Refractory Diarrhea: When Rotavirus Goes Rogue

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Disclosures

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History of Present Illness

- 6 week old healthy baby boy presented with 36 hours of profuse, watery diarrhea, 20+ episodes per day
- Non bloody, yellow colored, watery stools
- Afebrile, eating well. Breast fed with some formula supplementation.
- No vomiting. No sick contacts. No travel. No animal exposure.
- Born full term via primary C-section, serology/GBS negative, no complications

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Physical Exam

- **General**: No acute distress, social smile .
- **Eye**: Pupils are equal, round and reactive to light, Normal conjunctiva.
- **HENT**: Normocephalic, Oral mucosa is moist. Anterior fontanel depressed
- **Neck**: Supple.
- **Respiratory**: Lungs are clear to auscultation, Respirations are non-labored, Breath sounds are equal.
- **Cardiovascular**: Normal rate, Regular rhythm, No murmur.
- **Gastrointestinal**: Soft, Non-tender, Non-distended.
- **Genitourinary**: Normal genitalia for age and sex, No scrotal tenderness, circumcised.
- **Lymphatics**: No lymphadenopathy neck, axilla, groin.
- **Musculoskeletal**: Normal range of motion. Normal strength.
- **Integumentary**: Warm, Dry, Pink, Intact.
- **Neurologic**: Alert, Moves all extremities appropriately.

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Initial Labs

138	106	10	123
4.9	15	0.6	

15.6	13.5	767
	38.8	

Ca++: 9.7
AST: 48
ALT: 33
Alk Phos: 387
T.Bili: 1
Alb: 3.2

Neutrophil: 38
Bands: 6
Lymphocyte: 43
Eosinophil: 1

Ova and parasite: Negative

Stool culture: Moderate normal intestinal flora, no Salmonella, Shigella, or Campylobacter isolated.

Rotavirus Antigen: Positive

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Hospital Course

Admitted for severe dehydration with rotavirus positive gastroenteritis

- HD 2: transition from similac to soy based formula (concern for temporary lactase deficiency 2/2 rotavirus)
- HD 4: developed severe hypovolemia with metabolic acidosis, hyperkalemia and hyperchloremia; transferred to PICU.

Subsequent prolonged hospitalization for fluid management and TPN for nutritional support while work-up progressed. Hospital course complicated by persistent diarrhea, failure to thrive, hepatitis, hypoalbuminemia, anemia, recurrent infections.

- HD 10: normal IgG
- HD 15: normal lymphocyte subset panel, elevated IgA, IgE
- HD 22: dermatology consulted for rash consistent with miliaria
- HD 25: low-normal mitogen response panel
- HD 26: initial endoscopy with diffuse villous atrophy and inflammatory changes
- HD 25-39: development of diffuse xerosis, lichenification and scaling with progressive eosinophilia

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Immune Workup

- Newborn screens normal
- Immunoglobulins:

Lab test	Interpreted Result	Referance Interval
Immunoglobulin G and M	Normal	
Immunoglobulin E	High (2128 ku/L)	< =13
Immunoglobulin A	High (63 mg/dL)	3-47

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Immune Workup, continued

Lab test	Interpreted Result
Absolute and % CD2, CD3, CD4, CD8, CD19, CD45RA, CD45RO, HLA-DR	Normal
Absolute and % Natural Killer cells	Normal
Lymphocyte Ag and Mitogen Panel	Absent lymphocyte responses to Candida Low Lymphocyte responses to Tetanus Low-normal Lymphocyte responses to PHA Normal Lymphocyte responses to ConA Normal Lymphocyte responses to Pokeweed Mitogen
% CD4+CD25+CD127LowCD45RO+ (N Tregs)	Low (1.6 % of CD4+) [referance interval 2.4-8.7]

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image

- Will insert image from pathology

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Duodenal Mucosal Biopsy

- Diffuse epithelial injury and patchy necrosis
- Poor preservation of ultrastructural detail
- Marked reduction of microvilli on enterocytes
- Small to moderate sized vacuoles suggestive of lipid vacuoles in a few enterocytes
- No microvillous inclusions or diagnostic viral inclusions seen

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Regulatory T-Cell Panel

Regulatory T-Cell Panel
ARUP test code 2010172

% CD4+CD25+CD127Low (Tregs)	4.0 % of CD4+		(Ref Interval: 4.0-10.7)
Abs CD4+CD25+CD127Low (Tregs)	90.5 cells/uL		(Ref Interval: 20.0-126.3)
% CD4+CD25+CD127LowCD45RA+ (Nn Tregs)	1.1 % of CD4+		(Ref Interval: 0.4-4.5)
Abs CD4+CD25+CD127LowCD45RA+ (Nn Tregs)	25.0 cells/uL		(Ref Interval: 2.3-54.0)
% CD4+CD25+CD127LowCD45RO+ (N Tregs)	**1.6 % of CD4+**	L	(Ref Interval: 2.4-8.7)
Abs CD4+CD25+CD127LowCD45RO+ (N Tregs)	37.0 cells/uL		(Ref Interval: 14.3-88.3)

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Further Work-Up

- Anti-enterocyte antibodies: negative
- SCID panel: negative
- Very Early Onset IBD panel—takes 12 weeks to obtain results—returned after results of whole exome sequencing available—pathogenic variant detected

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Whole Exome Sequencing

Gene	Genomic Change	Zygosity	Coding Change	Protein Change	Classification	Inherited From
FOXP3	chrX:g.49111956_49111958delCTT	Hemizygous	NM_014009.3:c.748_750delAAG	p.Lys250del	Pathogenic	De novo

- De novo hemizygous variant in the FOXP3 gene
- Hemizygous c.748_750delAAG (p.Lys250del) variant in FOXP3 not previously observed in general population
- **Has been reported as disease causing for IPEX syndrome in one symptomatic individual.**

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Nutritional Course

- Admitted with EBM and Similac supplementation
- HD 2: transition to soy based formula (concern for temporary lactase deficiency secondary to rotavirus)
- HD 4: NPO on TPN
- HD 11: small trials of EBM
- HD 13: Flecks of blood in stool so EBM transitioned to Nutramigen
- HD 16: Retrialed EBM, blood returned, Neocate initiated
- HD 25: EBM resumes (goal to increase PO given poor taste for Neocate)
- HD 30: Neocate resumed, via NGT given poor PO (concern for disaccharidase deficiency given diffuse enteral inflammation)
- HD 35: trial of carbohydrate free formula, not tolerated due to emesis
- HD 36: Neocate resumed
- HD 38-40: NPO with bacteremia, blood in stool, no signs of pneumatosis on serial KUBs
- *ongoing profuse diarrhea as enteral feeds advanced. Large component of secondary osmotic diarrhea given diffuse villous atrophy from inflammatory changes
- HD 41: enteral feeds resume with Mead-Johnson 3232A (trickle feeds with 1-5ml/hr and very slowly advanced)
- No improvement, ultimately transitioned back to Neocate diluted to 10kcal/oz, never surpassing 5ml/hr without subsequent increase in output
- **HD 75: DIAGNOSIS**
- HD 79: Steroids and immunosuppression initiated
- Steady improvement in stool output, feeds advanced, Neocate slowly fortified
- HD 133: Solid foods [age 5.9 months)
- HD 140-145: TPN discontinued, IVF discontinued, central line removed
- HD 147: Discharged home on continuous Neocate feeds 20kcal/oz

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Immune Dysregulation
Polyendocrinopathy Enteropathy
X-linked Syndrome
(IPEX Syndrome)

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IPEX Syndrome

- Extremely rare monogenic primary immunodeficiency
- Caused by mutations in gene for T regulatory (Treg) cells-*FOXP3*
- Hallmark of disease is multi-organ autoimmunity due to malfunctioning T regulatory cells

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IPEX Syndrome

- Classic triad
  - Severe enteropathy
  - Endocrinopathy such as Type I DM
  - Dermatitis
- Published case reports are limited
  - Incidence and prevalence difficult to establish
- Poor prognosis

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IPEX presentation and course

- Birth typically unremarkable
- Symptom onset in 1st months of life
  - Chronic intractable diarrhea is common presentation
  - Type I DM and eczematous dermatitis can also be presenting symptoms
- Course characterized by disease flares/fluctuations
  - Variety of potential triggers
  - New symptoms can present over time

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Presentation and course

- Varied phenotypes
  - Autoimmune disease
  - Allergic inflammation
- Other clinical manifestations
  - Failure to thrive
  - Severe food allergy
  - Thyroiditis
  - Immune mediated cytopenias
  - Increased infections
    - Enterococcal and staphylococcal species, cytomegalovirus (CMV), and Candida
  - Nephritis

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Differential Diagnosis

- Diagnosis is challenging if patients present with single symptom
  - Vast differentials for infantile enteropathies and erythroderma
  - DDx for enteropathy includes infection, food allergy, celiac disease, inflammatory bowel disease, eosinophillic enteropathy
  - Multiple genetic syndromes associated with neonatal diabetes
- IPEX-like syndromes
- Other rare syndromes
  - NOMID/CINCA, ALPS, APS I or APECED
- Immune deficiency
  - SCID, Omenn syndrome, Netherton Syndrome

Evaluation and Diagnosis

- Extensive preliminary evaluation
  - CBC
  - Glucose, anti-islet cell antibodies
  - TFTs and thyroid antibodies
  - Antienterocyte antibodies
  - Immunoglobulins
  - Food hypersensitivity testing
  - Lymphocyte subsets and proliferation assays
- Advanced investigation
  - Endoscopy with biopsies
  - Skin biopsy
  - Regulatory T cell immunophenotyping and functional studies
- **Genetic sequencing of FOXP3 gene required for definitive diagnosis**

Management

- Immune suppression is hallmark of treating acute flares
  - Presence of invasive infections can pose a dilemma
- Enteropathy flares managed with gut rest and TPN
- Long term management
  - Glucocorticoids, calcineurin inhibitors or sirolimus
  - **Hematopoietic stem cell transplantation**
    - Only curative therapy for patients with IPEX
    - High morbidity and mortality
    - Early HSCT provides better outcomes
    - Endocrinopathies may persist after successful transplantation

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Pathogenic Autoantibodies Identified in Patients with IPEX

- Pancreas- GAD65, Insulin, Islet cell cytoplasmic antibodies (ICA), IA-2A
- Thyroid-Thyroglobulin, Thyroid peroxidase, TSH Receptor
- Liver= Liver/Kidney Microsomal (LKM), Smooth Muscle (SM), Mitochodondrial

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Pathogenic Autoantibodies Identified in Patients with IPEX

- GI- Anti-enterocyte, Anti-goblet cell
- Hematologic- Direct and Indirect Coombs
- Hypercoagulability-Anti-phospholipid antibody panel (Anti-β2 glycoprotein 1) , Anti-cardiolipin, Lupus anticoagulant (Dilute Russel Viper Venom Time, DRVVT)

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Lab test	Interpreted Result	Referance Interval
Anti-Thyroglobulin	<3.0	3.0-20.0
Thyroid Peroxidase-G	0.3	0-9
Anti-Islet Cell Ab	<1:4 (Normal low)	
Glutamic Acid Decarboxylase Antibody (GAD)	< 5 IU/mL	0.0-5.0
IA-2 Antibody	<0.8 U/mL	0.0-0.8
Insulin Antibody	< 0.4 U/mL	0.0-0.4
F-Actin (Smooth Muscle) Ab, IgG by ELISA	**21 units (weak positive) ***	**0-19**
Liver-Kidney Microsome-1 Ab, IgG by ELISA	1.6 units	0 – 24.9
Mitochondrial (M2) Antibody, IgG	5.3 units	0-20
Smooth Muscle Ab, IgG titer	**1:20 (weak positive) ***	**< 1:20**
Direct Coombs (DAT IgG)	**Positive ***	
Anti Enterocyte antibody	Negative	

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Current patient status

- Our patient was diagnosed on hospital day 75 and initiated on immunosuppression on hospital day 79
- He demonstrated steady improvement in stool output and feeds were very slowly advanced and fortified
- On hospital day 133 he was able to initiate solid foods (purees) at age 5.9 months of age
- On hospital days 140-145 labs were closely followed while TPN was discontinued, IVF were discontinued and his central line was removed
- He was discharged home 147 days after his initial presentation on tacrolimus and continuous Neocate feeds at 20kcal/oz with an HLA matched donor identified and plans for relocation to a specialty stem cell transplant center for HSCT

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Questions?

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