Primary VitreoRetinal Lymphoma

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UVEITIS WORK-UP

- Any uveitis work-up should exclude an infection and a masquerade syndrome

- as Intraocular lymphoma masquerade as chronic bilateral uveitis

- Most of Primary VitreoRetinal Lymphoma cases are diagnosed by Uveitis specialists
Rule out a Masquerade Syndrome

- Practical approach
- Should be considered in the evaluation of all patients with undiagnosed uveitis
- Before starting the work-up
- Before any treatment
Masquerade syndromes

- simulating a chronic idiopathic uveitis
- having an underlying primary cause
  . not immune mediated and
  . associated with an
- apparent clinical picture of intraocular inflammation
Masquerade syndromes

- are usually poorly, if not at all, responsive to corticosteroid Rx

- be suspicious when the apparent intraocular inflammation:
  . is unilateral
  . occurs in very young children or in the elderly
Masquerade syndromes

5% of referred uveitis patients

- malignancy in 48%
- nonmalignant conditions in 52%
Malignant disorders in adults

- Intraocular lymphoma
  - primary oculo-CNS NHL: PVRL
  - systemic NHL metastatic to eye
- Uveal malignant melanoma
- Metastasis (renal, lung, breast carcinomas, leukemia, cutan. malignant melanoma)
- Paraneoplastic syndromes (CAR, MAR, BDUMP)
Primary Oculo-CNS Non-Hodgkin’s Lymphoma
Primary VitreoRetinal Lymphoma (PVRL)

- Large B cell lymphoma
- Increased incidence
- Elderly patients
- Bilateral most of the time
- Ocular involvement may precede detectable lesions in other part of the CNS
Primary Cerebral Lymphoma (PCNSL)

• Aggressive B cell lymphoma that affects the CNS. Cerebral lymphoma represents 2% of the whole malignant lymphomas and 5% of the whole cerebral tumors.

• However, incidence has dramatically increased in the past 10 years by 4% per year with an incidence of 0.40/100 000 in 1982 vs 0.28/100 000 in 1988 in the USA.

• Ocular involvement is noticed in 13% of the patients affected by cerebral lymphoma (Ferreri AJ Ann Oncol 2002)

• Mean age is 55 to 65 year-old and the cerebral affection is more frequent in male.
Primary Cerebral Lymphoma (PCNSL)

- Incidence of $0.43 \pm 0.2$ per 100,000 person-years, with a mean age at diagnosis of 54 years (1500 cases/year in the USA).

- Knowledge of the incidence of PCNSL is useful, because it is estimated from multiple case series that intraocular involvement occurs in 15%–25% of cases (300 cases/year in the USA).

- Conversely, 65%–90% of patients presenting with PVRL develop CNSL, usually within 29 months.
Ocular involvement

- This lymphoma can initially involve the eye, then it is named Primary IntraOcular Lymphoma (PIOL) or Primary VitreoRetinal Lymphoma (PVRL)
- Frequently masquerades as a posterior uveitis
- Often misdiagnosed (suggestive clinical signs)
- “Apparent Intraocular inflammation”
  - Poorly responsive to corticosteroids
  - Bilateral cellular vitritis associated with subretinal infiltrates
  - Fluorescein angiography is highly suggestive
  - Sometimes associated with neurological signs
DIAGNOSIS

• Clinical features
• CNS involvement

• Fluorescein Angiography
• IL-10 levels in the AH

• Diagnostic Vitrectomy
• Brain biopsy
Primary Oculo-CNS Non-Hodgkin’s Lymphoma

- Blurred vision, floaters
- Non painful and white eyes
- Minimal or no anterior segt. Inflammation
  \textit{(No posterior synechia, normal LCFM)}
- Sheets of vitreous cells
- Subretinal infiltrates, vasculitis, CME
- Poorly responsive to corticosteroid Rx
Circumferencial cellular infiltration

Spider’s web
Sub retinal infiltrates

Retinal infiltration

RPE disturbances

Sub retinal infiltrates
After ChemoRx

Sub retinal mass
Fluorescein angiography

- RPE disturbances with pigment migrations, atrophic lesions (windows defects) associated with round hypofluorescent lesions (fluo blockade) in 50% = highly suggestive (leopard appearance)

- Vasculitis in 7% with vascular occlusions in 2%
- Cystoid macular edema 2%

- Normal fundus BUT abnormal fluorescein angiography in 5% of the cases
- Normal fluorescein angiography in 5%
Leopard skin = lymphoma
Hypofluorescent tumoral
Subretinal infiltrates

RPE alterations
Retinal optical coherence tomography manifestations of intraocular lymphoma

Tin Yan Alvin Liu & Mohamed Ibrahim & Millena Bittencourt & Yasir J. Sepah & Diana V. Do & Quan Dong Nguyen

Neurologic work up

- Cerebral involvement in 65%:
  - CNS tumor precedes ocular involvement in 25 % with an average of 54 months (3-144)
  - CNS tumor and ocular involvement at the same time in 21%,
  - Ocular involvement precedes CNS localisation in 55% after a mean time of 20 months (1-84)

- MRI: hypersignal in T2 enhanced by gadolinium
- LP: lymphoma cells, IL-10 levels
DIFFERENTIAL DIAGNOSIS

- HIV associated lymphoma
- Systemic NHL metastatic to the eye
- Other hematologic cancers
- Metastatic tumors
Systemic Non Hodgkin’s Lymphoma

- Metastatic to the eye
- infiltration of the choroid, exsudative retinal detachment
- Hypopion or hyphema in an uninflammed eye
Waldenstrom’s disease
Metastatic tumors

- Carcinomas (renal, lung, breast)

- Cutaneous Malignant Melanoma

- Leukemia (CLL, AML)
Non-Malignant Disorders

Endophthalmitis

- endogenous
- postoperative
Primary Oculo-CNS Non-Hodgkin’s Lymphoma (PVRL)

- Fluorescein angiography, MRI
- Elevated IL-10 levels in AH, vitreous, CSF
- Cytology +++, immunohistochemical staining for B and T cell markers and for kappa and lambda light chains
- IgH clonal gene rearrangement and translocation (microdissection + PCR)
IL-10 levels (AH, Vitreous, Spinal fluid)

- Vitreous and spinal fluid IL-10 levels are correlated with the clinical activity and the number of malignant cells (IL-10 / IL-6 quotient is above 1)

- Elevated IL-10 in the aqueous humor (> 50 pg/ml) : strong suspicion +++

- Demonstration of IL-10 and its mRNA in lymphoma cells (RT-PCR, nested PCR, microdissection)
Diagnosis

• IL-10 levels in AH >50 pg/ml and in vitreous body > 400 pg/ml or IL-10/IL-6 ratio

• Diagnostic vitrectomy
  – Cytology
  – Immunohistochemistry

• Microdissection + PCR (CC Chan)

• Retinal biopsy
• Stereotaxic brain biopsy
Presumed PIOL

MRI

Positive

Diagnostic vitrectomy

Cytology+
IL-10>50 pg/ml
PCR+

Lymphoma

Cytology negative
IL-10>50

Brain biopsy

Negative

HA tap

IL-10>50

Corticotherapy

corticoresistance

IL-10<50

Clinical response
Primary Oculo-CNS Non-Hodgkin’s Lymphoma (PVRL)

- Identification of malignant B lymphocytes in the vitreous, the CNS and the spinal fluid (morphology, immunostaining …)

- But: few malignant cells, presence of polyclonal reactive lymphocytes, necrotic debris …)
Vitreous specimen

Large lymphoid cells
Primary IntraOcular Lymphoma
Primary Oculo-CNS Non-Hodgkin’s Lymphoma (PVRL)

- Non-Hodgkin’s Diffuse Large B-cell lymphoma
- Rapidly detectable gene rearrangements by PCR
  - Clonal rearrangements in heavy chain Ig genes (FR3 region of IgH variable regions) *(non follicular)*
  - bcl-2 gene translocation t (14;18) *(follicular)*
- Diagnostic and therapeutic implications
  - benign hyperplasia
  - low grade lymphoma
  - high grade lymphoma
Framework Region 3
Reciprocal Chromosomal Translocation

- IgH (chromosome 14)
- bcl-2 (chromosome 18)
- Reciprocal translocation t (14;18) = bcl-2 gene contiguous to the IgH promoter
  - Gene fusion
  - Deregulation of bcl-2 gene
  - Expression of the bcl-2 protein = proto-oncogene
  - Apoptosis inhibition
- Accumulation of tumor cells (no increased proliferation nor differentiation block)
Reciprocal t(14;18) chromosomal translocation
Microdissection and PCR amplification

- Microdissection
  . Differentiate the suspected tissue from the background noise
  . Accurate diag. on previously fixed tissue
  . Guided sampling of few suspected cells

- PCR amplification
  . IgH clonal rearrangements : FR2A, FR3A
  . Bcl-2/IgH rearrangement associated with the t (14;18) translocation
  . Possible diag. In the absence of surface Ig
microdissection
microdissection
IgH clonal rearrangement
Primary Oculo-CNS Non-Hodgkin’s Lymphoma

- Differential diagnosis: Lymphoid hyperplasia of the uvea

- Treatment:
  
  systemic and intrathecal chemotherapy
  
  radiotherapy
  
  intravitreal chemotherapy ($MTX$ $400\mu g$, $Rituximab$ $1mg$)
  
  bone marrow transplantation (stem cells)
TREATMENT of PIOL

- Chemotherapy >>> radiation therapy
  *(systemic and intrathecal)*
  *(temoselomide p.o. + MTX i.v. – 5 /28 days)*

- Autologous bone-marrow transplantation

- Intravitreal chemotherapy
  *(400 microg MTX inj. Twice weekly for three weeks, thiotepa 2 mg once a week for three weeks, Rituximab anti-CD20 1mg/wk)*
cytology
pathology

AFTER CHEMORX
• **chemotherapy** \( n=15 \) : iv, PL MTX+aracytine
  – Deaths \( n=7 \) related to the NHL
  – Active eye disease \( n=4 \), active CNS disease \( n=2 \)
  – Complete Remission \( n=4 \)
  – **Median survival 13 months**

• **chemotherapy + radiation** (CNS + Eye) \( n=10 \)
  – Deaths \( n=5 \) related to the NHL
  – Active eye disease \( n=2 \), CNS \( n=0 \)
  – Complete Remission \( n=3 \)
  – **Median survival 11 months**

• **chemotherapy + autologous BM transplantation** \( n=12 \)
  – Deaths \( n=2 \) (unrelated to the NHL)
  – Complete Remission \( n=5 \)
  – évolutif œil \( n=5 \), SNC \( n=0 \)
  – **Median survival = 50 months**
MANAGEMENT of PIOL

Eye + CNS involv.

- CR CNS
  - CR Eye
    - monitoring
- CR CNS
  - active Eye
    - IVT-MTX
- PR CNS
  - active Eye
    - BM transplant.
MANAGEMENT of PIOL

Isolated ocular involv.

Karnofsky > 40
- conventional chemotherapy

Karnofsky < 40
- IVT-MTX
Murine models of PIOL

Intraocular T-cell lymphoma:

Assaf et al
Newborn mice (6-10 days postnatal)
Rev-2-T-6:syngeneic
Inoculation IP: developpement of PIOL and CNS lymphoma
Virchows Arch, 1997

Chan et al
Adult mice
Rev-2-T-6: syngeneic
Inoculation IVT
IOVS, 2005

RPE: a possible barrier in the limitation of tumor infiltration between the retina and the choroid?
Intraocular B-cell lymphoma:

*Li et al*

Adult mice
Xenogeneic (Human B-cell lymphoma cells)
Inoculation: IVT
SCID mice

*Can research, 2006*

Expression of chemokine receptors at the lymphomatous cell surface

- CXCR4
- CXCR5 (up-regulated in vivo)

Eradication of tumor colonization and invasion by a B-cell specific immunotoxin
Intraocular B-cell lymphoma:

Development of a new murine model of PIOL:

• **Adult mice:**
  - Mature immune system
  - Clinical relevance
• **Immunocompetent host:**
  - Complete cellular and molecular microenvironment
• **Syngeneic**
  - Avoid xenograft rejection
• **B-cell lineage (IIA1.6 cell line)**
  - Clinical relevance (>80% of PIOL are B-cell lymphoma)
  - Expression of MHC II and costimulatory molecules on B-cell that are absent on T-cell surface
Murine model of PIOL

Intravitreal injection of syngeneic lymphomatous B-cells in adult immunocompetent mice
Murine model of PIOL

Histological aspect of the retina

- lens
- Vitreous cavity
- Retina

Vitreous cavity
Retina
Murine model of PIOL

Histological aspect of the retina and the choroid:

- Normal retinal and choroidal cells
- Lymphomatous B-cells expressing Green Fluorescent Protein (GFP)
Murine model of PIOL

Detection of tumor cells by flow cytometry analysis
CONCLUSION

• Neuro-imaging +++
• Diagnosis based upon Diagnostic Vitrectomy
• Haematologist’s expertise +++
• AH IL-10 dosage and molecular techniques improve the yield of diagnostic vitrectomy.
• Multidisciplinary approach (diagnosis, treatment)
• Poor prognosis
• Animal model
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