T-cell: CD3
B-cell: CD20 (CD79a)
NK/T-cell: CD56

What are the tools?
- Flow cytometry
- Molecular studies
- Hematoxylin and Eosin Biopsy
- Immunohistochemistry

Flow cytometry
- Cell suspension
  1. Blood
  2. Tissue
- RPMI Media or Saline soaked gauze
- Count/sort cells using surface and cytoplasmic antigens (e.g. CD markers)
- Utility:
  1. B-cell lymphomas: tissue, low yield
  2. Sézary syndrome: blood

Molecular studies
- Polymerase chain reaction (PCR): technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence
- Preformed on:
  1. Paraffin embedded tissue
  2. Fresh tissue
- Utility:
  1. T-cell receptor (TCR) - molecule found on the surface of T lymphocytes that is responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules
  2. IgH Immunoglobulin heavy locus - region on human chromosome 14 that contains a gene for the heavy chains of human antibodies (or immunoglobulins)
H+E biopsy
- Formalin fixed and embedded in paraffin
- Two 4 mm punch OR 1 long shave and one 4 mm punch
- Can do molecular studies on paraffin embedded tissue
- Cannot do flow cytometry

**Extranodal NK/T cell lymphoma nasal-type**
Lethal midline granuloma, polymorphic reticulosis, ENKL, NTCL, midline malignant reticulohistiocytosis
Most common lymphoma of the nasal cavity
Most common presentation of ENKL is with upper aerodigestive tract (UAT) symptoms
Immunophenotype: CD2+, CD56+, CD3-, cytoplasmic CD3
EBV positive (EBER by in-situ hybridization or LMP-1 IHC)
REFER!

**Cutaneous B-cell Lymphomas**
Approximately 20-25% of all cutaneous lymphomas
Cutaneous lymphoid hyperplasia (CLH)
Shows considerable clinical overlap so repeat skin biopsies with review by dermatopathology and/or hematopathology is important
Bloodwork:
- Complete blood count (CBC) with differential
- Lactate dehydrogenase
- Comprehensive metabolic panel
- HIV
- Lyme ELISA/titer
Consider: Peripheral blood flow cytometry - lymphoma panel
Systemic evaluation:
Recommended for every patient even if only a solitary lesion to distinguish between primary cutaneous B-cell lymphoma versus secondary involvement of skin:
1. Computed tomography (CT) chest/abdomen/pelvis with and without contrast OR Positron emission tomography (PET) / CT scan
2. Bone marrow biopsy:
   a. No consensus for indolent cutaneous B-cell lymphomas
   b. Suspect a secondary CBCL or patient has abnormal bloodwork or systemic symptoms
Autoimmune associations:
- Helicobacter pylori
- Hepatitis panel
- Thyroid-stimulating hormone (TSH)
Molecular: Monoclonal rearrangement of IgH (50%)
Treatment:
- follow clinically
- topical/intralesional steroids
- XRT
- Rituximab infusions or IL Rituximab

**Primary cutaneous marginal zone lymphoma**
Papules and nodules on extremities, trunk
Histopathology
Patchy, nodular, or diffuse infiltrates of central nodular dark area with small reactive lymphocytes with or without germinal centers
Immunohistochemistry
CD20+, BCL2+, BCL6-

**Primary cutaneous follicle center lymphoma**
Papules coalescing into a plaque or plaques on scalp, face, back
Histopathology
Follicle center cell: Nodular or diffuse infiltrates of centroblast, centrocytes, smaller lymphocytes (follicular, diffuse or mixed patterns)
Immunohistochemistry
Follicle center cell: CD20+, CD10+, BCL6+, BCL2- (except when secondary cutaneous)

**Indolent Primary Cutaneous B-cell Lymphomas**
1. Primary cutaneous marginal zone lymphoma
2. Primary cutaneous follicle center
Once diagnosis is established can be followed clinically
REFER for diagnosis!

**Primary cutaneous diffuse large B-cell lymphoma, leg type**
Leg but can arise elsewhere, nodules that can ulcerate and can have lymphadenopathy
Histopathology
Dense diffuse infiltrates dominated by large cells with round nuclei (centroblast, immunoblasts)
Immunohistochemistry
CD20+, BCL2+, MUM1+
REFER!

**Cutaneous T-cell Lymphomas**
1. Mycosis Fungoides
2. Variant of Mycosis Fungoides
3. Sézary Syndrome
4. Other Cutaneous T-cell Lymphomas

**Other Cutaneous T-cell Lymphoma Variants**
1. Adult T-cell leukemia/lymphoma (ATLL)
2. Cutaneous gamma/delta T-Cell Lymphoma (CGD-TCL)
3. Subcutaneous panniculitic like T-cell lymphoma alpha/beta (SPTL)
4. CD30+ Lymphoproliferative Disorder
   a. Lymphomatoid Papulosis (LyP)
   b. Anaplastic large cell lymphoma (ALCL)

**Adult T-cell leukemia/lymphoma (ATLL)** Four clinically distinct entities: acute, chronic, smoldering and lymphomatous
Cutaneous manifestations are generally a presentation of disseminated disease and can occur in 43-72% of cases
ATLL can appear on the skin as erythroderma, infiltrated plaques, localized or generalized macules, papules, patches, nodules, or tumors.
The gold standard for the diagnosis of ATLL is a positive HTLV-1 serology

**Cutaneous gamma/delta T-cell lymphoma**
Previously described as:
SPTL with a gamma/delta phenotype
Primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma
Generalized pagetoid reticulosis (Ketron-Goodman type)
Adults affecting both sexes equally, rapidly growing disseminated plaques and/or ulceronecrotic nodules or tumors that occur mostly, but are not limited to, the extremities
Patients can present with systemic symptoms including lymphadenopathy, hepatosplenomegaly, cytopenias, and/or elevated liver function tests
Constitutional symptoms of fever, night sweats and fatigue are common
Hemophagocytic syndrome
There are three histologic patterns of involvement: epidermotropic, dermal and subcutaneous
Bone marrow examination may show histiocytic hyperplasia, hemophagocytosis or decreased cellularity

*The comprehensive criteria for diagnosis of CGD-TCL:*
1. prominent involvement of the dermis and epidermotropism
2. CD4-/-CD8- phenotype
3. aberrant expression of CD56
4. TCR gamma gene rearrangement
5. unresponsiveness to chemotherapy with an extremely poor prognosis
Immunophenotype: CD3 +, CD5 +, TCRGamma+, BetaF1-, CD4 -, CD8 -, TIA-

**Subcutaneous panniculitic like T-cell lymphoma alpha/beta**
Cytotoxic lymphoma characterized by a subcutaneous, nodular or diffuse, atypical lymphoid infiltrate mimicking a lobular panniculitis
Immunophenotype: alpha/beta+ (BetaF1+), CD3+, CD4-, and CD8+.
Patients are commonly misdiagnosed as having lupus erythematosus panniculitis (lupus profundus) since their clinical features may overlap and some patients have autoimmune syndromes: positive ANA, hematologic changes (anemia, neutropenia), renal changes, and positive DIF on lesional skin
If associated hemophagocytic syndrome worse prognosis
Patients without hemophagocytic syndrome may be treated with oral steroids
Diagnosis of lupus panniculitis should be carefully made
REFER! (evolving entity)

**CD30+ Lymphoproliferative Disorder**
30% of all CTCL
Subtypes:
1. lymphomatoid papulosis (LyP)
2. anaplastic large cell lymphoma (ALCL)
The diagnosis of primary cutaneous ALCL versus LyP is based on clinical presentation and disease course
Immunophenotype: CD3+, CD4+, CD30+
**Lymphomatoid Papulosis**

Occurs mostly in young adults as violaceous to reddish-brown papular, papulonecrotic, and/or nodular skin lesions at different stages of development

Mostly on the trunk and proximal extremities

Usually less than 1 cm in size, self-healing, and may disappear within 3-12 weeks leaving behind superficial scars

Time interval between eruptions is variable, ranging from weeks to several years

Histology is divided into three (main) different subtypes

Type A: wedge-shaped and have evidence of scattered or small clusters of large CD30+ cells mixed with histiocytes, small lymphocytes, neutrophils, and/or eosinophils.

Type B: uncommon (less than 10%) and are characterized by a wedge-shaped or sometimes band-like infiltrate of epidermotropic small atypical cells with cerebriform nuclei, similar to the histopathology observed in MF

Unlike the other types, these cells do not express the CD30 antigen

Type C: nodular infiltrate with sheets of large CD30+ T-cells

Cannot distinguish from ALCL histologically!

At risk for a second cutaneous or nodal lymphoid malignancies, including mycosis fungoides, cutaneous or nodal anaplastic large-cell lymphoma and Hodgkin lymphoma

4%-25% of affected patients; occur before, concurrent with, or after the onset of LYP

Staging: complete history, including previous lymphoid neoplasms (in particular Hodgkin lymphoma and MF)

B-symptoms

Physical examination (lymph node)

Laboratory studies: CBC, CMP, LDH

Treatment:

None of the therapies for LYP has been proven to alter course of disease or to prevent LYP-associated second lymphomas

Few lesions: clinically follow or topical steroids

Numerous, disseminated:

Phototherapy especially PUVA

Methotrexate

REFER if not a straight forward case due to complexity of diagnostic work up

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**Primary Cutaneous Anaplastic Large Cell Lymphoma**

Occurs mainly in adults with a higher prevalence in men than women

Solitary or localized violaceous to reddish-brown nodules or tumors with ulceration multifocal 20%

Can regress, but more often result in a partial rather than complete resolution and leaves scarring

Immunophenotype: CD30+, CD4+, CD3+

Most lesions demonstrate a clonal rearrangement of the T-cell receptor

Distinction of primary cutaneous ALCL from systemic ALCL is imperative given that skin is the most common extranodal site of disease:

1. cutaneous variant expresses cutaneous lymphocyte antigen (CLA), while epithelial membrane antigen (EMA) and anaplastic lymphoma kinase (ALK) are absent

2. the (2;5)(p23;q35) translocation is rarely seen in cutaneous ALCL

Treatment: XRT or Excision if skin only

REFER!
**Mycosis Fungoides**
Most common type of cutaneous T-cell lymphoma
Classic Alibert-Bazin
Patch, plaque, tumor
Double covered areas
Higher risk of secondary malignancy
Especially Hodgkin and non-Hodgkin lymphoma
Non hematologic malignancy
Associated with LYP
Morphology small cerebriform cells, epidermotropism is common, Darier’s nests (Pautrier’s microabscesses)
Immunophenotype: CD2+, CD3+, CD4+, CD5+, CD8-, CD30 may be positive in large cell transformation
Molecular: TCR 40-50%

**Diagnosis**
Biopsies: 1 punch and 1 H+E
Immunohistochemistry
TCR
If patches/plaques and tumors sample both types of lesion
Multiple sequential biopsies often needed
Sun protected, double covered areas, untreated for 2-3 weeks

**Staging**
Physical exam for estimation of tumor burden
Computed tomography (CT) chest/abdomen/pelvis with and without contrast OR Positron emission tomography (PET) / CT scan
Laboratory studies: CMP/CBC/LDH
Blood for flow cytometry / Sézary cell count
Refer for staging

**Long-term Outcome of 525 Patients With Mycosis Fungoides and Sézary Syndrome**
T1: 2% die of their disease
T2: 15% die of their disease
T3 or T4: 24% die of their disease
Common cause of death during the tumor phase is sepsis from Pseudomonas aeruginosa or Staphylococcus aureus caused by chronic skin infection and subsequent systemic infections

**Long-term Outcome of 525 Patients With Mycosis Fungoides and Sézary Syndrome**
Risk of progression @ 5, 10, and 20 years
T1: 10%, 13%, and 16%
T2: 22%, 32%, and 40%
T3: 56%, 72%, and 81%
T4: 48%, 57%, and 78%
Risk of progression for extracutaneous disease @5, 10, and 20 years
T1: 1%, 2%, and 2%
T2: 8%, 9%, and 9
Risk for disease progression worsened with more advanced T classification

**Treatment**
Stage dependent
Early nbuvb/PUVA, topical steroids, topical chemotherapy, imiquimod
Bexarotene, renbuvb/rePUVA, HDAC inhibitor, photophoresis, xrt
Allogenic stem cell transplantation
Total body electron beam irradiation: Limited availability

*Topical Corticosteroids for Mycosis Fungoides Experience in 79*

T1: 94%
T2: 82%
Patch/ plaque

Side effects:
Reversible depression of serum cortisol levels occurred in 10 (13%)
Minor skin irritation occurred in 2 patients and localized, reversible skin atrophy in 1

Potency: temperature, hydration, occlusion
*Arch Dermatol.* 1998;134(8):949-954

**Mechlorethamine**

Gel 0.016%(VALCHLOR®)
Accredo Specialty Pharmacy
Compounded ointments 0.02% - 0.04%
ACD 8%
CR 50%
Aqueous 0.02%
ACD 67%
CR 63%

Alkylating agent: cytotoxic to T-cells, immune effect?
Chemotherapy in Cutaneous T-cell Lymphoma Positive Results of a Randomized, Controlled, Multicenter Trial Testing the Efficacy and Safety of a Novel Mechlorethamine, 0.02%, Gel in Mycosis Fungoides *JAMA Dermatol.* 2013;149(1):25-32.

**Bexarotene (Targetine®) 1% Gel**

CTCL (1A and 1B) refractory or failing other therapies
Second generation retinoid RXR
Inhibits cell growth and induces apoptosis
Minimal systemic absorption unless >40% BSA
Open label ORR 54-63%
QD escalating to 3-4x day
20 weeks


**Nbuvb**

311nm
Use 1999
DNA damage, apoptosis of cells in epidermis, immunomodulation
Induction/Clearing, Conservation 1-3 months, Maintenance
3x/wk (2x/wk longer to clear)
1.5 to 14 months
20-52 treatments
CR 54-91% 77%
Patch vs plaque some difference
Maintenance therapy:
Short term vs long term maintenance: no difference
Maintenance therapy does decrease relapse <12 months
Side effects:
>10% erythema, pruritus
Idiopathic guttate hypomelanosis
Photo-aging, conjunctivitis
Photo carcinogenicity
no significant association between NB-UVB treatment and BCC, SCC or melanoma
small increase in BCCs amongst those also treated with PUVA

**PUVA**
8-MOP interacts with pyrimidines to form stable DNA monoadducts -> crosslinks -> transcription inhibited
Efficacy: 1A median 90% / 1B median 77%
Side effects
Short term: Erythema, pigmentation, blistering, nausea
Long term: Actinic damage, NMSCA, melanoma

**Mycosis Fungoides subtypes:**
1. Folliculotropic/Epidermotropic mycosis fungoides
2. Pagetoid reticulosis (Woringer-Kolopp)
3. Erythrodermic MF
4. Hypopigmented CD8+ (overlap with vitiligo)
5. Granulomatous MF ‘histology’
6. Granulomatous slack skin

**Sézary Syndrome**
Pruritic erythroderma, generalized lymphadenopathy and circulating malignant T-lymphocytes
Palmoplantar hyperkeratosis, alopecia, onychodystrophy
1000 circulating Sézary cells/mm³
Rule out erythrodermic MF
25% 5-year survival
Immunophenotype CD2+, CD3+, CD4+, CD7-, CD8-
Photophoresis
REFER!

**Additional References**


