmed by sequencing for both targets.

Conclusions: In ten-year retrospective study of ocular MALT lymphomas in our institution, we could not identify *C.psittaci* by molecular studies. Our results correlate well with the current literature that *C.psittaci* vary widely between geographic regions and even within the same country. The prevalence range varies from 11-50%. Further collaborative large international study is still necessary to clarify the role of *C.Psittaci* in the pathogenesis of the disease.

Pancreas

1838 Gallbladder Pathology in IgG4-Related Sclerosing Disease

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Background: Originally recognized as autoimmune pancreatitis (AIP), IgG4-related sclerosing disease (ISD) is now well established as a systemic autoimmune process that can involve various organs and is characterized by increased number of tissue IgG4 plasma cells (PCs). Gallbladder (GB) manifestations of this disease have not yet been fully elucidated.

Design: Patterns of inflammation in 29 GBs from patients with proven ISD were contrasted with those in 2394 cholecystectomies with various etiologies. IgG4 immunostaining was performed in 156 (Table). The number of IgG4+ PCs was graded as negative (<10/HPF), low (10-29), moderate (30-49), or high (≥50).

Results: 7/29 GBs from ISD patients revealed a distinctive pattern of inflammation associated with delicate fibrosis akin to that seen in AIP (Fig.1). IgG4+ PC grade in these patients was low in 1, moderate 1, high 5. Of the remaining 22, 10 revealed mucosal-predominant lymphoplasmacytic cholecystitis, 2 had eosinophilic chronic cholecystitis, and 10 had non-specific chronic inflammation. Overall, high numbers (≥50/HPF) of IgG4+ PCs were seen in 24% of ISD GBs. However, they were also seen in GBs with non-ISD etiologies, including 10% of GBs with obstructive tumor in the CBD (Table). Among the 10 non-ISD patients with moderate/high numbers of IgG4+ PCs, 5 had diabetes mellitus and 1 had hypothyroidism.

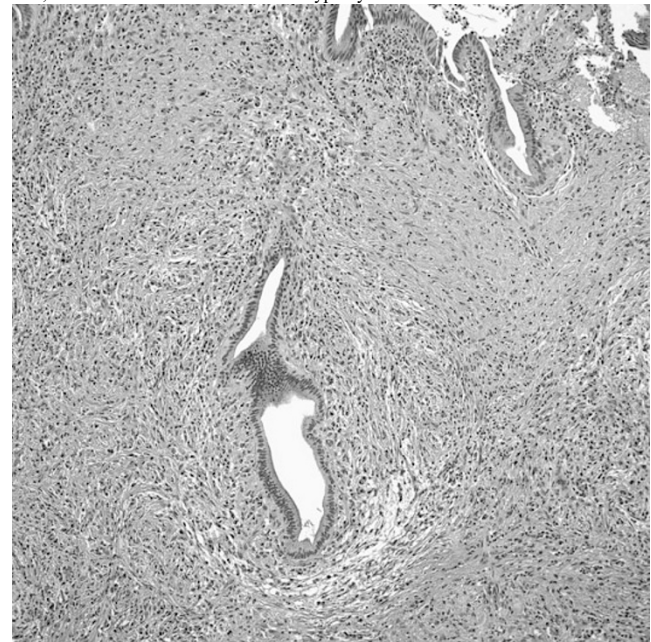


Table: IgG4 positive plasma cell scores (HPF) compared to the etiology

ETIOLOGY (n,%)	NEGATIVE (<10)	LOW (10-29)	MODERATE (30-49)	HIGH (≥50)
Autoimmune Pancreatitis/ISD (29, 18%)	16 (55%)	4 (14%)	2 (7%)	7 (24%)
Primary sclerosing cholangitis (20, 13%)	14 (70%)	6 (30%)	0	0
Obstructive tumor in the CBD (31, 20%)	23 (74%)	4 (13%)	1 (3%)	3 (10%)
Ordinary cholecystitis with plasma cells (76, 49%)	66 (87%)	4 (5%)	5 (7%)	1 (1%)