Peripheral Nerve Sheath Tumors in Neurofibromatosis

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Disclosures

• I have no relevant financial relationships to disclose
Learning Objectives

1) Explain the role of neuropathology in the clinical care and diagnosis of neurofibromatosis patients

2) Apply the appropriate diagnostic workup using immunostains for differential diagnosis of schwannomas from neurofibromas in tumors with hybrid morphology

3) Apply the appropriate diagnostic workup using immunostains and molecular data for diagnosis of neurofibromas with atypical features
Introduction: The Neurofibromatoses

• Three distinct disorders:
  – NF1 (von Recklingausen, peripheral NF)
  – NF2 (Wishart, central NF)
  – Schwannomatosis

• Genetically determined disorders characterized by multiple peripheral nerve sheath tumors

• Distinguished by distinct spectrum of nerve sheath tumors, other tumors and non tumor manifestations
Historical Timeline

Clinical descriptions of NF1

Late 1700

Early 1800

Clinical descriptions of NF2

1880

Early 1930s

Von Recklinghausen disease

NIH diagnostic criteria for NF1 and NF2

Characterization of NF2

Clinical descriptions of schwannomatosis

1987

1990

1993

1980s

NF2 gene

Diagnostic criteria for NF2

1990

NF1 gene

Diagnostic criteria for Schwannomatosis

1993

2005

2007

2014

2005

2021-2022

SMARCB1 gene in schwannomatosis

LZTR1

Diagnostic criteria for NF1, NF2 and Schwannomatosis

Late 1800
Multiple PNSTs - What type of NF?

- Neurofibromatosis 1
- Neurofibromatosis 2
- NF2 related schwannomatosis (2022)
- SMARCB1 related schwannomatosis
- LZTR1 related schwannomatosis
- 22q LOH related schwannomatosis (2022)
NF1

- NF1 most common of the neurofibromatosis (1 in 3,000 individuals).

- Autosomal dominant

- Germline mutations in the *NF1* gene (chromosome 17) encodes neurofibromin, a Ras-GTPase activating protein

- Half of the patients are founders - have a sporadic mutation and negative family history
Neurofibromas in NF1

Dermal neurofibromas:
• Post natal
• Increase in puberty and pregnancy
• Hormonal role (?)

Plexiform neurofibroma
• Congenital – second week of gestation
• May be associated with soft tissue overgrowth
• Risk of malignant transformation
Other tumors

• CNS: Optic gliomas (15% of patients), pilocytic astrocytomas elsewhere, diffuse gliomas (diffuse, high grade)

• other types of malignancies including pheochromocytoma, leukemia, MPNST, sarcomas, breast cancer, Gastro intestinal stromal tumor.
Other Non tumor manifestations

• Cutaneous manifestations (café au lait, freckling)
  • Café au lait patches are very early manifestation (birth)
  • Freckling – age 3 years

• Ocular
  • Lisch nodules (melanocytic hamartomas),
  • choroidal plaques

• Characteristic skeletal dysplasia (long bone dysplasia, tibial bowing; sphenoid wing dysplasia)

• Cognitive impairment– present in 50% of NF1 patients: learning disabilities, attention deficit disorder, behavior disorders

• Cardio-Vascular: fibromuscular dysplasia of renal artery
Revised diagnostic criteria for NF1 (2021)

• Patient who does NOT have a parent diagnosed with NF1 needs 2 of the following
  – 6 or more café au lait (5 mm in pre pubertal; >15 in post pubertal)
  – Axillary or inguinal freckling
  – 2 or more neurofibromas of any type OR 1 plexiform neurofibroma
  – Optic pathway glioma
  – 2 or more Lisch nodules OR 2 or more choroidal abnormalities (patches)
  – Bony abnormalities (sphenoid dysplasia, anterolateral bowing of the tibia)
  – Germline pathogenic NF1 variant in 50% of normal tissue

• Patient that HAS a parent diagnosed with NF1 – needs only 1 of the above criteria

*Genetics in Medicine (2021)*
The challenges....

• Diagnostic signs appear over time – most difficult diagnoses are in children
• Overlap between NF1 and Legius syndrome (*SPRED1*; cutaneous manifestations only)
• 30% patients have germline mosaicism – only subpopulation of cells have mutation-
  segmental NF (cell type involved or location)
• Dosage analysis (copy number variants) and DNA based sequencing detects pathological
  variants in 90% of classic non founder NF1 patients (neurofibromas and pigmentary
  cutaneous findings)
• Presence of *NF1* pathogenic variant alone is NOT enough for diagnosis
• Few genotype/phenotype correlations:
  – There is wide range of phenotypic variability, even within family members and
    progress is unpredictable over time
  – Large deletions are associated with severe forms
  – Missense or splicing mutations are associated with spinal form

_Kayes, 2004_  
_Korf, 2005_
NF2 (NF2 related schwannomatosis)

- NF2 affects 1 : 25,000 individuals
- Autosomal dominant disorder
- 50% are de novo mutations; no affected parent
- Germline mutation in the *NF2* gene, chromosome 22
- 17 coding exons, Most mutations are in exons 1-8
- encodes merlin, a scaffold protein, regulates cell proliferation by interaction with multiple intracellular signaling pathways.

Baser, Neurology 2002
Tumors in NF2

- Schwannomas (cranial nerves; spinal, peripheral)
- Meningiomas – lifetime risk 80%, often multiple, pediatric
- Ependymomas (spinal) – lifetime risk 30%

Although histologically benign, tumors are associated with significant morbidity.

Risk of early mortality from brainstem compression and other complications is significant.
Non Tumor manifestations in NF2

• Ocular features
  – posterior cataract,
  – retinal hamartomas,
  – epiretinal membrane

• Neuropathies

• Meningioangiomatosis

• Glial micro hamartomas

• May have café au lait spots (2%)
Schwannomas in NF2

- Most common in VIII cranial nerve (bilateral vestibular schwannomas)
- Can be plexiform
- 30% have histological features of schwannoma/neurofibroma hybrid tumors
- Can be multiple along a single nerve; some tumors are a conglomerate of multiple separate tumors (polyclonality)

Klekamp M, Neurosurgery, 1998
Dewan R. Neuro Oncol, 2015
Mehta GU ClinC Neurol and Neurosurg 2020
• (A) An axial section of the right temporal bone through the cochlea (C). A large schwannoma is seen in the internal auditory canal (IAC) and a separate schwannoma arising from the saccular macula (SM) within the vestibule medial to the stapes footplate (FP).

• (B) An axial section through the right temporal bone at the level of the round window (RW). A small intracochlear schwannoma (S) is present within the fluid space of the scalatypmani (ST) and another schwannoma is present at the ampullated end of the posterior semicircular canal (PSCA). An additional, separate schwannoma arises within the descending segment of the facial nerve.

Diagnostic criteria for NF2 related schwannomatosis (NF2)

• An individual can be diagnosed as NF2 in 3 ways:
  • 1) has 1 of the following:
    – Bilateral vestibular schwannomas
    – NF2 pathogenic variant AND 2 distinct tumors OR
    – NF2 pathogenic variant in unaffected tissue AND an affected parent
  • 2) has 2 major criteria
    – Unilateral vestibular schwannoma
    – Parent or offspring diagnosed with NF2
    – 2 or more meningiomas
  • 3) has 1 major criteria and 2 minor criteria
    – Ocular features of NF2
    – 2 or more tumor types (peripheral schwannoma, meningioma, ependymoma)
Challenges...

• Genetic analysis of constitutional NF2 with next generation sequencing supplemented with multiplex ligation dependent probe amplification to detect exon 1 and high resolution karyotyping to identify translocations interrupting the NF2 locus – 96%

• Mosaic forms of NF2 are common (60% of sporadic) Manifestations are segmental, some of the clinical criteria may be late in developing or absent

• Overlap of NF1 and NF2 – hybrid tumors
• Overlap of NF2 and schwannomatosis – same tumor types
• Pathogenic variant of NF2 is not enough for diagnosis
( NON NF2 related ) Schwannomatosis

• Rare with estimated prevalence of 1:40-1:70,000.

• Only 15% are familial; Most cases (85%) are sporadic – no family history

• Characterized by predisposition to develop multiple peripheral schwannomas and in some cases meningiomas (5%)

• Segmental forms – common (30%). Thought to be mosaics? Modifiers?

• Pain – tumors often associated with debilitating neuropathic pain which over time becomes generalized.

• Clinical overlap with NF2
Schwannomas in Schwannomatosis

- Peripheral nerves and spinal nerves
- Unilateral VS may occur (LZTR1)
- Schwannomas can be discrete or plexiform (uncommon)
- Rare MPNST (reports of malignant transformation of schwannomas in familial schwannomatosis)
- Hybrid histology is common
Schwannomatosis Genes

- Schwannomatosis was shown to be distinct from NF2 as there are NO NF2 **germline** mutations.

- Known germline mutations:
  - **SMARCB1**: 48% of familial forms; 10% of sporadic forms; 22q (non-truncating mutations)
    - Component of SI/SNF chromatin remodeling complex
    - Involved in ATRT
  - **LZTR1**: 38% of familial forms; 30% of sporadic forms; 22q
    - A Golgi complex protein, interacts with CUL3-based ubiquitin ligase complex
    - Altered in some glioblastomas
  - Unknown gene/genes: 15% of familial; 60% of sporadic forms

- 4 hit hypothesis:
  - Germline SCHW gene (1)
  - LOH of 22q (2) – SCHW 2nd allele and (3) NF2;
  - Somatic mutation of remaining NF2 gene 2nd allele (4)

- Risk of transmission: 50% if germline mutation is identified (AD)
- NON fully penetrant – having the gene does not mean symptoms/signs will develop
Diagnostic criteria for non NF2 related schwannomatosis

• Diagnosis of schwannomatosis can be made when:

1) 1 pathologically confirmed schwannoma or hybrid peripheral nerve sheath tumor AND SMARCB1 or LZTR1 pathogenic variant in an unaffected tissue

2) 2 tumors (schwannomas and/or hybrid peripheral nerve sheath tumors) AND shared SMARCB1 or LZTR1 pathogenic variant in the tumors.

3) 22q related schwannomatosis:
   1) no germline NF2, SMARCB1, LZTR1 pathogenic variant AND
   2) LOH of the same chromosome 22q markers in 2 tumors
   3) Different pathogenic variants of NF2 in each tumor
Case Number 1

Schwannoma or Neurofibroma?
Clinical History

- A 64 year old woman
- Presented with progressive enlargement of a painful mass in the left foot
- Skin fold freckling in the right torso (front and back), stops at midline; No other dermal lesions
- There is no family history of any type of neurofibromatosis
- A previous surgery for resection of a mass from the left leg diagnosed as a schwannoma (2013)
- The current biopsy was seen in an outside hospital and diagnosed as schwannoma.
So - what’s the problem?
Clinical considerations

- This patient has multiple peripheral nerve sheath tumors and therefore has an underlying syndrome: NF1, NF2 or schwannomatosis

- Her clinical and pathological findings do not fit any of the NF syndromes
  - Skin fold freckling are s/o NF1,
  - multiple schwannomas are c/w schwannomatosis or maybe early NF2
Neurofibroma or Schwannoma?

• Different forms of NF
  – Management of the patient
  – Different risk for offsprings
  – Different risks for malignant transformation: cellular parts of a schwannomas may be confused with MPNST
Immunohistochemistry for workup of schwannoma/neurofibroma hybrid tumor:

- S100, SOX10 – Schwann Cells
- CD34, SMA – fibroblasts/myofibroblasts
- Perineurial cells – Glut1, claudin, EMA
In summary

• A benign peripheral nerve sheath tumor with areas of solid Schwann cell proliferation

• Mixed cell population (Schwann cells, myofibroblasts, perineurial like cells)

• Diagnosis: neurofibroma with hybrid features
Hybrid Nerve Sheath Tumors

WHO (2016) definition:
Benign peripheral nerve sheath tumors with combined features of more than one conventional type (neurofibroma, schwannoma and perineurioma)
3 conceptual hypotheses

• 1) Hybrid tumors are collision tumors

• 2) A distinct entity –biologically different; clonal alteration?

• 3) a phenotype of schwannoma or neurofibroma
Hybrid Tumors

Difficult to classify – have mixed features

- Neurofibroma/perineurioma
- Schwannoma/Perineurioma
- Schwannoma/neurofibroma
Neurofibroma/perineurioma

- Rare
- Most cases are associated with NF1
- Described as areas of perineurial differentiation in plexiform neurofibromas
- Risk of malignant transformation (2 reported cases in the literature)

Kacervoska D. Am J Dermatopathol, 2013
Michal M et al. Vrichows Arch 2004
Schwannoma/perineurioma

- Most cases reported in the literature are sporadic (not associated with NF)
- Young adults
- Digits and subcutaneous tissue; may occur in unusual sites (colon, nasopharynx, stomach)
- Diagnosis based on classic histology of Schwannoma and of perineurioma as well as immunohistochemistry
- Recent study found frequent VGLL3 rearrangement (14/18 tumors)

Dickson BC et al. Mod Pathol 2021
Hybrid schwannoma/neurofibroma

• Can have 2 distinct components or the two components that are intermingled (immunohistochemistry is essential)

• May be difficult to classify; some will remain unresolved - true hybrids

• Schwannoma with hybrid features (myxoid schwannoma)
• Neurofibroma with hybrid features (Schwann cell rich neurofibroma)
• Neurofibroma/schwannoma Hybrid tumor; suggestive of neurofibroma (or schwannoma)
Schwannoma with hybrid features
Review of the pathology files: 41 hybrid tumors from 23 patients (neurofibroma/schwannoma)

- 61% (14/23) – syndromic (39% no information)

- Syndromic:
  - 26% - NF2
  - 17% - schwannomatosis
  - 9% - NF1

A retrospective review of 43 nerve sheath tumor surgical specimens from NF2 patients (NIH)

11 specimens from 11 (26%) patients were found to be benign nerve sheath tumors exhibiting hybrid features

Harder et al, 2012; Am J Patho
• However, 71% of tumors from schwannomatosis patients have hybrid schwannoma/neurofibroma histology

• This data suggests that while most or all neurofibroma/schwannoma hybrid tumors are associated with NF
  – Most are associated with NF2 or schwannomatosis
  – A small subset that are associated with NF1

Yamasaki O et al, European Journal of Dermatology 2015;
Studies focused specifically on hybrid tumors

– 31 tumors from 16 patients with multiple PNSTs (only 6 patients with known type of NF)

– NO evidence of collision tumor (analysis of different regions)

ERBB2 mutations in Hybrid schwannoma/neurofibroma tumors

• Recently, ERBB2 mutations were identified in hybrid schw/neurofibroma tumors associated with non NF2 related schwannomatosis (4/8)
• Analysis of hybrid S/N tumors from 6 NF2 patients was negative.
• Methylation analysis of the 19 tumors – sporadic schwannomatosis tumors cluster and NF2 associated tumors cluster.
• This is in contrast with the methylation profiling study of the Heidelberg group in which NF2 and non NF2 schwannomatosis tumors (5 of each) did not form separate subgroups.
Molecular data on hybrid tumors

Methylation based classification study (Heidelberg group) of
- a large cohort (171 tumors) of benign and malignant peripheral nerve sheath tumors

7 hybrid neurofibroma/schwannoma tumors
- The 7 hybrid tumors clustered as one of the clusters of schwannomas
- Interestingly, so did the atypical neurofibromas

The authors suggest that this reflects the tumors’ cell composition; as hybrid tumors and atypical neurofibromas have higher % of Schwann cells than conventional neurofibromas.

Bottom line - Clinical practice

• Immunohistochemical workup may aid in the pathological designation of a tumor with mixed features, but in some cases that is difficult or impossible and the designation as “hybrid tumor” is appropriate.

• In cases that molecular or histological workup has led to classification of the tumor – it is still helpful to include hybrid in the diagnosis – “schwannoma with hybrid features” – as implies it may be associated with NF.
Case Number 2

Benign or Malignant?
Clinical History

• 33 year old man with NF1
• History of previous spine surgeries for benign tumors
• Large mass in left thigh, painful
IHC workup for a neurofibroma with atypical features

Cell population
• S100, SOX10, CD34, Glut1, Claudin, EMA

Proliferation:
• Ki67

Molecular
• P16
• p53
• H3k27me3
H3K27me3
Diagnosis?

• Atypical Neurofibromatous Neoplasm of Uncertain Biologic Potential: **ANNUBP**
ANNUBP: Atypical Neurofibromatous Neoplasm of Uncertain Biologic Potential

Recently published consensus proposed criteria for tumors with worrisome histological features – ANNUBP (Consensus meeting, NIH, 2016)

1) Integrated criteria for the diagnosis of ANNUBP
2) Replacement of “atypical neurofibroma” term.

Markku Miettinen et al.
Human pathology 67, 2017
Evaluating an unusual neurofibroma in a NF1 patient

1) The transition from a plexiform neurofibroma (benign) to an MPNST is a histological spectrum. The definition (by clear criteria) of different stages in the process will provide more consistent diagnoses.

2) The term “atypical neurofibroma” – confusing because:
   – Used by radiologists
   – Used by clinicians (growing, painful)
   – The histological criteria are not clear and there is inconsistency in the diagnosis
MPSNT in NF1

• Lifetime risk of developing MPNST is 8-16%
• Most common in 30s and 40s
• Prognosis is poor; especially if unresectable or metastatic (50% in 5 year survival)

Clinical concerning features:
  – Pain
  – Rapid growth
  – MRI-rapidly growing nodules, especially if associated with PET avid
Concerning characteristics in a plexiform neurofibroma
Rapid growth of selected nodules and increased uptake of FDG-PET
The entities (histological spectrum of transition)

1) Neurofibroma with 1 atypical feature:
   cellular atypia or hypercellularity

2) Atypical neurofibromatous tumor with uncertain biologic potential (ANNUBP)
   2 or more atypical features (atypia, hypercellularity, mitoses less than 3/10 hpfs, loss of architecture)

3) MPNST
   Necrosis; mitoses 3 or more/10 hpfs; loss of H3K27me3
Neurofibroma with cytological atypia OR increased cellularity
**Cellular neurofibroma**  
**Neurofibroma with cytological atypia**

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<td>Increased cytological atypia only (degenerative?)</td>
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<td>Biological behavior is uncertain May progress with time Close follow up advised</td>
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Molecular markers

A deletion at 9p21.3, which includes genes CDKN2A/2B, 
-15/16 (94%) ANF and in 
-16/23 (70%) high-grade MPNST but not in PN.

This makes early detection and management of ANF a possible strategy to prevent MPNST

Carrio et al.: correlation of genetic alterations and histology 
- Analysis of different regions of 8 neurofibromas. 
- Focal atypical changes correlated to haploinsufficiency and homozygous deletions of p16.

Changes associated with malignancy: H3K27me3 loss is diagnostic of MPNST (mutations of EED and SUZ12 and inactivation of the PRC2 complex)

Beert E et al., Genes Chromosomes Cancer 2011 
Carrio et al., Human mutation, 2018 
Cleven AHG et al. Mod Pathol 2016
Multiple studies have shown that resection of ANF (or ANNUBP) is effective **without wide margins**. Some cases recur.

63 patients had a total of 76 pathologically confirmed ANF. 61% were symptomatic (pain) and on MRI, nodules with increased $^{18}\text{F}-\text{fluorodeoxyglucose}$ (FDG) avidity.

57 ANF were resected **without wide margins and have not recurred on follow-up**, 2 ANF that were only partially resected have shown regrowth. 4 ANF transformed into MPNST. median follow-up, 4.1 y; range, 0–14.)

*Christine S Higham et al. Neuro-Oncology, 2019*
• Gross total resection of nodules with PET avidity

• 11 patients; 16 resected targeted neurofibroma nodules
  – 14 (67%) – NF with degenerative atypia
  – 3 (14%) – ANNUBP
  – 3 (14%) – benign NF
  – 1 (5%) – Low grade MPNST

• None of the resected tumors has recurred (1-4 years)

*Nelson CN et al. Safe marginal resection of atypical neurofibromas in NF1. J Neurosurg 2019*
Bottom line - Clinical practice

• The spectrum of changes from benign plexiform neurofibroma to high grade MPNST includes lesions with intermediate histology which are challenging to classify. Use of uniform criteria will be helpful to the field.

• The designation of “atypical neurofibromatous neoplasms of UNCERTAIN biologic potential” is for tumors with some worrisome features but not sufficient for diagnosis of MPNST.

• This may reflect the biology of the tumor, or sampling problem.
Conclusion

• NF associated lesions present unique challenges to the pathologist

• The correct diagnosis has important implications for clinical management and follow up

• There is continuing evolution of our understanding of these lesions, as new tools become available.
Thank You!

Q/A