What to do with FGFR3 Antibodies in Neuropathy?

CARRRELL-KRUSEN NEUROMUSCULAR SYMPOSIUM, FEBRUARY 23, 2018

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Fibroblast growth factors and their receptors

- FGFs = key peptides that control multiple cell processes
- FGFs bind to receptors (FGFR), which effect various intracellular changes
- 23 known members of FGF family but only 4 FGFR
Fibroblast Growth Factor Receptors (FGFR)

- Play a key role in cell proliferation and migration
- Germline mutations lead to developmental anomalies
- Somatic mutations have been described in various cancers

Structure of FGFR

Ig-like = immunoglobulin like domain
HSPG = heparan sulfate proteoglycans

The text in the figure is difficult to read. I wonder if we can add a larger text on top of the image or make the image bigger and cut down on the overall text.
Srikanth Muppidi, 02/01/2018
FGFR3

- 2 main isoforms of FGFR3
  - **FGFR3b**: epithelial cells; linked to skin lesions and urothelial malignancies
  - **FGFR3c**: chondrocytes; implicated in bone formation and several skeletal dysplasias

- FGFR3 in the **nervous system**:
  - **CNS**:
    - Self-renewal of cortical stem cells
    - Neural migration signaling
  - **PNS (mouse/rat model)**:
    - Expressed in dorsal root ganglia and sensory neurons
    - Upregulated in sensory ganglions after sciatic nerve injury
    - FGFR3-deficient mice seem to be resistant to neuronal death in their dorsal root ganglia after sciatic nerve injury

Furusho et al.. J Neurosci. 2009;29:1608-1614
FGFR3 antibodies in patients with sensory neuropathy

Antoine et al. searched for potential antibodies in patients with likely immune-mediated sensory neuropathy and found antibodies against the intracellular domain of FGFR3.

- ELISA to screen for antibodies
- Confirmation with cell-based assay
- Poor sensitivity
- High specificity for clinically presumed immune-mediated sensory neuropathy
FGFR3 immunoreactivity on sensory neurons

Figure 4: Immunoreactivity of antifibroblast growth factor receptor 3 (FGFR3) purified IgGs on sensory neurons. Digitally enhanced pictures using the serum of a patient with anti-FGFR3 Abs and a control subject on in vitro cultivated rat sensory neurons doubly labelled with either an anti-CRMP5 antibody which specifically recognise sensory neurons (left) or an antibody directed towards the intracellular domain of FGFR3 (FGFR3) (right). Upper row: DAPI (4',6-diamidino-2-phenylindole) staining of nuclei. Middle row: rabbit anti-CRMP5 or anti-FGFR3 antibodies. Lower row: purified IgGs. Note that FGFR3 is expressed in the nucleus and cytoplasm of sensory neurons but that the patients IgGs only reacted with the cytoplasm and did not reach the nucleus.
Patient characteristics in Antoine’s cohort

Figure 1. Flow diagram of the study. SNN, possible sensory neuronopathy according to published criteria; Non-SNN, neuropathies not fulfilling the SNN criteria; ID, idiopathic; Dys, dysimmune origin; other, other aetiologies. AB+ indicates the number of patients found to have antifibroblast growth factor receptor 3 (FGFR3) antibodies by ELISA.
Patient characteristics in Antoine’s cohort

- 16 patients (10 women, 6 men); age 18-73
- Onset: acute in 2, subacute in 4; progression in 10 at follow-up
- Examination: Neuropathy was non-length dependent in 13 patients and fulfilled criteria for sensory neuronopathy in 9 patients
- Pain was present in seven patients and was prominent in five
- EMG/NCS (only performed in 11 patients): sensory neuronopathy in 10 patients, normal in 1 patient
- Nerve biopsy in six patients showed moderate to severe myelinated fiber loss without regenerating clusters
- 10/16 patients had no known autoimmune disease at the time the sample was taken, 3 developed autoimmune disease later on

Figure 2. Superficial cutaneous nerve biopsy from three patients with anti-FGFR3 Abs. A: Immunohistochemical staining with an anti-CD3 antibody of a frozen section of the nerve biopsy of patient 16 counterstained with toluidine (40) showing T cell infiltration around epineural blood vessels. B and C. Toluidine-stained semithin sections of the nerve biopsy of patients B (B) and 12 (C) (x30) showing moderate (B) or severe (C) fibre loss without regenerating clusters.
FGFR3 was screened for as part of the WashU sensory neuropathy/neuronopathy panel in clinic patients with sensory neuropathy.
## Our patient cohort – clinical characteristics

<table>
<thead>
<tr>
<th>Pt #</th>
<th>Age</th>
<th>Sex</th>
<th>Initial FGFR3 titer</th>
<th>F/u FGFR3 titer</th>
<th>Onset</th>
<th>Progression</th>
<th>Neuropathic pain</th>
<th>Clinical examination</th>
<th>NIS-LL score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>74</td>
<td>F</td>
<td>35,000</td>
<td>0</td>
<td>Acute</td>
<td>Mild</td>
<td>Yes</td>
<td>Mild distal symmetric sensory loss, mild weakness of ankle and toe dorsiflexion (4/5)</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>F</td>
<td>27,000</td>
<td>0</td>
<td>Subacute</td>
<td>Mild</td>
<td>Yes</td>
<td>Patchy distal sensory loss</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>M</td>
<td>65,000</td>
<td>37,000</td>
<td>Acute</td>
<td>Mild</td>
<td>No</td>
<td>4/5 proximal and distal upper and lower extremity weakness, distal symmetric sensory loss</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>71</td>
<td>F</td>
<td>18,000</td>
<td>N/A</td>
<td>Subacute</td>
<td>No</td>
<td>Yes</td>
<td>Mild distal symmetric sensory loss</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>M</td>
<td>4,000</td>
<td>N/A</td>
<td>Acute</td>
<td>Mild</td>
<td>Yes</td>
<td>Normal neurological exam except for mildly reduced sensation in feet</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>M</td>
<td>5,500</td>
<td>N/A</td>
<td>Subacute</td>
<td>No</td>
<td>No</td>
<td>Mild distal symmetrical sensory loss</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>81</td>
<td>M</td>
<td>9,000</td>
<td>N/A</td>
<td>Subacute</td>
<td>No</td>
<td>Yes</td>
<td>Mild to moderate distal symmetric sensory loss, mild weakness toe dorsiflexion weakness (4/5)</td>
<td>12</td>
</tr>
</tbody>
</table>

Normal FGFR3 titer = <3,000
### Our patient cohort – clinical characteristics

<table>
<thead>
<tr>
<th>Pt #</th>
<th>Age</th>
<th>Sex</th>
<th>Initial FGFR3 titer</th>
<th>F/u FGFR3 titer</th>
<th>Ataxia</th>
<th>Paraproteinemia</th>
<th>Autonomic symptoms</th>
<th>Comorbidities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>74</td>
<td>F</td>
<td>35,000</td>
<td>0</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>F</td>
<td>27,000</td>
<td>0</td>
<td>No</td>
<td>No</td>
<td>Yes (orthostatic lightheadedness, alternating constipation and diarrhea, urinary incontinence)</td>
<td>Sarcoidosis DMII</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>M</td>
<td>65,000</td>
<td>37,000</td>
<td>No</td>
<td>Elevated kappa (22.3 mg/dl) and lambda (8.5 mg/dl) free light chains</td>
<td>No</td>
<td>DMII</td>
</tr>
<tr>
<td>4</td>
<td>71</td>
<td>F</td>
<td>18,000</td>
<td>N/A</td>
<td>Yes (mild)</td>
<td>No</td>
<td>No</td>
<td>Polycythemia vera</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>M</td>
<td>4,000</td>
<td>N/A</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>M</td>
<td>5,500</td>
<td>N/A</td>
<td>No</td>
<td>No</td>
<td>Yes (orthostatic symptoms). Delayed orthostatic hypotension on Tilt table testing</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>81</td>
<td>M</td>
<td>9,000</td>
<td>N/A</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Pre-diabetes</td>
</tr>
</tbody>
</table>
## Our patient cohort – diagnostics

<table>
<thead>
<tr>
<th>Pt #</th>
<th>Age</th>
<th>Sex</th>
<th>Initial FGFR3 titer</th>
<th>F/u FGFR3 titer</th>
<th>NCS/EMG/Other testing</th>
<th>Biopsy</th>
<th>Other autoantibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>74</td>
<td>F</td>
<td>35,000</td>
<td>0</td>
<td>Sensorimotor axonal polyneuropathy</td>
<td>N/A</td>
<td>TS-HDS 37,000 (40,000 on repeat testing) Histone H3 1,300 (negative on repeat testing)</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>F</td>
<td>27,000</td>
<td>0</td>
<td>Normal</td>
<td>N/A</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>M</td>
<td>65,000</td>
<td>37,000</td>
<td>Severe demyelinating polyneuropathy with secondary axonal features</td>
<td>N/A</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>71</td>
<td>F</td>
<td>18,000</td>
<td>N/A</td>
<td>Sensorimotor polyneuropathy with axonal and demyelinating features</td>
<td>N/A</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>M</td>
<td>4,000</td>
<td>N/A</td>
<td>Mild slowing across the right elbow</td>
<td>N/A</td>
<td>TS-HDS 37,000</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>M</td>
<td>5,500</td>
<td>N/A</td>
<td>Normal large fiber function. Decreased epidermal fiber density on skin biopsy</td>
<td>N/A</td>
<td>TS-HDS 24,000</td>
</tr>
<tr>
<td>7</td>
<td>81</td>
<td>M</td>
<td>9,000</td>
<td>N/A</td>
<td>Sensorimotor axonal polyneuropathy</td>
<td>N/A</td>
<td>None</td>
</tr>
</tbody>
</table>
7 patients (4 men, 3 women); age: 44-81 years

**Initial testing:**
- 4 patients had **high titers**: 18,000 – 65,000
- 3 patients had low titers: 4,000 – 9,000

**Follow-up antibody testing:**
- Undetectable in 2 patients
- Significant reduction in 1 patient
- Not performed in the 4 patients with the lowest titers

Onset: acute in 3, subacute in 4; mild progression in 4 at follow-up

Examination: **spectrum of neuropathic manifestations** (small fiber neuropathy to moderate large fiber sensory neuropathy); 1 patient had ataxia; **NIS-LL scores: 2-28**

EMG/NCS:
- Normal large fiber function in 3 patients
- Mild to moderate sensory polyneuropathy in 3 patients
- Severe demyelinating features in 1 patient

Two patients had autonomic symptomatology and one patient had delayed orthostatic hypotension on tilt table testing
What to do with FGFR3 antibodies?

- Antoine et al. used multiple steps to confirm the presence of FGFR3 antibodies:
  1) Screen with human protein microarray
  2) Confirmation of a positive result by ELISA
  3) A second confirmatory step with a cell-based assay (HEK293 cells transfected with eGFP)
     → Confirmed presence of antibodies only in patients with high titers.

- Current commercially available testing only uses ELISA with no confirmatory assay (either cell-based or by protein microarray)

Figure 2. Serum immunoreactivity against FGFR 3 in the protein array or ELISA. (A) Protein array with patients and control sera. (C) ELISA detail of the sensory neuropathy group. SN, sensory neuropathy not fulfilling the SSN criteria; SNN, sensory neuronopathy; DYS, dysimmune origin; ID, idiopathic; OTH, other origin. The titer of anti-FGFR3 antibodies is expressed as a Z score.
What to do with FGFR3 antibodies?

If FGFR3 antibodies are identified as the sole etiology of neuropathy, one should consider immunotherapy…. **BUT**

- Diverse group of neuropathies with acute or subacute onset
- No clear relationship between the titers and the severity of neuropathy
- Are FGFR3 really causative of the symptoms?

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**Autonomic “myasthenia”: the case for an autoimmune pathogenesis**

Daniel B. Drachman

Department of Neurology and Neuroscience, Johns Hopkins School of Medicine, Baltimore, Maryland, USA


**Five criteria for recognizing antibody-mediated autoimmune disease**

1) Autoantibodies are present in patients with the disease
2) Antibody interacts with the target antigen
3) Passive transfer of antibody reproduces features of disease
4) Immunization with antigen produces a model disease
5) Reduction of antibody levels ameliorates the disease

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Stanford University
Take home points

1) FGFR3 antibodies can be seen in patients with a spectrum of neuropathies ranging from small fiber to moderate axonal or demyelinating neuropathies

2) There may be a variability in FGFR3 antibody titers on repeat testing without any intervening immunotherapy

3) Clinicians need to be cautious in interpreting pathogenicity of FGFR3 antibody titers in an individual
Thank you

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