INC
CRITICAL PATH INSTITUTE
International Neonatal Consortium

Second Annual Neonatal Scientific Workshop at the EMA

Welcome Day 2

September 12th – 13th, 2016

CRITICAL PATH INSTITUTE

Agenda – September 13th, Afternoon

1:00 p.m.	**Session VI:** *Necrotizing Enterocolitis* RON PORTMAN, INC CO-DIRECTOR (NOVARTIS) & MICHAEL CAPLAN (UNIVERSITY OF CHICAGO), CO-CHAIRS
3:00 p.m.	*Concluding Remarks* MARK TURNER, INC CO-DIRECTOR
3:15 p.m.	WORKSHOP ADJOURNED
4:00 – 8:00 p.m.	SATELLITE WORKGROUP SESSIONS TO BE HELD AT THE MARRIOTT WITH A WORKING DINNER *Workgroup Session I on BPD* *Workgroup Session II on Data* *Workgroup Session III on Seizures*

Agenda – Necrotizing Enterocolitis

1:00 – 3:00 p.m.

Session VI: *Necrotizing Enterocolitis*

RON PORTMAN, INC CO-DIRECTOR (NOVARTIS) &
MICHAEL CAPLAN (UNIVERSITY OF CHICAGO), CO-CHAIRS

NEC: State of the Art
MICHAEL CAPLAN (UNIVERSITY OF CHICAGO)
Biomarkers and Barriers: Opportunities and Challenges in NEC
KARL SYLVESTER (STANFORD UNIVERSITY)

Session VI Panel:
TAHA KEILANI (SIGMA TAU)
IRJA LUTSAR (PDCO)
PAOLO MANZONI (S. ANNA HOSPITAL, TORINO)
TOKUO MIYAZAWA (SHOWA UNIVERSITY, JAPAN)
JOSEPH NEU (UNIVERSITY OF FLORIDA - GAINESVILLE)
JENNIFER CANVASSER (NEC SOCIETY & PPA)

Neonatal Necrotizing Enterocolitis (NEC)

- NEC is an acute inflammatory and coagulative necrosis of any part of the bowel affecting primarily premature infants in a NICU setting
- NEC has a worldwide incidence varying between 6-15% (2-22% in individual NICU's) of babies <1500 grams at birth and is associated with a high mortality and morbidity with often devastating long-term sequelae
- Pathogenesis is poorly understood but seems to be mostly related to alterations in dysregulation of the inflammatory system and abnormal intestinal bacterial colonization pattern
- Diagnostic criteria are variable and have poor correlation to prognosis; no commonly accepted biomarkers for diagnosis or treatment outcome have as yet been accepted for clinical use.
- No treatment strategy has been clearly effective as yet
- Focus has been largely on prevention including preventing premature birth and the use of human milk enteral feedings
- Regulatory science approaches for treatment and prevention have also been challenging

Caplan MS: Necrotizing Enterocolitis: Colloquium Series on Integrated Systems Physiology
2014 Morgan& Claypool Life Sciences

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INC
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International Neonatal Consortium

Applying Regulatory Science to Neonates

Second Annual Scientific Workshop at EMA
Session VI: Necrotizing Enterocolitis

Michael S. Caplan, MD
September 13, 2016

CRITICAL PATH INSTITUTE

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

NEC: State of the Art - Objectives

- Define the scope of the problem
- Discuss the pathophysiology of NEC
- Identify approaches to early diagnosis
- Opportunities for prevention and/or treatment
- What are the barriers to efficient product development?

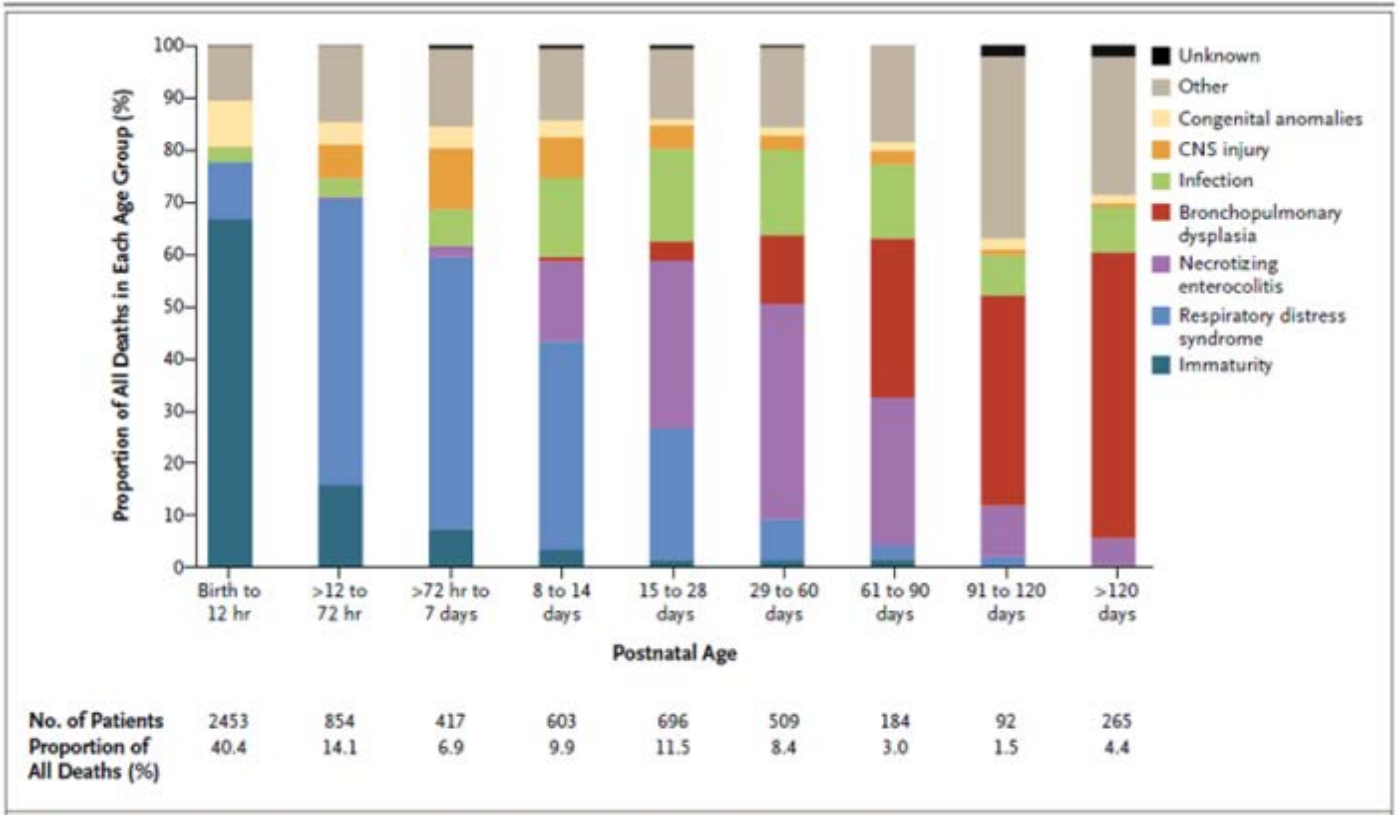
Changes in mortality etiology over time in premature infants: 2000-2011

Table 2. Overall Causes of Death among Extremely Premature Infants, 2000–2011.*

Variable	2000–2003	2004–2007	2008–2011	All Years	P Value
Total live births	7440	7684	7124	22,248	
Total deaths	2043	2193	1839	6075	
Overall mortality rate per 1000 live births (95% CI)†	275 (264–285)‡	285 (275–295)‡	258 (248–268)	273 (267–279)	0.003
Cause-specific mortality rate per 1000 live births (95% CI)†§					
Congenital anomalies	16 (13–19)	14 (12–17)	13 (10–16)	14 (13–16)	0.31
Respiratory distress syndrome	65 (60–71)‡	69 (63–75)‡	56 (51–62)	64 (60–67)	0.02
Bronchopulmonary dysplasia	18 (15–21)	15 (13–18)	12 (10–15)	15 (13–17)	0.09
Pulmonary: respiratory distress syndrome plus bronchopulmonary dysplasia	83 (77–90)‡	84 (78–90)‡	68 (63–74)	79 (75–82)	0.002
Infection	22 (19–25)	24 (21–28)	19 (16–22)	22 (20–24)	0.20
Necrotizing enterocolitis	23 (20–27)‡	29 (25–33)	30 (27–34)	28 (25–30)	0.04
CNS injury¶	7 (5–9)	11 (9–14)	10 (8–13)	9 (8–11)	0.21
Immaturity	86 (80–93)‡	81 (75–88)	81 (75–88)	83 (79–87)	0.04
Other	37 (33–42)	40 (36–45)	34 (30–39)	37 (35–40)	0.20

Patel et al, NEJM 2015

Mortality etiology depends on post-natal age in premature infants

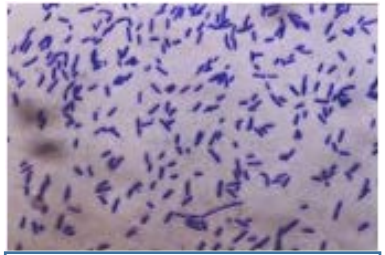


	Birth to 12 hr	>12 to 72 hr	>72 hr to 7 days	8 to 14 days	15 to 28 days	29 to 60 days	61 to 90 days	91 to 120 days	>120 days
No. of Patients	2453	854	417	603	696	509	184	92	265
Proportion of All Deaths (%)	40.4	14.1	6.9	9.9	11.5	8.4	3.0	1.5	4.4

Figure 1. Proportionate Mortality for Major Causes of Death, According to Postnatal Age.

CNS denotes central nervous system.

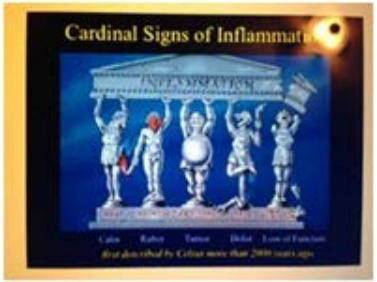
NEC Pathophysiology (in the preterm infant)



Altered intestinal microbiome

↑ TLR4, TLR2, et al

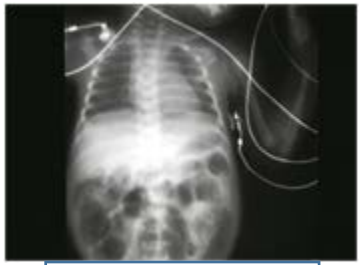
↓ human milk feeds (volume, dose, activity)



Unbalanced inflammatory response

Gut hypoperfusion?

Cellular injury



NEC

NICU environment, catheters, etc

Preterm microenvironment, impaired host defense

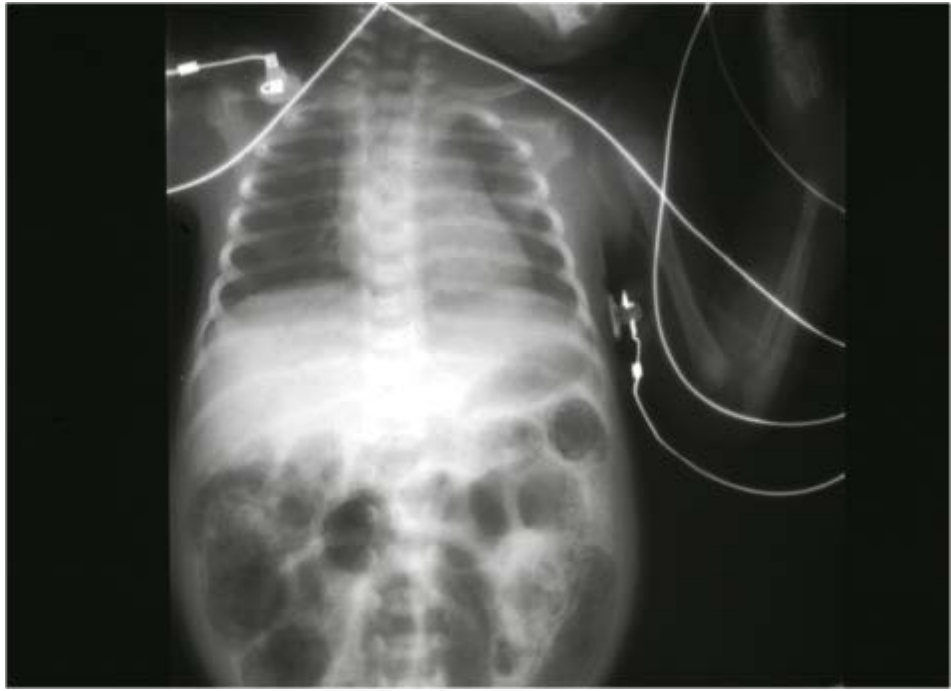
Delayed feeding and bacterial colonization, lack of breast exposure

Accentuated pro-inflammatory signaling/prematurity

↑ IL-8
↓PAF-AH
↓IkB/↑NFkB
Genetic factors

Can we diagnose NEC early so therapy might influence outcome?

- Frequent radiographs
- Abdominal ultrasound/MRI
- Blood biomarkers
- Stool biomarkers
- Urine biomarkers
- Breath hydrogen or other markers
- Heart rate variability algorithms



Opportunities for NEC prevention and/or treatment

- Human milk
- Exclusive human milk
- Probiotics
- Lactoferrin
- Growth factors
- Human milk oligosaccharides
- Other factors that alter cell injury/permeability/inflammation, etc
    - PUFA, PAF-AH, Inter-alpha inhibitor protein, TLR4 antagonists, etc

Bioactive Factors in Human Milk that Modulate NEC Pathogenesis

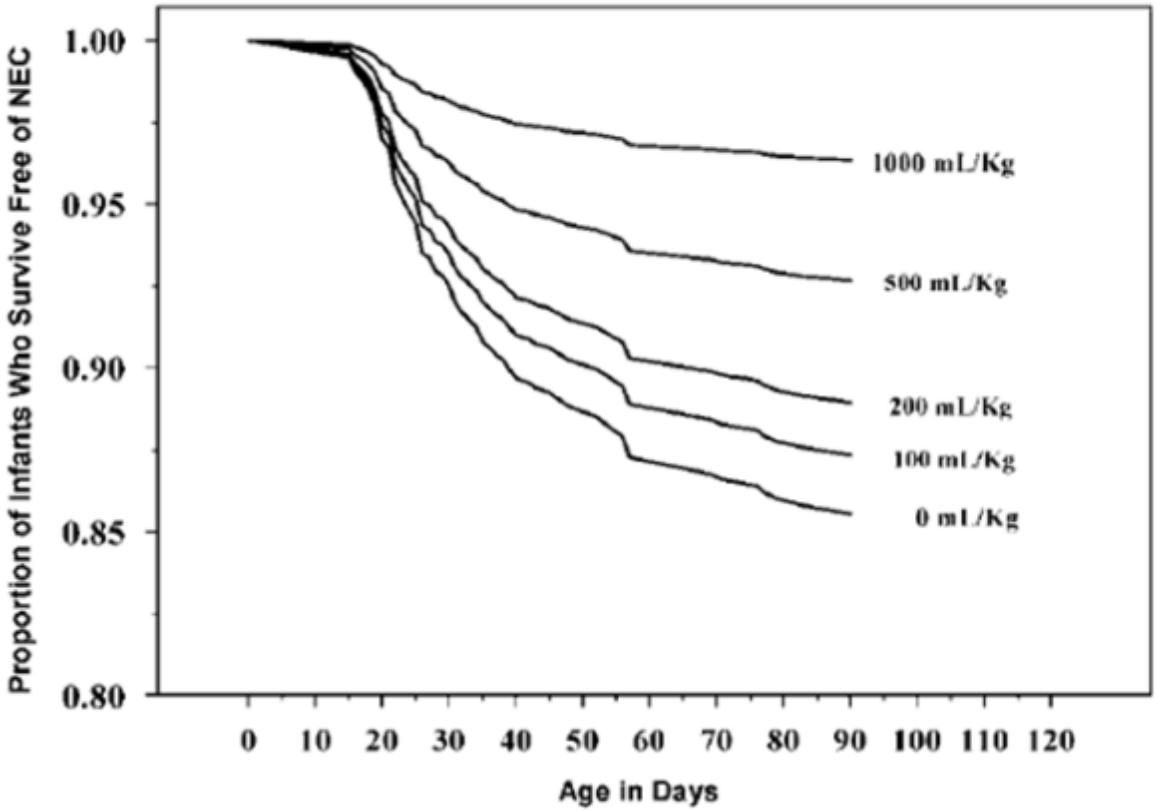
- Leukocytes
- Immunoglobulins (IgA, etc)
- Oligosaccharides
- PUFA
- Growth Factors (EGF, HBEGF, TGFβ, EPO, NRG-4)
- Lactoferrin
- Cytokines
- Enzymes (PAF-AH, lysozyme)
- Probiotics

Prospective Trial: Human milk and NEC incidence

Gestational age (weeks)	Formula	Human Milk
25-27	14%	8%
28-30	6%	3%
31-33	4%	0.4%
34-36	9%	0

Randomized patients: 5% formula vs 1% human milk:
Odd's ratio 4.7, $p > 0.05$.
Lucas and Cole, Lancet 1990:336;1519

Human milk: dose-dependent decrease in NEC or death



Meinzen-Derr et al, J Perinatol, 2009

Exclusive Human Milk-based diet reduces NEC

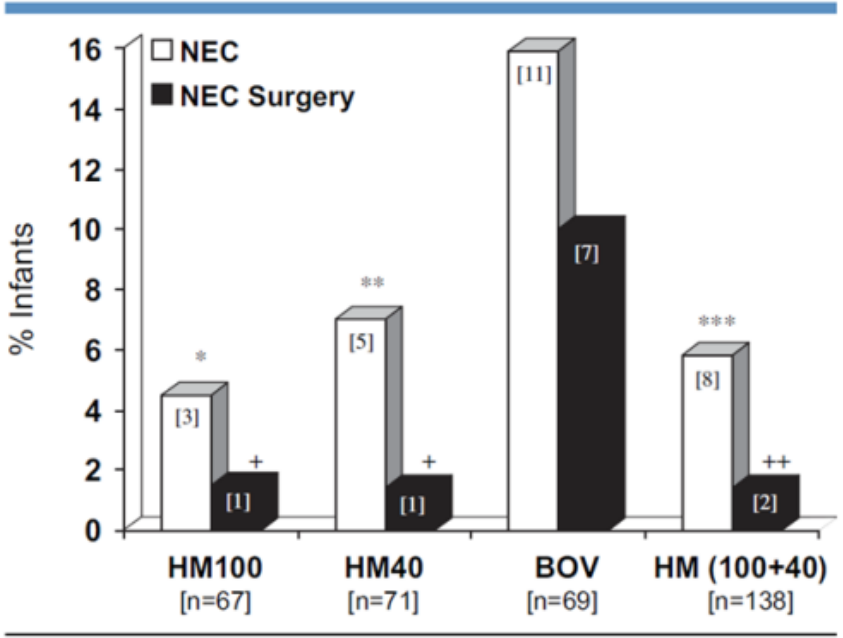


Figure 2. NEC and NEC surgery in study infants. There were significant differences in NEC among the 3 groups ($P = .05$), $^{*}P = .04$ vs BOV, $^{**}P = .09$ vs BOV, $^{***}P = .02$ vs BOV. There were significant differences in NEC requiring surgical intervention among the 3 groups ($P = .02$), $^{\dagger}P = .03$ vs BOV, $^{\dagger\dagger}P = .007$ vs BOV. [ ] refers to number of infants.

Study powered to identify reduction in TPN time; no difference found in primary outcome

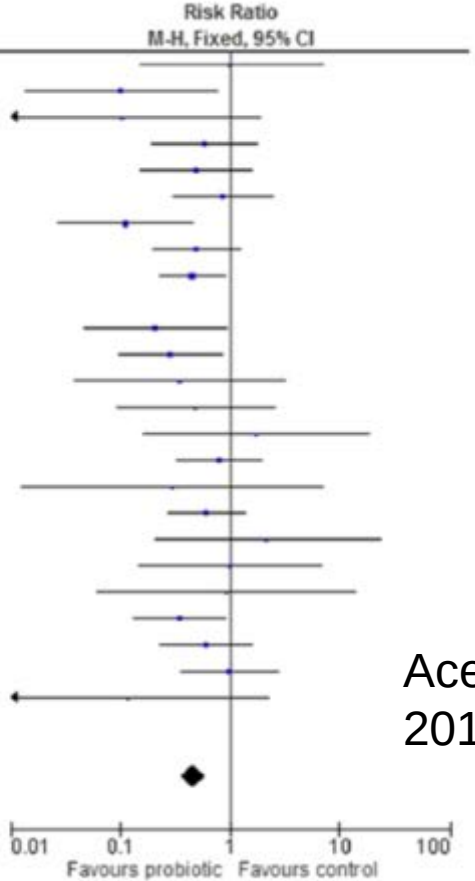
Sullivan et al,
J Peds, 2010

Probiotics and NEC: meta-analysis

a

Study or Subgroup	Probiotic Events	Probiotic Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Al-Hosni, 2012	2	50	2	51	1.0%	1.02 [0.15, 6.96]
Bin-Nun, 2005	1	72	10	73	5.2%	0.10 [0.01, 0.77]
Braga, 2011	0	119	4	112	2.4%	0.10 [0.01, 1.92]
Costalos, 2003	5	51	6	36	3.7%	0.59 [0.19, 1.78]
Dani, 2002	4	295	8	290	4.2%	0.49 [0.15, 1.61]
Demirel, 2013	6	135	7	136	3.7%	0.86 [0.30, 2.50]
Dilli, 2015	2	100	18	100	9.5%	0.11 [0.03, 0.47]
Fernandez-Carrocera, 2013	6	75	12	75	6.3%	0.50 [0.20, 1.26]
Jacobs, 2013	11	548	24	551	12.6%	0.46 [0.23, 0.93]
Kitajima, 1997	0	45	0	46		Not estimable
Lin, 2005	2	180	10	187	5.2%	0.21 [0.05, 0.94]
Lin, 2008	4	217	14	217	7.4%	0.29 [0.10, 0.85]
Manzoni, 2006	1	39	3	41	1.5%	0.35 [0.04, 3.23]
Mihatsch, 2010	2	84	4	82	2.1%	0.49 [0.09, 2.59]
Mohan, 2006	2	37	1	32	0.6%	1.73 [0.16, 18.20]
Oncel, 2013	8	200	10	200	5.3%	0.80 [0.32, 1.99]
Patole, 2014	0	74	1	66	0.8%	0.30 [0.01, 7.19]
Rojas, 2012	9	372	15	378	7.8%	0.61 [0.27, 1.38]
Rouge, 2009	2	45	1	49	0.5%	2.18 [0.20, 23.21]
Roy, 2014	2	56	2	56	1.1%	1.00 [0.15, 6.85]
Saengtawesin, 2014	1	31	1	29	0.5%	0.94 [0.06, 14.27]
Samanta, 2009	5	91	15	95	7.7%	0.35 [0.13, 0.92]
Sari, 2011	6	110	10	111	5.2%	0.61 [0.23, 1.61]
Serce, 2013	7	104	7	104	3.7%	1.00 [0.36, 2.75]
Stratiki, 2007	0	41	3	34	2.0%	0.12 [0.01, 2.23]
Totsu, 2014	0	153	0	130		Not estimable
Total (95% CI)		3324		3281	100.0%	0.47 [0.36, 0.60]
Total events	88		188			

Heterogeneity: Chi² = 20.26, df = 23 (P = 0.63); I² = 0%
Test for overall effect: Z = 6.08 (P < 0.00001)



Aceti et al, Ital J Peds, 2015

Changing risk ratio/NNT over time on probiotic protection against NEC: meta-analysis results

Risk ratio: probiotic v control	Number needed to treat (NNT	Author	Year
0.32	21	Alfaleh et al	2008
0.33	22	Wang et al	2012
0.41	29	Alfaleh et al	2014
0.47	32	Aceti et al	2015
0.58	37	Include PiPS (Costeloe et al, 1310 patients, B. breve, p=NS)	2016

Answered Question: effect size significant, probiotics reduce NEC rate

- UNANSWERED QUESTIONS:
- Safety in large study with long-term f/u? limited data, so remains unclear
- Best strain(s), species combination, dose? Combination preparations>*Bifidobacteria*>*Lactobacilli*
- Are all populations the same as the meta-analyses? In US, perhaps not
- Effect on infection and mortality? Varying results
- Do meta-analyses predict large RCT results? 30% of the time, large RCT finds opposite results!
- Appropriate quality control of available product? Key factor in US from the FDA perspective

Compelling preventive strategies with pre-clinical efficacy

- Growth Factors (intestinal maturation and anti-inflammatory effects)
  - EGF
  - HB-EGF
  - TGF-β
  - Neuregulin-4
- Human milk oligosaccharides (n-disialyllacto-N-tetraose)
- Products that reduce cellular injury or inflammation
  - Inter-alpha inhibitor protein, PAF-AH
- Products that alter mucosal permeability/tight junctions

Effect of HB-EGF on NEC in neonatal rats and mice

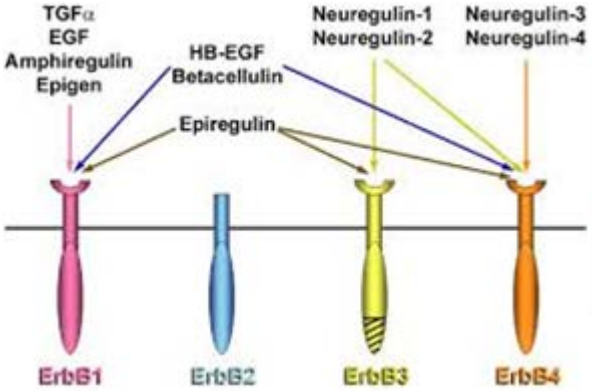
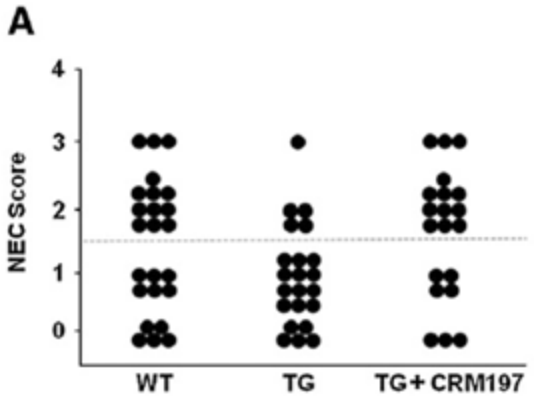


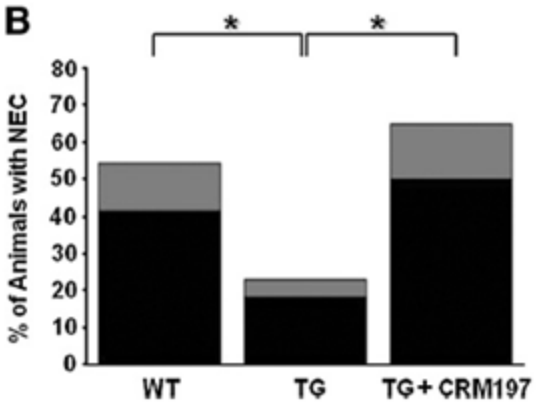
Table 1 Effect of HB-EGF on the incidence and severity of NEC in newborn rats

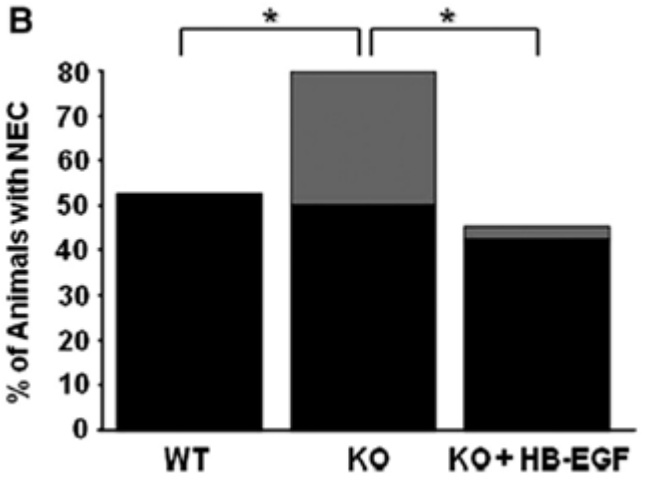
Group	Incidence of NEC (%)	Histological injury score (median)	Survival rate (%)	Median survival time (h)
BF	0 (0/10)	0		
HTF	10 (1/10)	0.3		
HHHTF + LPS	65 (13/20)*	2*	25% (5/20)	59
HHHTF + LPS + HB-EGF	27.3 (6/22)**	1.1**	63.6% (14/22)**	73**

* $P < .01$ compared with BF or HTF.
** $P < .05$ compared with HHHTF + LPS.

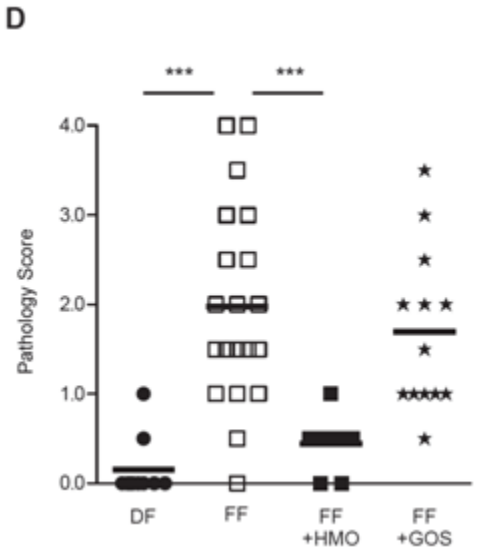
Besner Lab, J Ped Surg 2006, 2010

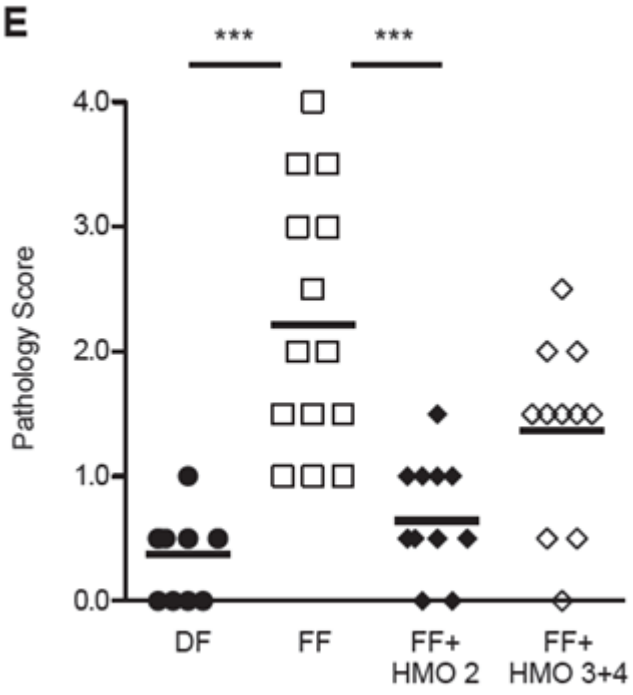






Human milk disialyllacto-N-tetraose protects against NEC in neonatal rats





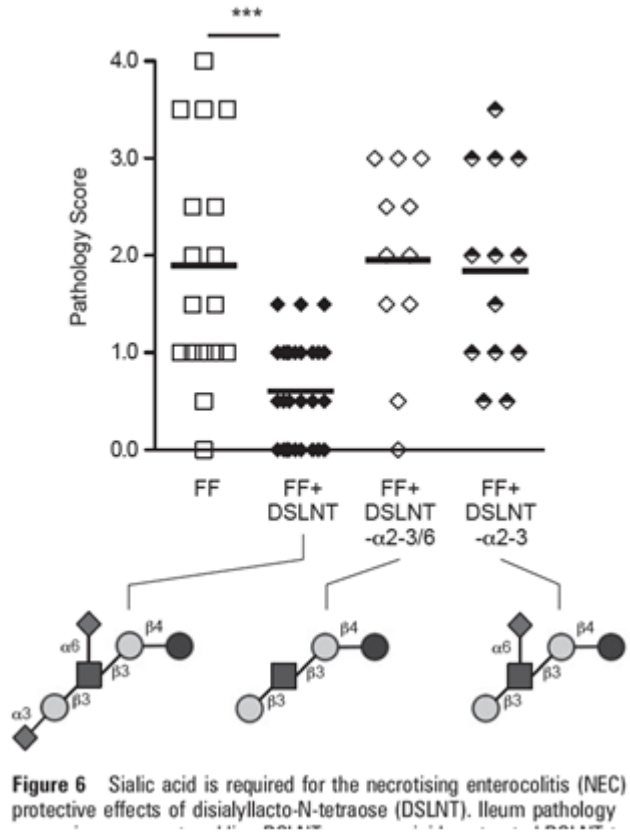
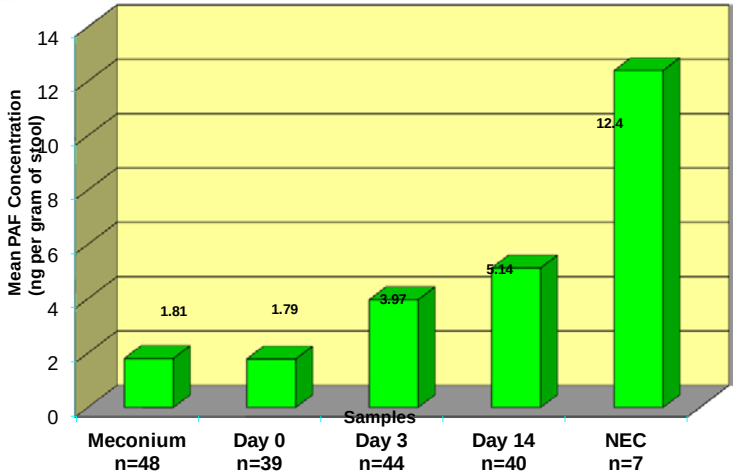


Figure 6 Sialic acid is required for the necrotising enterocolitis (NEC) protective effects of disialyllacto-N-tetraose (DSLNT). Ileum pathology

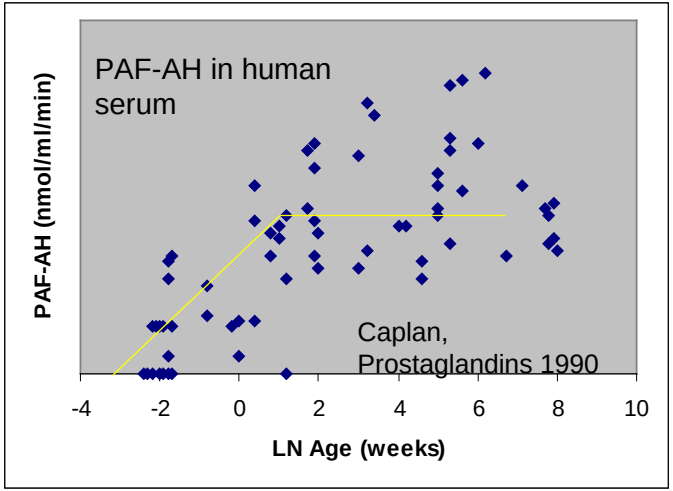
Jantsher-Krenn and Bode et al; IBD 2014

PAF and PAF-AH in NEC



Stool [PAF] in preterm infants over time

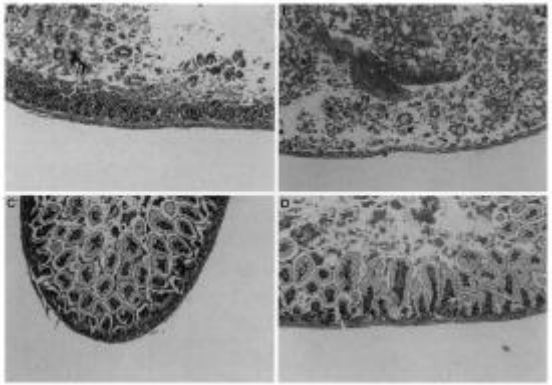
Amer et al, Biol Neo 2004



	NEC	Death
Control	19/26	21/26
PAF-AH	6/26 *	7/26 *

Caplan et al, Peds Res 1997

Controls (top) , rPAF-AH (bottom)



- PAF is important mediator in intestinal necrosis
- PAF-AH is deficient in newborns
- PAF receptors are plentiful in gut epithelium
- PAF-AH ko mice develop NEC
- PAF-AH supplementation prevents NEC in newborn rats
- PAF-AH is present in human milk
- PAF-AH could be developed for NEC prevention

NEC prevention: Barriers to efficient product development

- Clarifying/confirming the diagnosis
- Better understanding of the pathophysiology
- NIH and other extramural support for investigators to pursue innovation
- Challenges with powering clinical trials
- FDA challenges
- Orphan drug status
- Pharmaceutical company interest/balance sheet/market assessment

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Applying Regulatory Science to Neonates
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Session VI: Necrotizing Enterocolitis

Karl G. Sylvester, M.D.
September 13, 2016

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EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Topics

- BIOMARKERS
- Clinical Challenges with NEC and Biomarkers
- What is the landscape of known biomarkers
- What are the challenges of discovering and validating biomarkers
- UNIFYING HYPOTHESES.
- Reflect pathophysiology of NEC
- Biomarkers Diagnosis and Screening
- Biomarkers and Prevention

Clinical Spectrum of NEC

- Bell's I Suspected
  - Limited mucosal injury

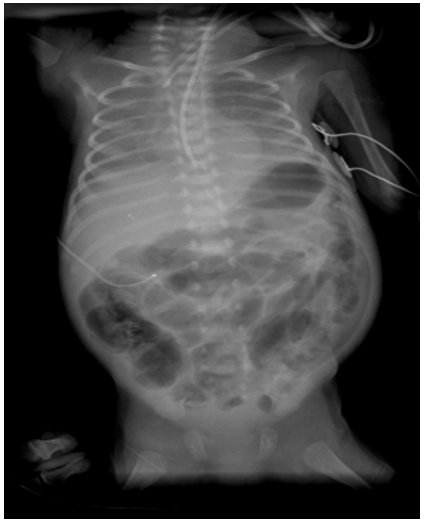
  v. Sepsis

- Bell's II Confirmed
  - Progressive Injury

  Transfer? early OR?

- Bell's III Advanced
  - Irreversible injury

  too late!



High specificity (>90%, poor sensitivity <50%)

Pitfalls: under-treated, over-treated, misdiagnosed

Alternative: objective molecular indicators based upon patient disease biology for tailored / individualized Rx

The problem

Lack of objective diagnostic and prognostic parameters

INSPIRE Network

Glaser – Gerber, Prospective NEC Consortium:

1. Stanford-LPCH
2. Ohio State Univ., NCH
3. Yale New Haven Children's Hospital
4. Baylor-Texas Children's Hospital
5. Univ. of Penn., CHOP
6. Johns Hopkins Children's Hospital

UCSF Children's Hospital
UCLA, Mattel Children's Hospital
Boston Children's Hosp., Harvard

Directors
Larry Moss, MD
Karl Sylvester, MD
Nurse Coordinator
Corinna Bowers
Site PI
Research Nurse

Biologic Studies
Stanford Univ.

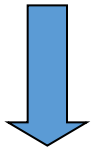
Epidemiologic DB
NCH Informatics

Clinical Parameters as Predictors?

Clinical Parameters do not adequately predict outcome in necrotizing enterocolitis: a multi-institutional study

RL Moss, LA Kalish, C Duggan, P Johnston, ML Brandt, JCY Dunn, RA Ehrenkranz, T Jaksic, K Nobuhara, BJ Simpson, MC McCarthy, KG Sylvester

Journal of Perinatology 28:665-674, Oct 2008



Biologic Studies

CRP does NOT correlate with Bell's Stage

CRP Performed and Results by Bells Stage

N = 631

Bells Stage	Total N	CRP Done	% with CRP	Mean	(Min, Max, 95% CI)	p-value
IA	246	41	16.7	4.1	(0, 40, 1.7-6.6)	0.904
IB	71	15	21.1	4.8	(0.1, 16.7, 1.4-8.3)	P-value for the mean
IIA	209	30	14.4	3.0	(0.1, 22.0, 0.9-5.1)	
IIB	14	3	21.4	2.5	(0.6, 6.3, -5.6-10.6)	
IIIA	52	7	13.5	2.9	(0.1, 11.3, -0.7-6.6)	
IIIB	39	6	15.4	2.0	(0.9, 8.5, -1.3-5.5)	

ANOVA test was used for this table.

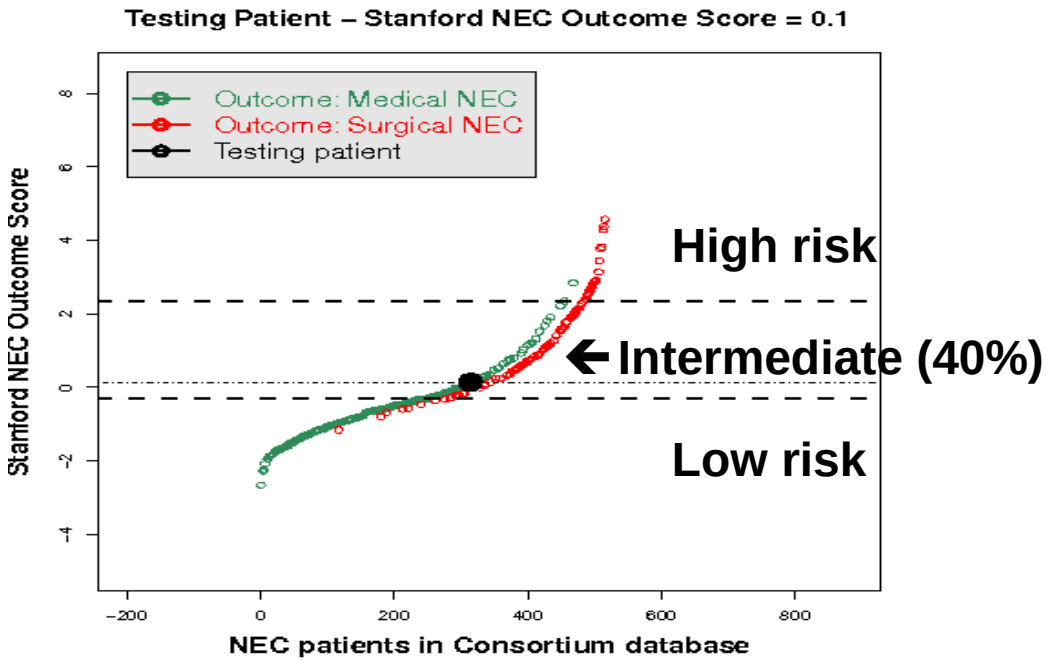
- CRP was not done frequently, averaging between 14-21% of infants for each Bell stage.
- CRP values do not differ significant among all stages.

Clinical parameters can stratify the patients, but not adequately predict NEC outcomes

Clinical parameters:

- Patient demographics
- Laboratory tests
- Radiographic analysis
- Medical history
- Physical exam

NEC outcome prediction



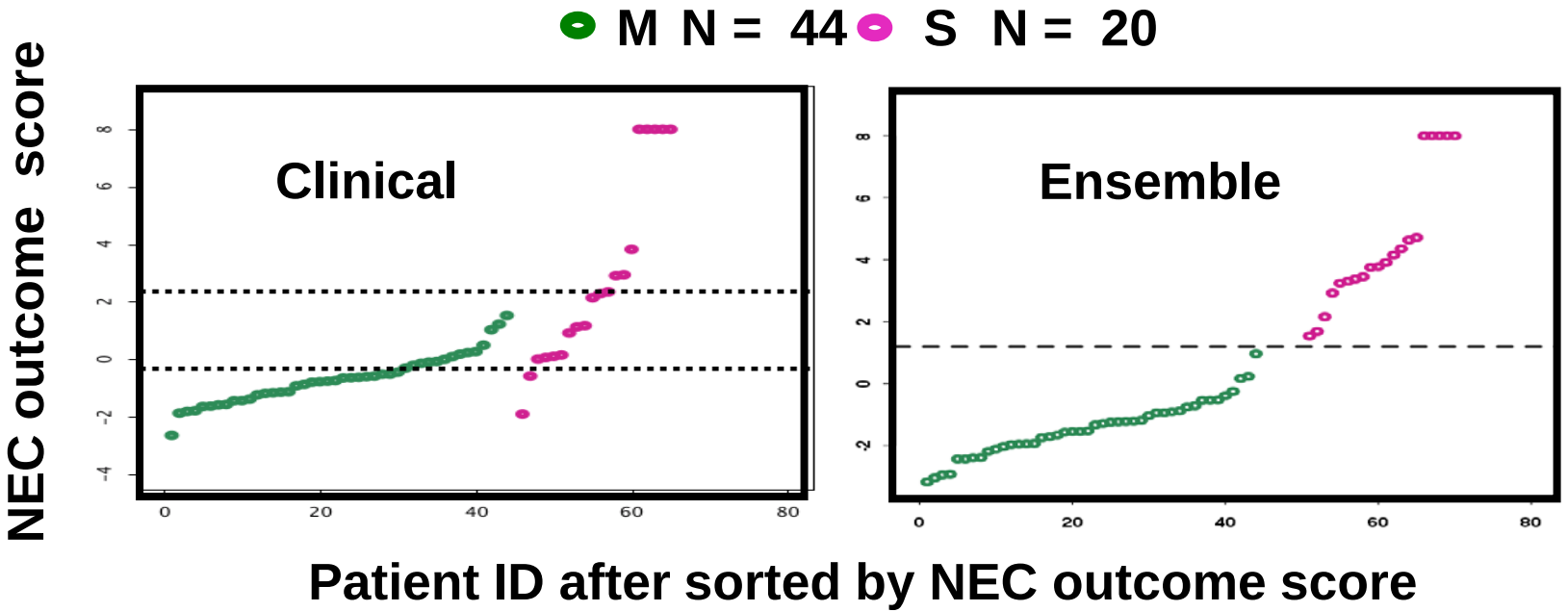
Ling XB, Sylvester KG. PloS One, 9(2), e89860- Feb, 2014.

Ensemble – Integrated Model: Clinical and Molecular Findings

Sylvester *et al*. Gut. 2014 Aug;63(8):1284-92

Clinical parameters

Urine peptide markers:
FGA1826;FGA1823,FGA 2659



Biomarker – **BEST (Biomarkers, EndpointS, and other Tools) Resource**

FDA-NIH Biomarker Working Group.

A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions. Molecular, histologic, radiographic, or physiologic characteristics are types of biomarkers. A biomarker is not an assessment of how an individual feels, functions, or survives.

- susceptibility/risk biomarker
- ✔ diagnostic biomarker
- monitoring biomarker
- prognostic biomarker
- predictive biomarker
- pharmacodynamic/response biomarker
- safety biomarker

January 28,2016

REFERENCE- Citations of Biomarkers for NEC and or Sepsis

1. Jurges ES, Henderson DC: **Inflammatory and immunological markers in preterm infants: correlation with disease**. *Clin Exp Immunol* 1996, **105**(3):551-555.
2. Pourcyrous M, Korones SB, Yang W, Boulden TF, Bada HS: **C-reactive protein in the diagnosis, management, and prognosis of neonatal necrotizing enterocolitis**. *Pediatrics* 2005, **116**(5):1064-1069.
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4. Isaacs D, North J, Lindsell D, Wilkinson AR: **Serum acute phase reactants in necrotizing enterocolitis**. *Acta Paediatr Scand* 1987, **76**(6):923-927.
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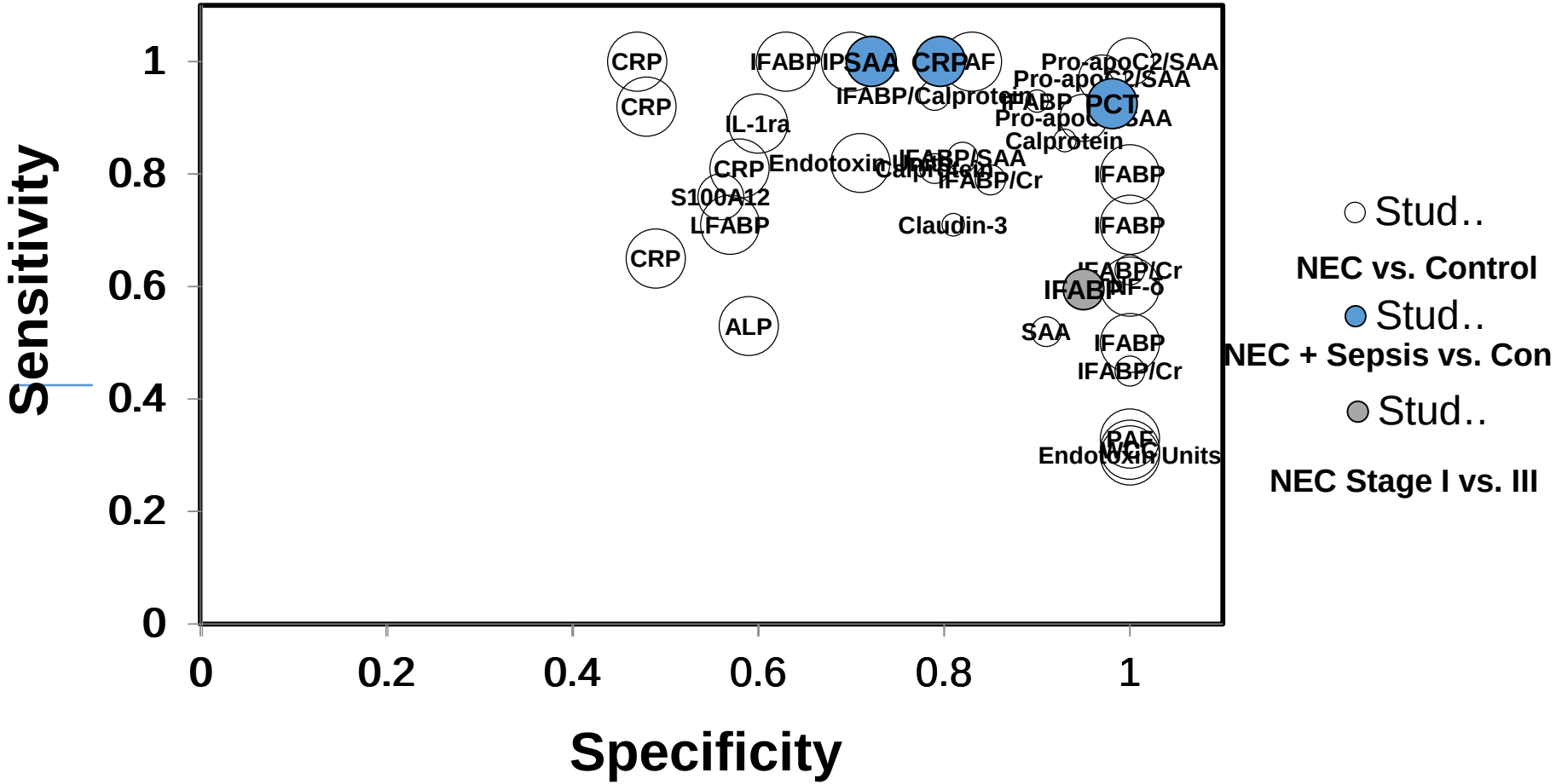
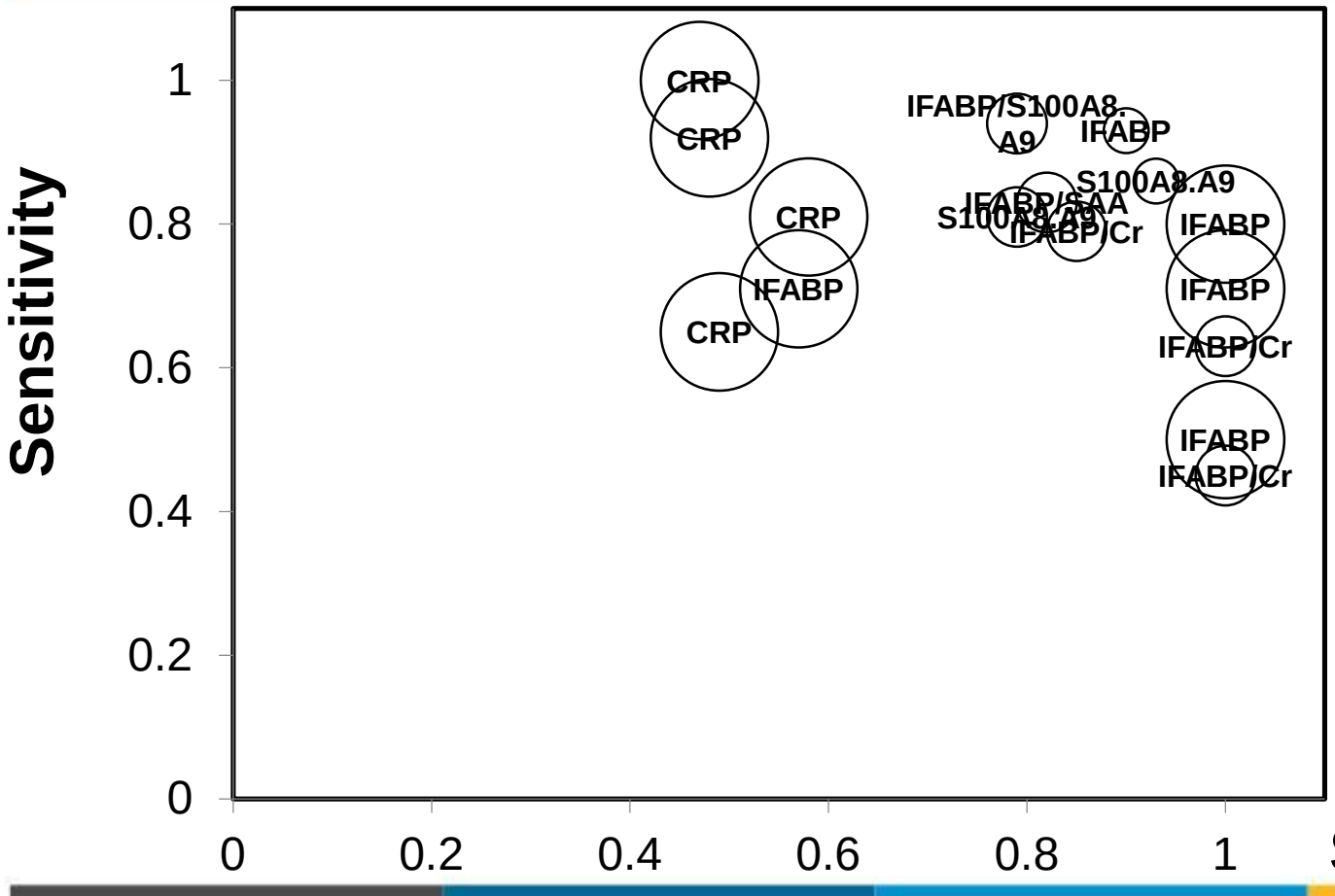
6. Edelson MB, Sonnino RE, Bagwell CE, Lieberman JM, Marks WH, Rozycki HJ: **Plasma intestinal fatty acid binding protein in neonates with necrotizing enterocolitis: a pilot study**. *Journal of pediatric surgery* 1999, **34**(10):1453-1457.
7. Lieberman JM, Sacchettini J, Marks C, Marks WH: **Human intestinal fatty acid binding protein: report of an assay with studies in normal volunteers and intestinal ischemia**. *Surgery* 1997, **121**(3):335-342.
8. Rabinowitz SS, Dzakpasu P, Piecuch S, Leblanc P, Valencia G, Kornecki E: **Platelet-activating factor in infants at risk for necrotizing enterocolitis**. *The Journal of pediatrics* 2001, **138**(1):81-86.
9. Caplan MS, Sun XM, Hseuh W, Hageman JR: **Role of platelet activating factor and tumor necrosis factor-alpha in neonatal necrotizing enterocolitis**. *The Journal of pediatrics* 1990, **116**(6):960-964.
10. Sharma R, Tepas JJ, 3rd, Hudak ML, Mollitt DL, Wludyka PS, Teng RJ, Premachandra BR: **Neonatal gut barrier and multiple organ failure: role of endotoxin and proinflammatory cytokines in sepsis and necrotizing enterocolitis**. *Journal of pediatric surgery* 2007, **42**(3):454-461.
11. Scheifele DW: **Role of bacterial toxins in neonatal necrotizing enterocolitis**. *The Journal of pediatrics* 1990, **117**(1 Pt 2):S44-46.
12. McLachlan R, Coakley J, Murton L, Campbell N: **Plasma intestinal alkaline phosphatase isoenzymes in neonates with bowel necrosis**. *J Clin Pathol* 1993, **46**(7):654-659.
13. Edelson MB, Bagwell CE, Rozycki HJ: **Circulating pro- and counterinflammatory cytokine levels and severity in necrotizing enterocolitis**. *Pediatrics* 1999, **103**(4 Pt 1):766-771.
14. Ng PC, Li K, Chui KM, Leung TF, Wong RP, Chu WC, Wong E, Fok TF: **IP-10 is an early diagnostic marker for identification of late-onset bacterial infection in preterm infants**. *Pediatric research* 2007, **61**(1):93-98.
15. Ragazzi S, Pierro A, Peters M, Fasoli L, Eaton S: **Early full blood count and severity of disease in neonates with necrotizing enterocolitis**. *Pediatric surgery international* 2003, **19**(5):376-379.
16. Thuijls G, Derikx JP, van Wijck K, Zimmermann LJ, Degraeuwe PL, Mulder TL, Van der Zee DC, Brouwers HA, Verhoeven BH, van Heurn LW *et*

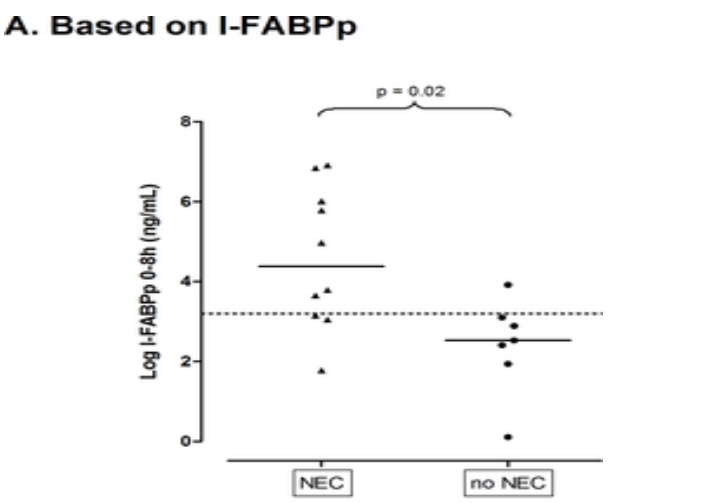
Published Biomarkers for NEC

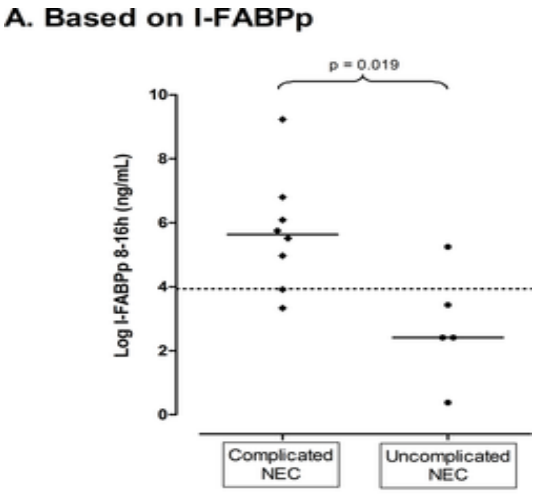


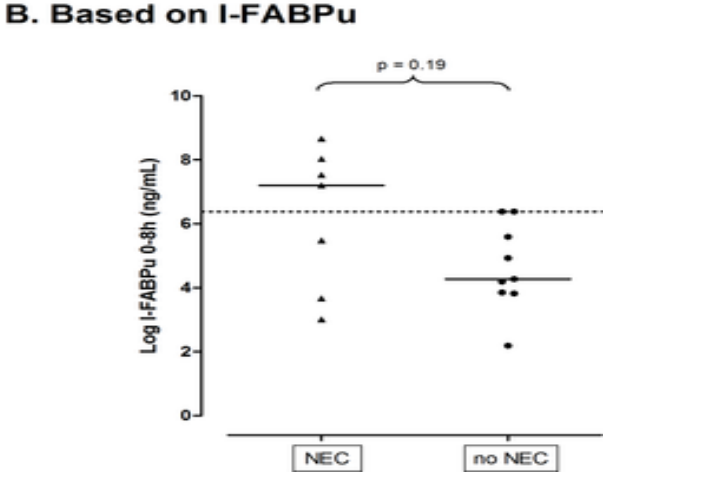
CRP, IFABP, Calprotectin (S100A8,12)



NEC vs. Control
Single time point







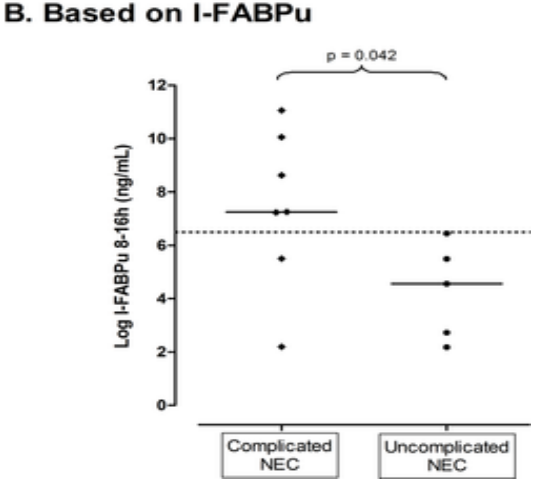


Fig 2. Median I-FABP values (after logarithmic transformation), measured from 0–8 h in plasma (A) and urine (B), of 22 NEC versus 15 no-NEC patients.

Schurink M, et al. (2015) Intestinal Fatty Acid-Binding Protein as a Diagnostic Marker for Complicated and Uncomplicated Necrotizing Enterocolitis: A Prospective Cohort Study. PLoS ONE 10(3): e0121336. doi:10.1371/journal.pone.0121336

Evennett N., et al. A Systematic Review of Serologic Tests in the Diagnosis of NEC. J of Ped Surgery 44:2192-2201, 2009

Table 3 Pooled estimates of diagnostic accuracy and global performance

Characteristic	Diagnostic test		
	CRP	I-FABP	
Sensitivity (95% CI)	0.85 (0.76-0.91)	0.62 (0.4	
Heterogeneity, P for χ^2 test	.02	.40	
Heterogeneity, I^2	69%	0%	
Sensitivity (95% CI)	0.60 (0.57-0.64)	1.00 (0.7	
Heterogeneity, P for χ^2 test	.00	1.00	
I^2	84%	0%	21%
LR+ (95% CI)	1.78 (1.55-2.03)	6.58 (1.41-30.62)	5.38 (3.81-7.59)
Heterogeneity, P for χ^2 test	.97	.94	.53
I^2	0%	0%	0%
LR− (95% CI)	0.32 (0.16-0.63)	0.47 (0.30-0.73)	0.15 (0.01-3.67)
Heterogeneity, P for χ^2 test	.14	.60	.00
I^2	45%	0%	86%
DOR (95% CI)	5.82 (3.21-10.53)	17.60 (2.68-115.78)	43.54 (7.04-269.27)
Heterogeneity, P for χ^2 test	.44	.97	.50
I^2	0%	0%	0%
AUC (±SEM)	0.70 ± 0.06	0.88 ± 0.16	0.92 ± 0.05
Q index (±SEM)	0.65 ± 0.05	0.81 ± 0.16	0.86 ± 0.06

The positive likelihood ratio is calculated as

L R + = sensitivity /1-spec

or

LR+ = Pr (T+/D+)

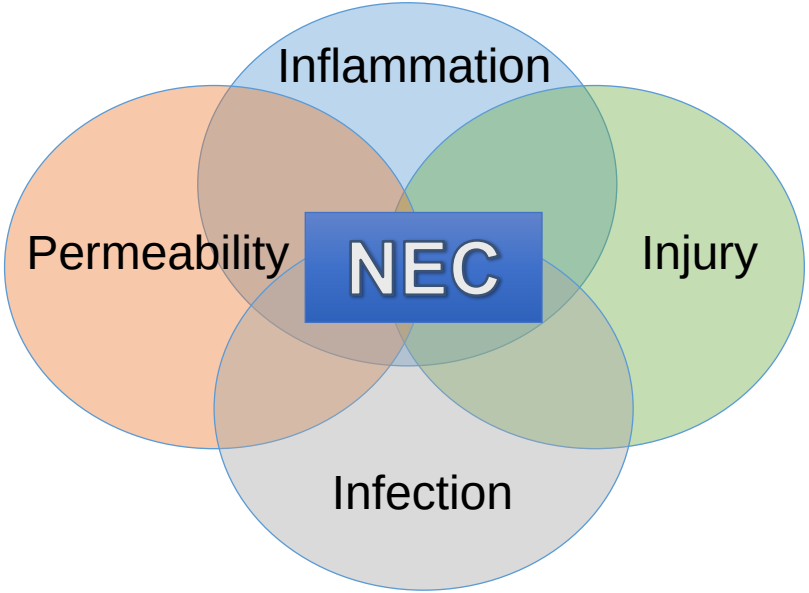
Pr (T+/D-)

Relevant Challenges: Treating and Preventing NEC

- Small subject number studies
  - Different controls
  - Different time collections and biologic samples
- Screening studies, baseline values, and biology
- Defining NEC by what criteria; clinical, radiographic, laboratory, treatment
- Contamination by other similar presentation diseases; SIP
- Low prevalence disease
- Multi-center studies
- Ivory tower & study effects of investigating rare diseases–
  - are there significant differences in risk and exposure(s) for NEC in academic and non-academic centers?
  - Generalizable
  - Adoption

Domains of NEC Biology & Biomarkers

CRP, Calprotectin, iFABP, IL8

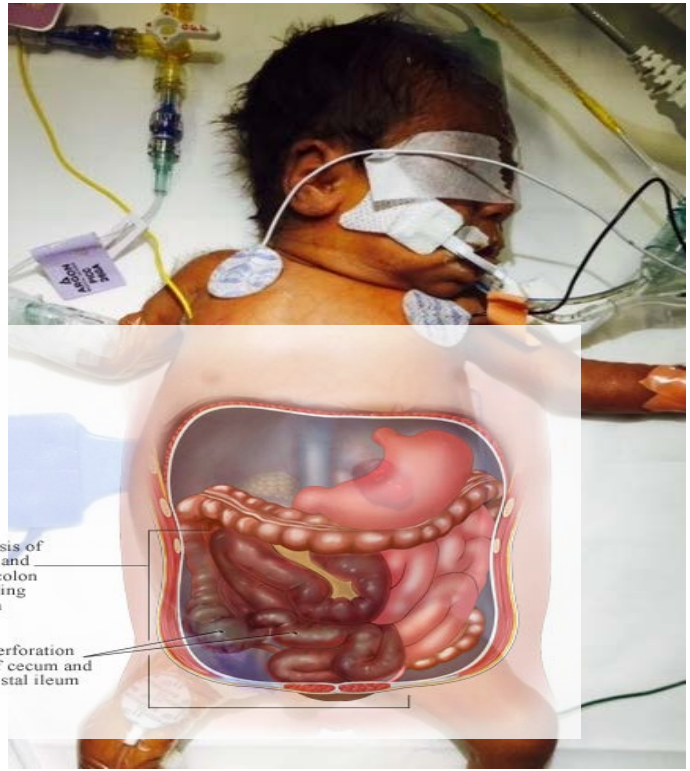


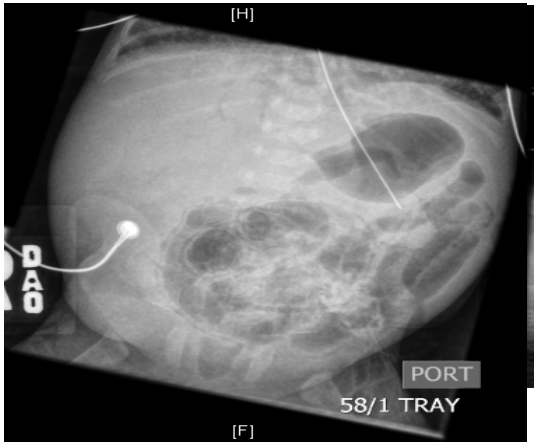
Citrulline
iFABP,
Claudins
Pneumatosis

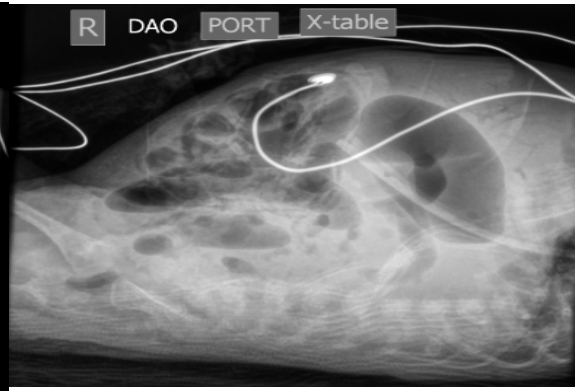
iFABP

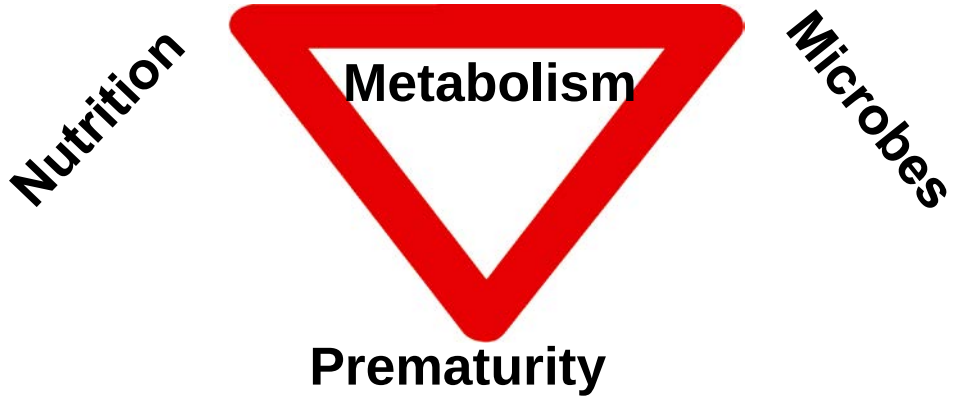
CRP, Calprotectin, iFABP
microbiome

NEC – Clinical Presentation









Prevention Strategies

- Feeding Strategies
  - (early v late, slow v. fast)(MBM v formula, banked) (TPN and lipids)
- Probiotics
  - (composition, off target effects, all v some or high risk)

Needs Assessment

A minimally invasive method to detect intestinal mucosal injury that precedes the onset of fulminant NEC

That reflects the degree of injury

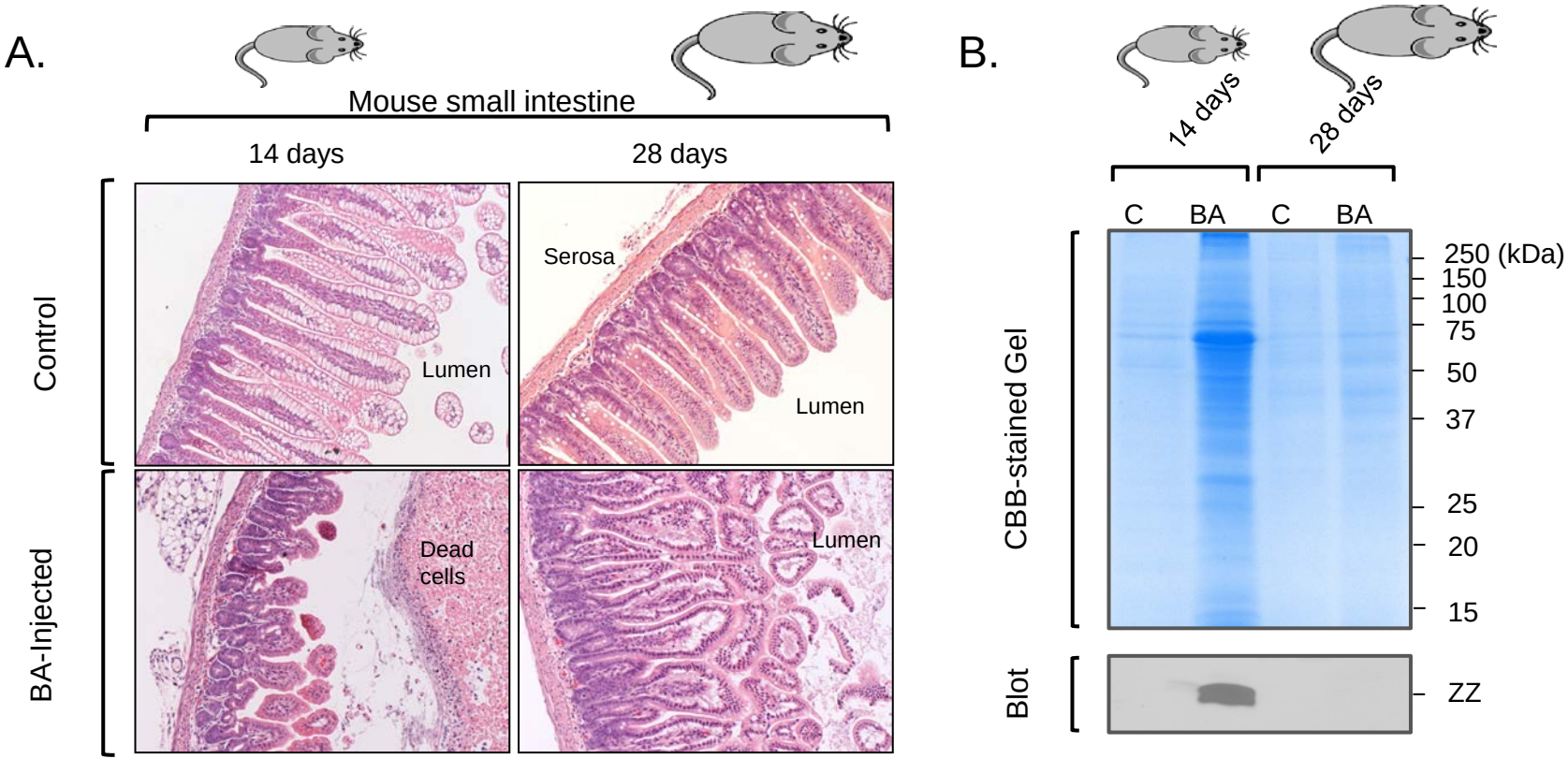
That reflects response to and guides therapy

Newborn Enteropathy

- Metabolic Panel for assessing risk of acquired newborn disease, *i.e.* Necrotizing Enterocolitis

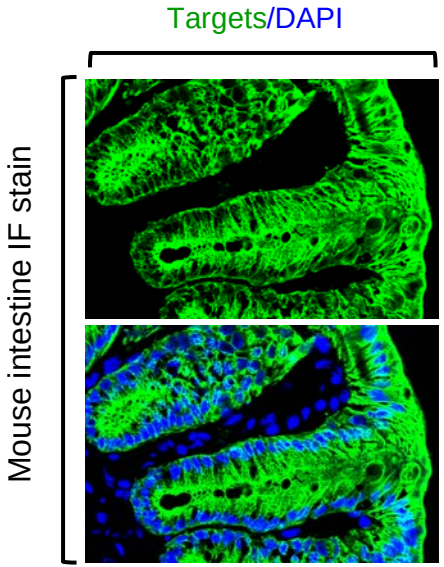
- Assay of mucosal health

Intestinal injury in neonate vs. juvenile mice after luminal BA-injection

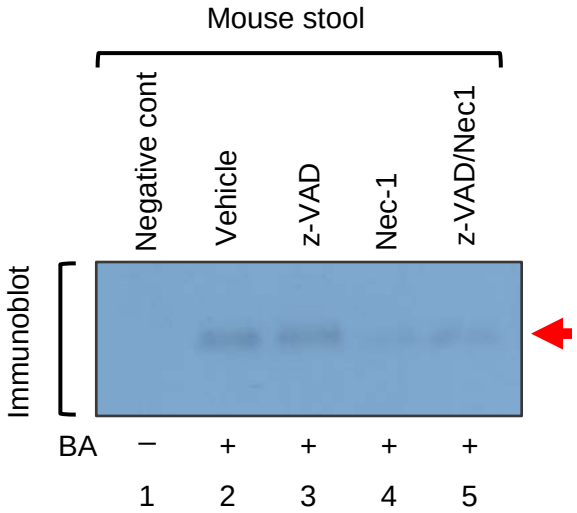


Target proteins are abundantly and specifically localized in enterocytes and can be detected in stool if intestines are injured

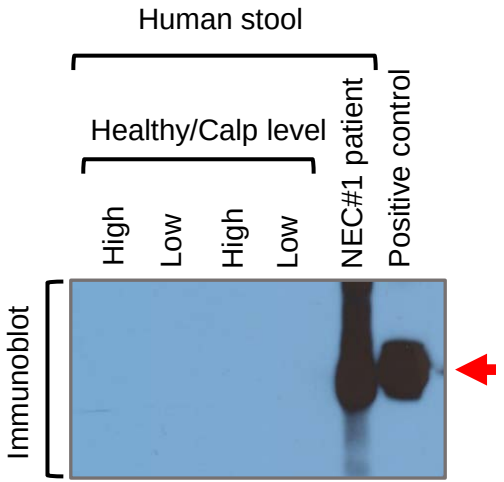
A.



B.

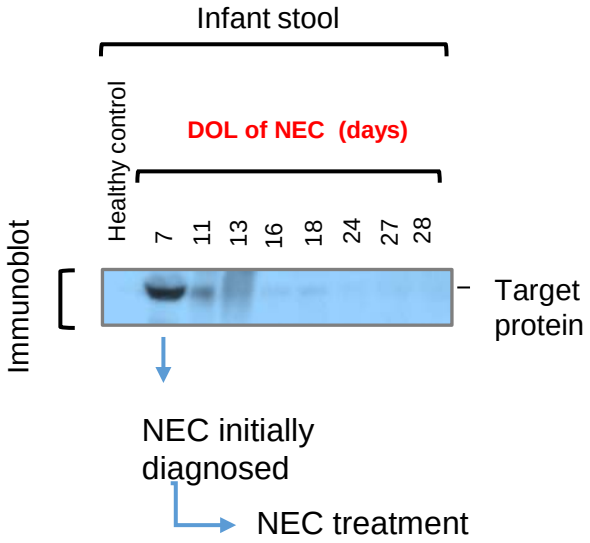


C.

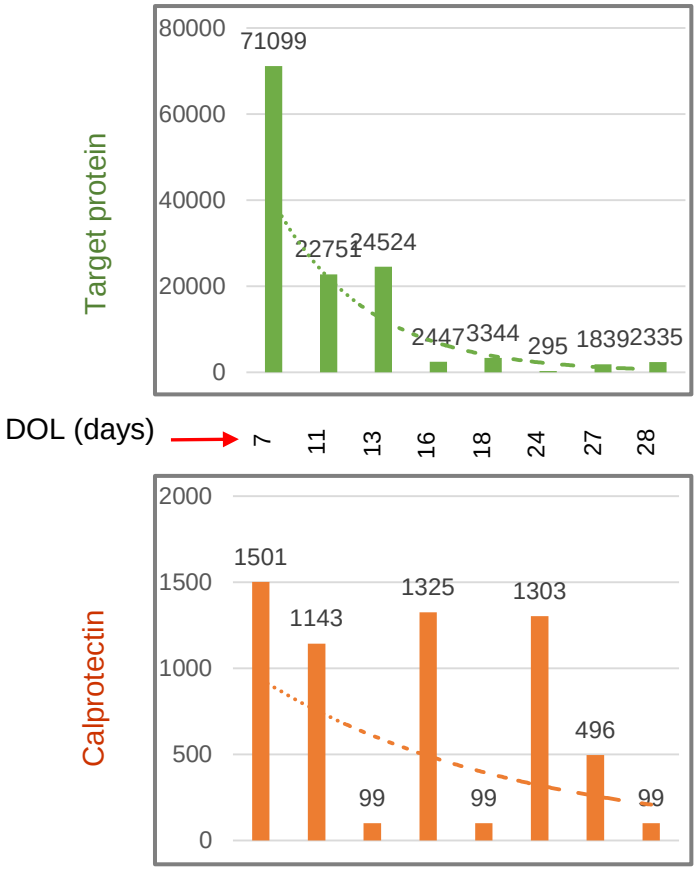


Comparison of time-course assays: fecal proteins for a NEC-patient

A.



B.



However, we caution filing on biomarkers under current patent law. Based on the facts summarized above, an important component for the utility of the invention is its use as a biomarker for diagnostic purposes. **There have been some broad changes in the approach that the USPTO takes in the review of such methods since Supreme Court decisions in 2012** and later. In the last few years **it has been our experience that it is extremely difficult to persuade Examiners to allow diagnostic claims that were previously routinely granted, and that the lower courts have confirmed the restrictions on patentability**.

USPTO, Legal Decisions affecting Biomarker Development

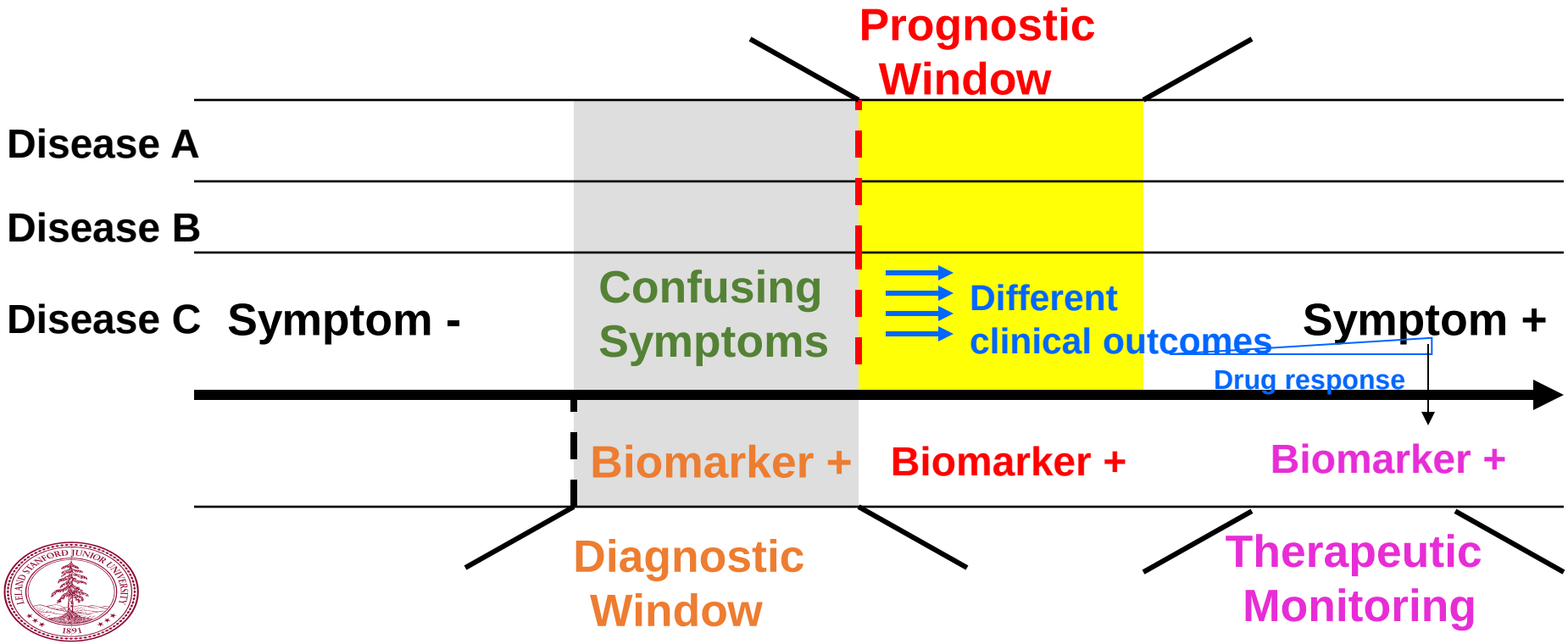
In *Mayo v. Prometheus*, the U.S. Supreme Court found that claims reciting methods for detecting a correlation between a metabolite and the likelihood of responding to a drug, without "more," are not patentable. 132 S. Ct. 1289 (2012).

In *Association for Molecular Pathology v. U.S. Patent & Trademark Office and Myriad Genetics* ("*Myriad*"), the Court of Appeals for the Federal Circuit found certain method claims ineligible because they were drawn to mental processes. *In Myriad, one stricken method claim was directed to screening for cancer-predisposing mutations with no further non-mental steps, while another was directed to a method comprising the single step of comparing a gene sequence to a control to identify a certain mutation*.

In practice what this has meant is that a **claim directed to a novel correlation for diagnostic or theranostic purposes, which claim uses known reagents and methods, is likely to be rejected** as being drawn to ineligible subject matter. It has been our experience that **only claims with a novel reagent or analytic process; or a claim including treatment steps, are currently considered to be patent eligible**.

Biomarker Clinical Utility

Diagnostic, Prognostic, Monitor Rx Response



Projects to consider for a NEC in Neonates Workstream

Potential Projects for Furthering Research in Necrotizing Enterocolitis in Neonates

1) Identification and utilization of biomarkers for the early diagnosis of NEC; are there candidates available and what additional investigation is needed?

2) Identification and utilization of biomarkers for the response to treatment of NEC; possibly prognostic indicators.

3) Detailed review and meta-analysis of current methods to prevent and treat NEC in high risk neonates leading to prioritization and study of leading candidates

4) Epidemiologic study of NEC across the globe

5) Determination and clarification of NEC diagnosis: are there different categories that should be considered?

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Thank you

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EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Agenda – Necrotizing Enterocolitis

1:00 – 3:00 p.m.

Session VI: *Necrotizing Enterocolitis*

RON PORTMAN, INC CO-DIRECTOR (NOVARTIS) &
MICHAEL CAPLAN (UNIVERSITY OF CHICAGO), CO-CHAIRS

NEC: State of the Art
MICHAEL CAPLAN (UNIVERSITY OF CHICAGO)
Biomarkers and Barriers: Opportunities and Challenges in NEC
KARL SYLVESTER (STANFORD UNIVERSITY)

Session VI Panel:
TAHA KEILANI (SIGMA TAU)
IRJA LUTSAR (PDCO)
PAOLO MANZONI (S. ANNA HOSPITAL, TORINO)
TOKUO MIYAZAWA (SHOWA UNIVERSITY, JAPAN)
JOSEPH NEU (UNIVERSITY OF FLORIDA - GAINESVILLE)
JENNIFER CANVASSER (NEC SOCIETY & PPA)

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Sigma-Tau Pharmaceuticals, Inc.
Live Biotherapeutics
STP206

Taha Keilani, MD
V.P., Chief Medical Officer
September 13, 2016

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STP (Sigma-Tau Pharmaceuticals) Experience

- Introductions
- Study Drug
- IND
  - Pre-IND activities
  - Manufacturing and product release
  - Clinical assays
  - Clinical development Plan
- Current status and plan

STP206

- Contains 2 commonly known and used bacteria in food production *(Lactobacilli and Bifidobacteria)*
- These bacteria are normal inhabitants of the human gastrointestinal tract, oral cavity, skin, and the vagina
- Associated with a long history of safe use in humans
- Integral to the production of fermented foods and have been consumed safely as part of these foods for millennia
- Are generally considered to be harmless and thus are afforded the generally recognized as safe (GRAS) status

IND

- Pre-IND activities
  - Preclinical testing
  - Toxicology
  - Discovering the road to test STP206 in target population
  - The need to test the product in older population first?
  - Implication on the Clinical Development Plan
- Manufacturing considerations
  - Finding the manufacturing vendor
  - cGMP conditions
  - Releasing the product
- Clinical Assay development and validation (for identifying the STP206 strains)

IND

- Proposed indication
  - Prevention of Necrotizing Enterocolitis (NEC) in premature babies with birthweight <1500 grams
- The IND submitted (May 18th, 2009)
- Main issues identified:
  - Additional and extensive release testing for objectionable organisms
  - Clinical assay issues
  - Other protocol issues
  - Develop manufacturing process (cGMP)
  - Optimize manufacturing process to obtain target viable count
- IND cleared for the healthy volunteer study on Feb. 12th, 2010

STP206-002

- This study initiated to include the target population
- First introduction and discussion of STP206-002 study protocol with FDA was in July, 2011
- Protocol was finalized in Dec. 2012
- In March, 2013, more pathogens were added for product release testing

Overall Experience and Current Status

Challenges:

- Very long time to agree on the IND (started in 2008)
- Communication and corresponding with FDA
- Manufacturing challenges
- At the time of IND submission, no clear Regulatory guidance was available for Live Biotherapeutics

Current Status:

- Currently focusing on completing the STP206-002 study
- STP is eager to propose and discuss an expedited path forward for approval

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NEC and Regulatory Science

Irja Lutsar MD, PhD
PDCO
University of Tartu, Estonia

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SCIENCE MEDICINES HEALTH

Background and current status

- Which disease category is NEC?
  - Infectious disease and treated with antibiotics
    - guidelines for antibiotics
  - Gastroenteral disease
    - guidelines GI medicines
  - Both?
- No diseases with similar mechnisms in adults or older children
- Medicines/drugs could be used and thus regulated
  - For prevention of NEC
  - For treatment of NEC

Current status

- Pathomechanims and thus management of NEC largerly unknown
- No regulatory guidelines on development medicines for NEC
- No PIPs submitted with the indication of prevention or treatment of NEC
- 16 PIPs agreed/under review for antibiotics for LOS (NEC not mentioned)
- No biomarkers identified
  - For diagnosis
  - For treatment
- NEC not mentioned in the neonatal guidelines

Probiotics and lactoferrin for NEC

- No clear position
  - Is it probiotic or pharmabiotic
  - Lactoferrin – drug or dietary supplement
  - Who should regulate approval - EFSA or EMA
    - Food/diatery supplements are regulated by EFSA
    - Medicines are regulated by EMA
- Current regulatory status
  - Probiotics have been presented for scientific advice
  - 1 or 2 PIPs for probiotics (not for NEC)
  - PIP for fecal transplantation (not for NEC)
  - Several academic trials on NEC completed ongoing but no PIPs or regulatory submission

Future directions

- Define management of NEC
- Initiate discussion on regulatory approach on NEC
- If medicines are needed for NEC the regulatory path should be developed
- Regulation of biomarkers for NEC
  - Diagnostic measurement
  - Outcome measurement

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The Current Situation of Necrotizing Enterocolitis in Japan

Tokuo Miyazawa
Department of Pediatrics
Showa University School of Medicine

SHOWA University

CRITICAL PATH INSTITUTE

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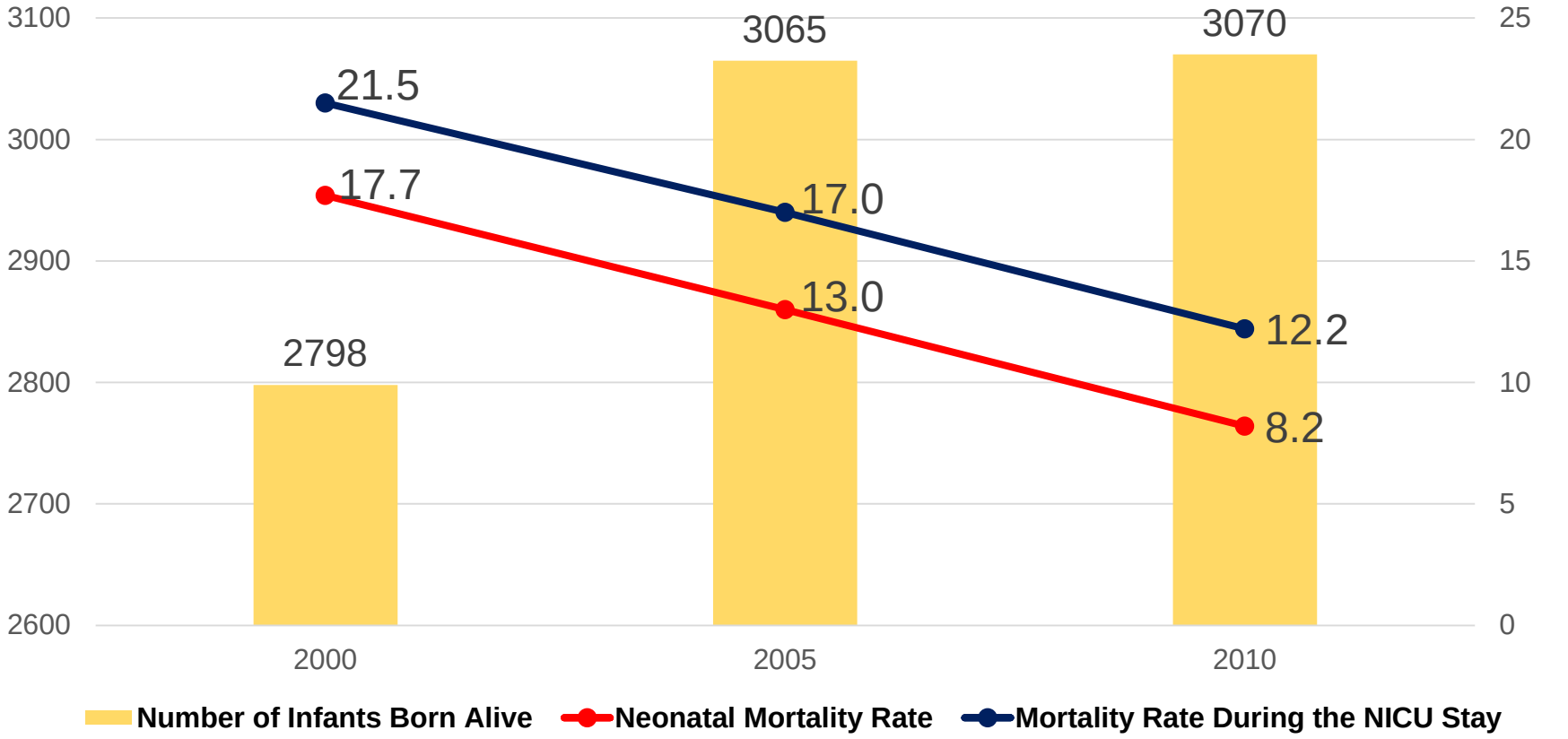
SCIENCE MEDICINES HEALTH

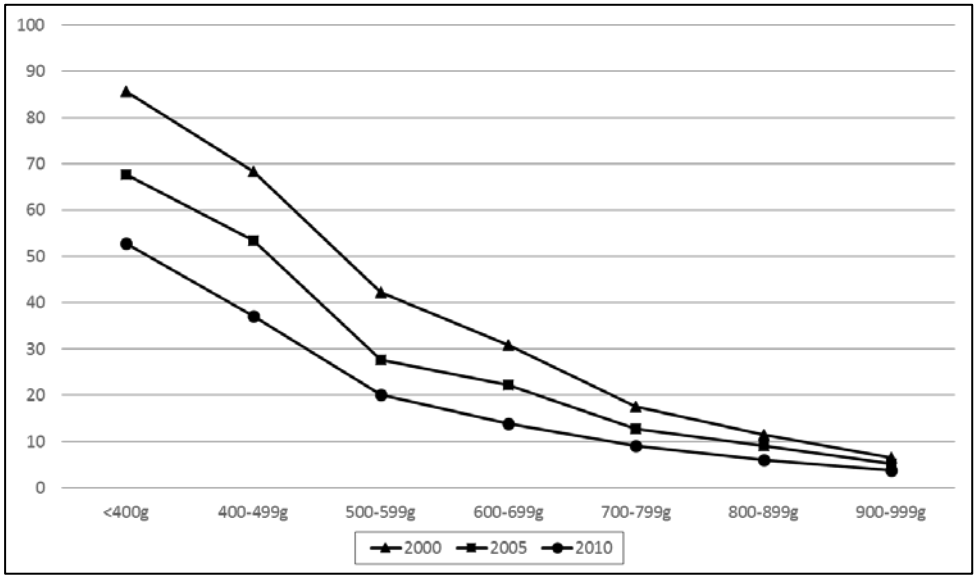
Mortality Rates of ELBW infants between 2000 and 2010

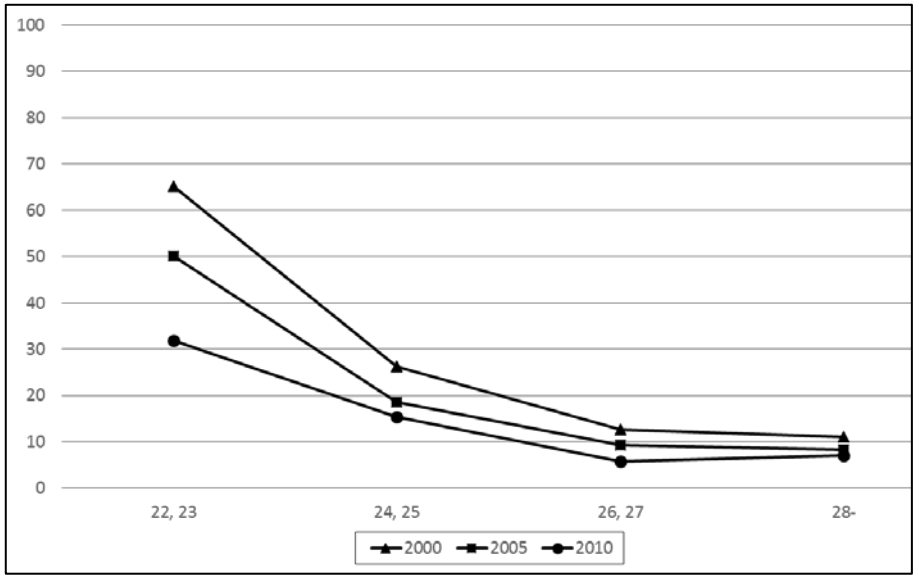
(National Survey by Committee of Neonatal Medicine, Japan Pediatric Society)



(This national survey covers over 95% of ELBWI reported in the maternal and health statics in Japan in each year)

Mortality Rates of ELBW infants between 2000 and 2010
(National Survey by Committee of Neonatal Medicine, Japan Pediatric Society)





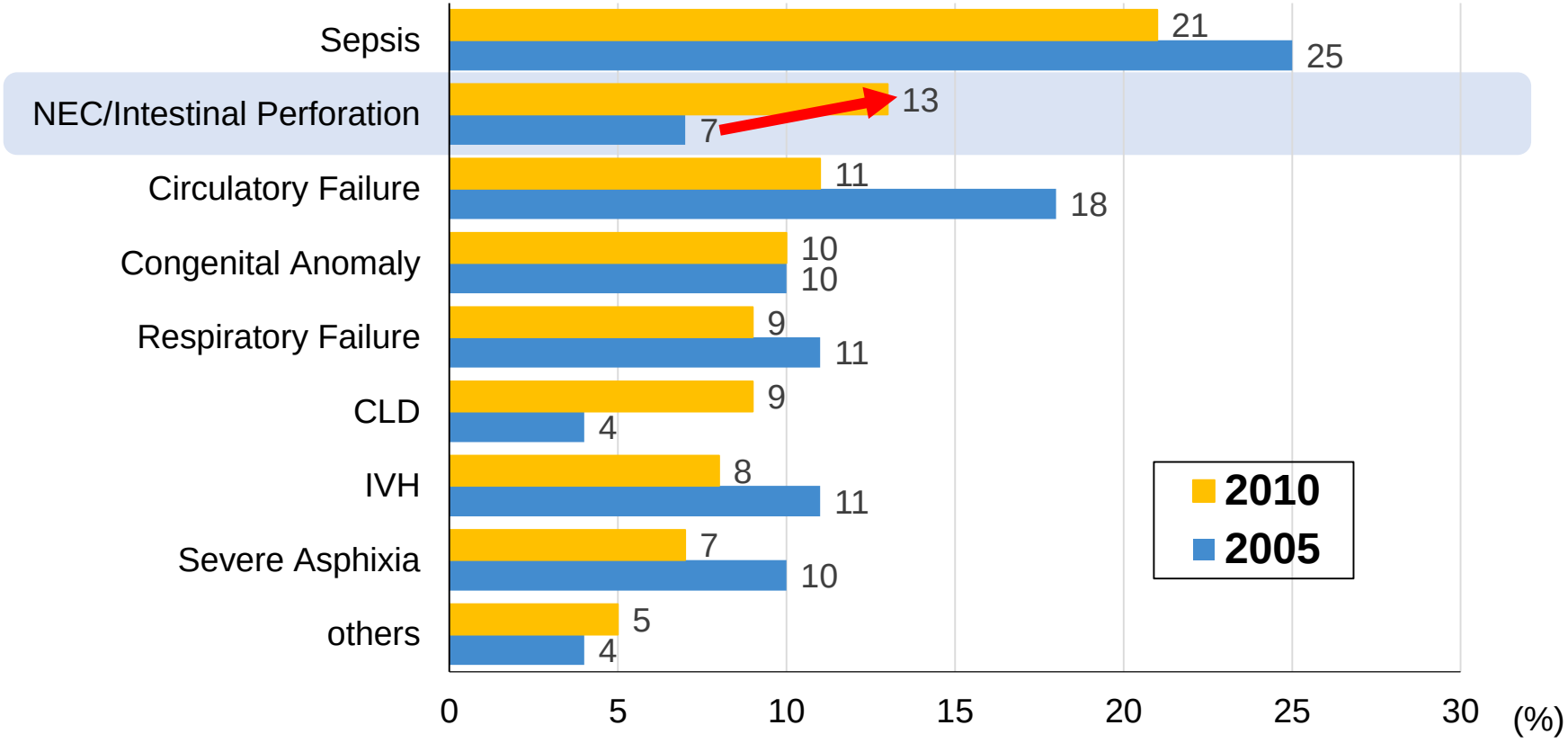
Comparison according to BW

Comparison according to Gestational Age

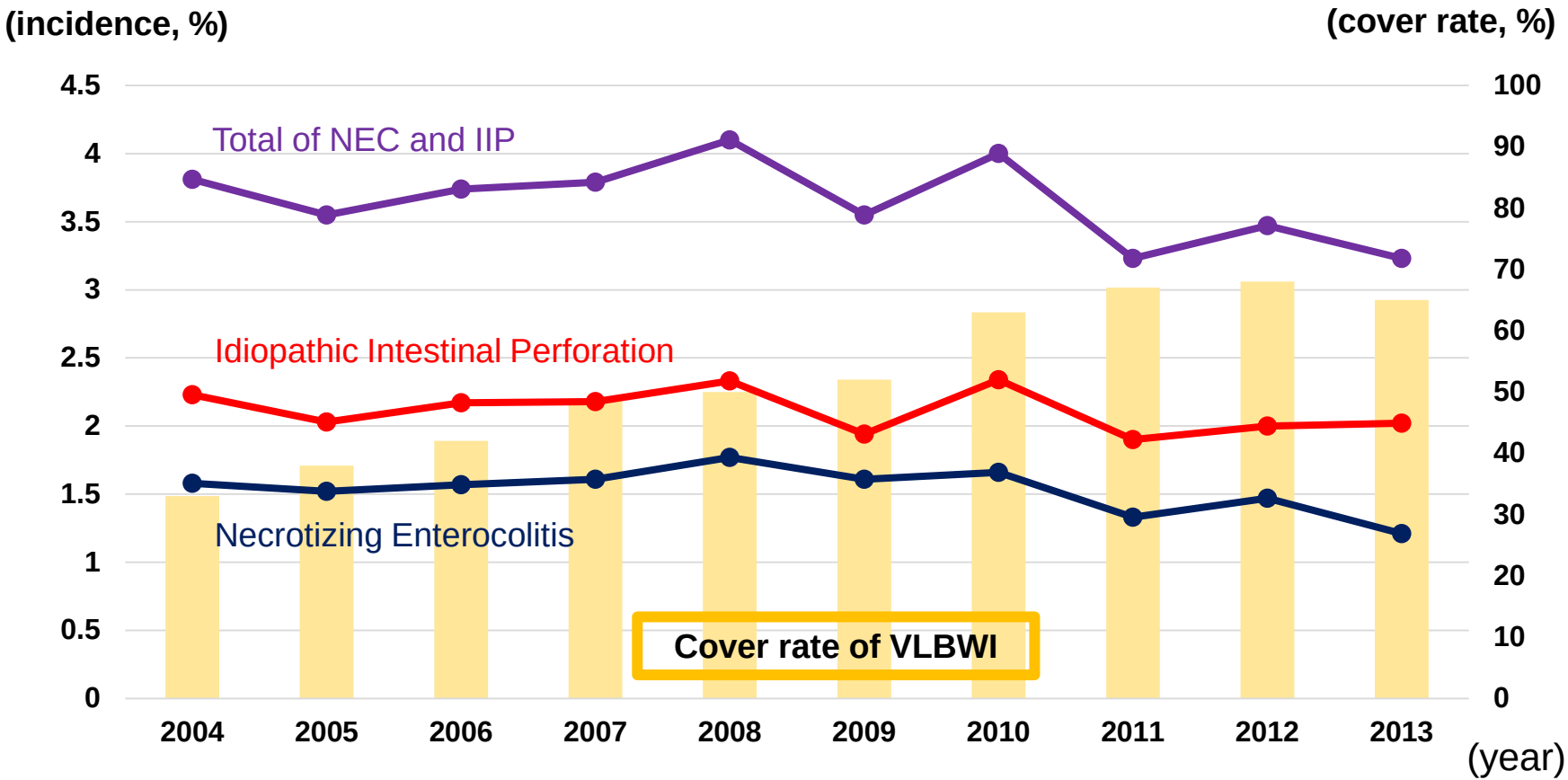
(This national survey covers over 95% of ELBWI reported in the maternal and health statics in Japan in each year)

Ranking of Causes of Death during the NICU stay

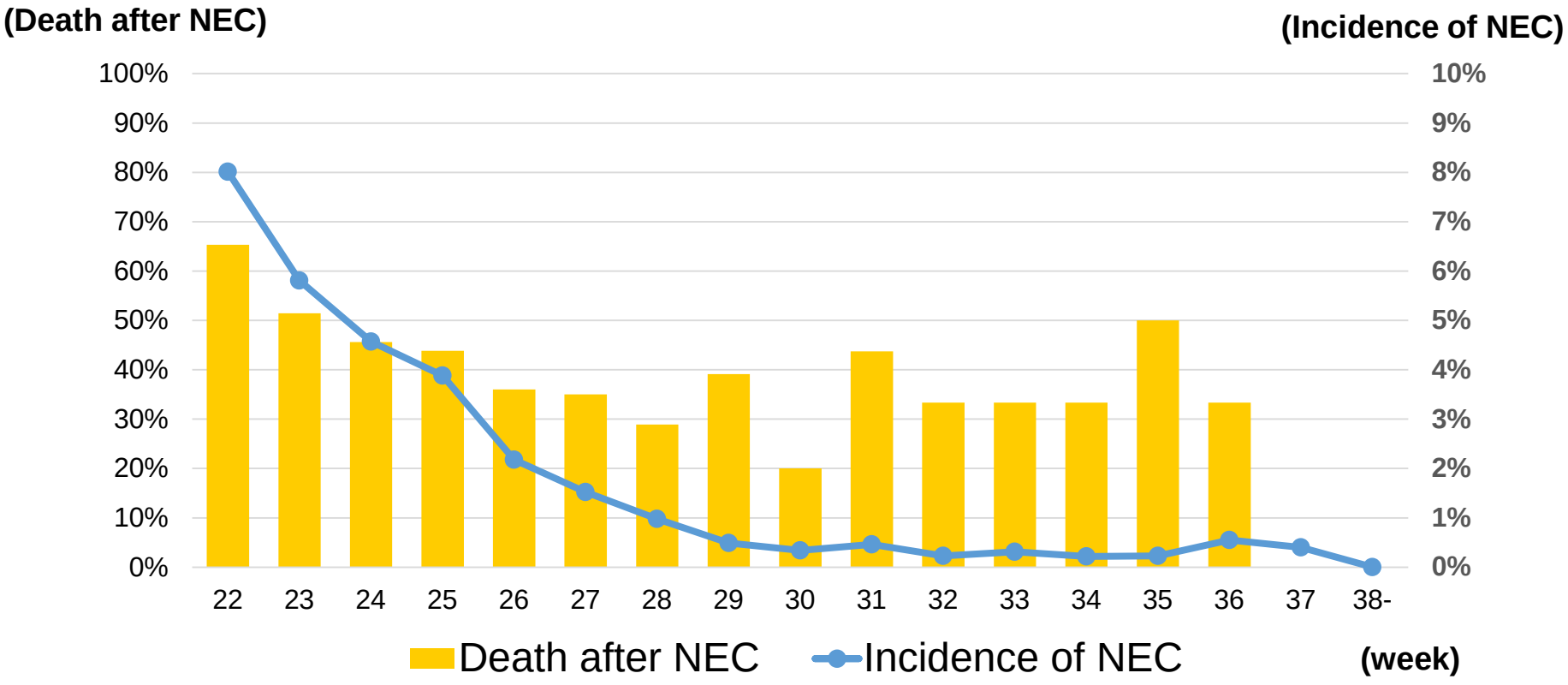
(National Survey by Committee of Neonatal Medicine, Japan Pediatric Society)



Incidence of NEC (from NRN Japan)



Incidence of NEC and Rate of Death after NEC according to GA(NRN Japan 2003-2012)



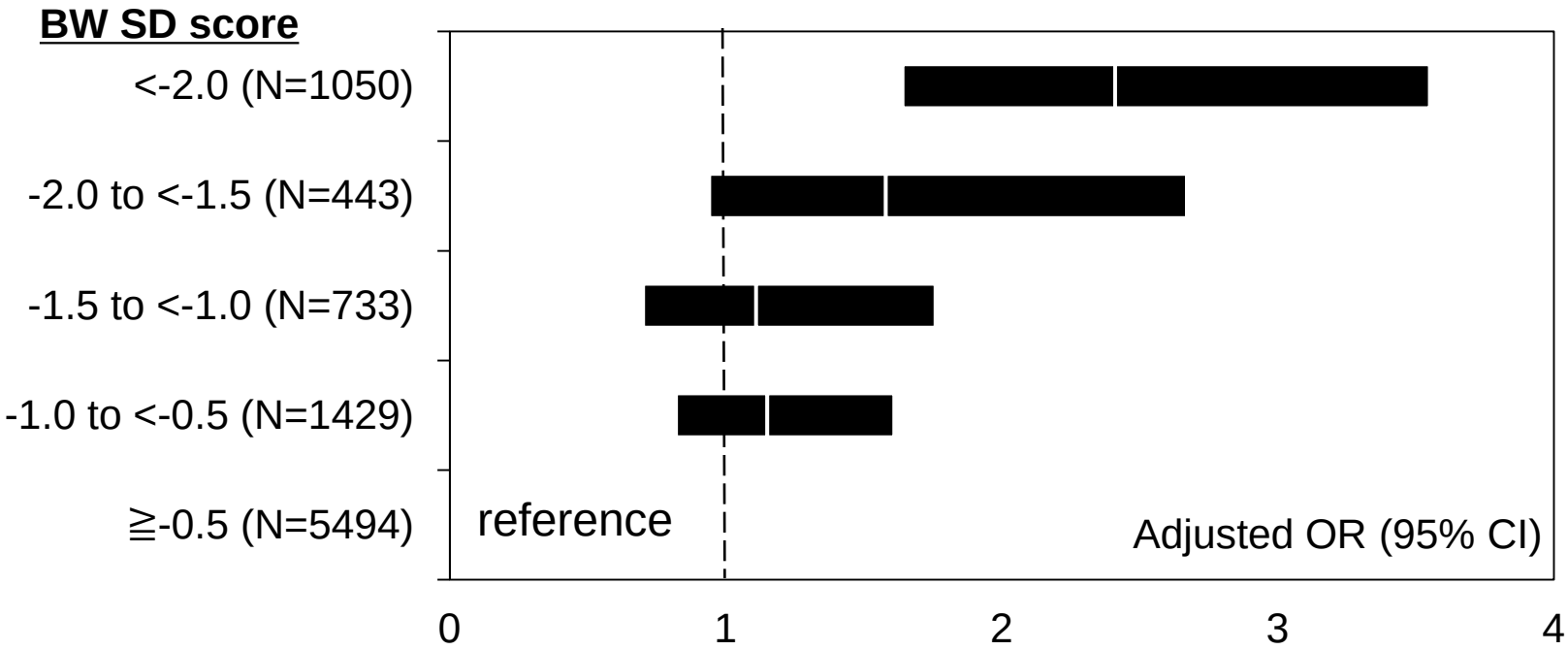
Risk factors affecting to NEC (multivariable analysis, NRN Japan 2003-2012)

	Adjusted OR	95% C.I.
Gestational Age (1wk)	0.82	0.75-0.86
Birth Weight (100g)	0.82	0.76-0.89
Gender (male)	1.46	1.22-1.75
Cesarean Section	1.06	0.85-1.31
Out Born	0.97	0.63-1.49
Multiple Birth	1.07	0.86-1.32
SGA	1.05	0.75-1.48

	Adjusted OR	95% C.I.
Maternal Hypertension	0.78	0.57-1.05
P-PROM	0.88	0.72-1.07
Antenatal Corticosteroids	1.03	0.86-1.23
Apgar Score 1min	0.94	0.89-0.99
Apgar Score 5min	1.05	0.98-1.11
RDS	1.44	1.13-1.83
PPHN	1.54	1.18-2.03
Indomethacin for PDA	1.48	1.23-1.78

Subjects: birth weight below 1500g
Exclusion: Congenital anomaly, infants with unknown gestational age or defected data

Morbidity risk of NEC vary with birth weight SD score in SGA-ELBWI (NRN japan)



OR adjusted for gestational age, sex, plurality, multiple birth, delivery modes, maternal hypertension, clinical chorioamnionitis, and antenatal steroids

Yamakawa T, Itabashi K, Kusuda S. Ear Hum Dev 92:7-11, 2016

Nutritional Management and Prevention of NEC (from Cochran Review)

Intervention	Control	OR	95%C.I.	Revision
Formula milk	**Donor milk**	2.77	1.40-5.46	Quigley, 2014
Trophic feeding	Enteral fasting	1.07	0.67-1.70	Morgan, 2013
Delayed advancement (after Day 5~7)	Early advancement (within Day 4)	0.93	0.64-1.38	Morgan, 2014
Slow advancement (15-20ml/kg/day)	Fast advancement (30-40ml/kg/day)	1.02	0.64-1.62	Morgan, 2015
Continuous milk feeding	Intermittent bolus milk feeding	1.09	0.58-2.07	Premji, 2011
Human Milk Fortification	No Fortification	1.57	0.76-3.23	Bown, 2016
Probiotics	Placebo	0.43	0.33-0.56	AlFeleh, 2014
Restricted water intake	Liberal water intake	0.43	0.21-0.87	Bell, 2014

Nutritional Management and Prevention of NEC (from Cochran Review)

Management in JAPAN

Intervention	Control	OR	95%C.I.	Revision
Formula milk	**Donor milk**	2.77	1.40-5.46	Quigley, 2014
Trophic feeding	Enteral fasting	1.07	0.67-1.70	Morgan, 2013
Delayed advancement (after Day 5~7)	Early advancement (within Day 4)	0.93	0.64-1.38	Morgan, 2014
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Restricted water intake	Liberal water intake	0.43	0.21-0.87	Bell, 2014

Feeding Policy for VLBWI

- **Trophic Feeding**
  - To avoid gut atrophy, colonize normal microbiota, prevent NEC, PNAC and infections.
  - Start with own mother's milk (if possible), at least within 72 hours after birth.

- **Advancement of Enteral Feeding**
  - Start at 10ml/kg/d and increase daily by 10-20ml/kg/d, up to 150-160ml/kg/d

- **Use of Donor Milk**
  - **The official human milk banking program is not available in Japan.**
    In 2014, the first human milk bank is established at Showa Univ. Koto Toyosu Hospital. It does not provide donor milk outside of their NICU yet.
  - 25% of the NICUs traditionally use **unpasteurized donor milk** after screening for pathogens by checking serum antibodies of the donor mother.
    (Mizuno K. Pediatr Int 57: 639-644, 2015)
  - If OMM is not available, preterm infant formula is applied in general case.

Other Characteristic (experimental) Management in Japan

- Examination of **C-reactive protein (CRP)** as a biomarker of infectious disease and necrotizing enterocolitis

  Pourycyrous M. Pediatrics 2005;116:1064-1069

- Screening of PDA with **daily echocardiography by neonatologists**

  Roze JC. JAMA 2015;313:2441-2448

- Routine **administration of enema** to prevent feeding intolerance
  - 1ml/kg/dose, 1 to 3 times per day

- Comparatively **Restricted Water Intake**
  - Start at 60ml/kg/day and increase daily by 10ml/kg/day
  - Increase up to 120(enteral and parenteral)-150(enteral feeding only)

CRP rapid assay instrument

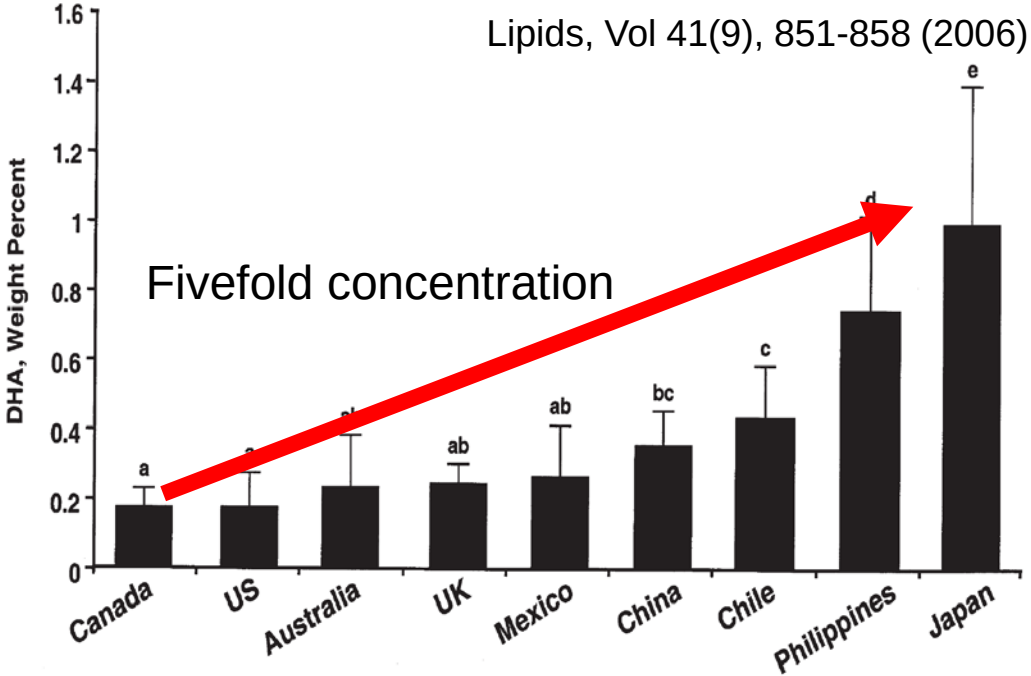
Screening echocardiography by neonatologist

High Concentration of DHA Level in Human Milk of Japanese Mothers

Human Milk Fatty Acid Composition from Nine Countries Varies Most in DHA

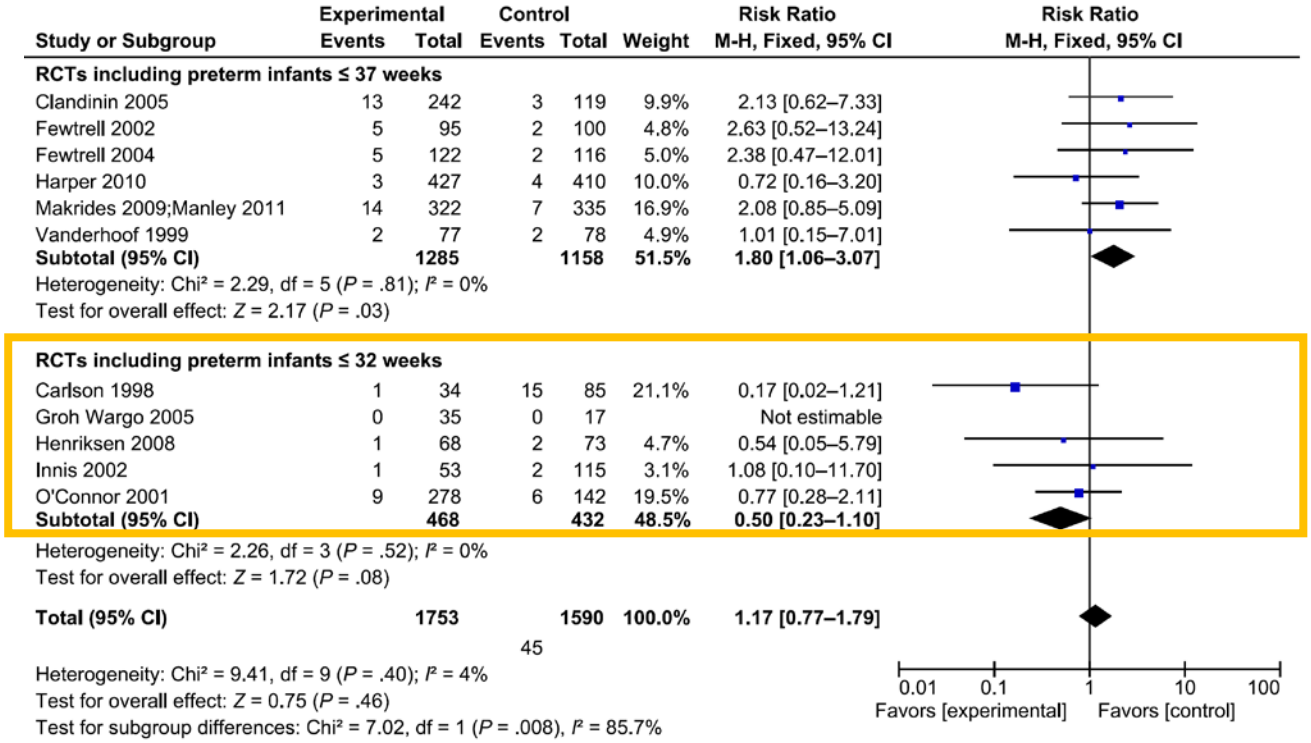
Rebecca Yuhas*, Kathryn Pramuk, and Eric L. Lien
Department of Nutrition Research, Wyeth Nutrition, 500 Arcola Road, Collegeville, Pennsylvania 19426

Lipids, Vol 41(9), 851-858 (2006)



Subjects:
Healthy, nonsmoking mothers (age 14 to 41yr), exclusively breastfeeding single-birth, full-term infants aged 1 to 12 month. Approximately 50 samples were collected from each countries.

Omega-3 LC-PUFA supplementation and NEC



Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
RCTs including preterm infants ≤ 37 weeks						
Clandinin 2005	13	242	3	119	9.9%	2.13 [0.62–7.33]
Fewtrell 2002	5	95	2	100	4.8%	2.63 [0.52–13.24]
Fewtrell 2004	5	122	2	116	5.0%	2.38 [0.47–12.01]
Harper 2010	3	427	4	410	10.0%	0.72 [0.16–3.20]
Makrides 2009;Manley 2011	14	322	7	335	16.9%	2.08 [0.85–5.09]
Vanderhoof 1999	2	77	2	78	4.9%	1.01 [0.15–7.01]
Subtotal (95% CI)		**1285**		**1158**	**51.5%**	**1.80 [1.06–3.07]**
Heterogeneity: Chi² = 2.29, df = 5 (*P* = .81); *I*² = 0%						
Test for overall effect: *Z* = 2.17 (*P* = .03)						
RCTs including preterm infants ≤ 32 weeks						
Carlson 1998	1	34	15	85	21.1%	0.17 [0.02–1.21]
Groh Wargo 2005	0	35	0	17		Not estimable
Henriksen 2008	1	68	2	73	4.7%	0.54 [0.05–5.79]
Innis 2002	1	53	2	115	3.1%	1.08 [0.10–11.70]
O'Connor 2001	9	278	6	142	19.5%	0.77 [0.28–2.11]
Subtotal (95% CI)		**468**		**432**	**48.5%**	**0.50 [0.23–1.10]**
Heterogeneity: Chi² = 2.26, df = 3 (*P* = .52); *I*² = 0%						
Test for overall effect: *Z* = 1.72 (*P* = .08)						
Total (95% CI)		**1753**		**1590**	**100.0%**	**1.17 [0.77–1.79]**
			45			
Heterogeneity: Chi² = 9.41, df = 9 (*P* = .40); *I*² = 4%						
Test for overall effect: *Z* = 0.75 (*P* = .46)						
Test for subgroup differences: Chi² = 7.02, df = 1 (*P* = .008), *I*² = 85.7%						

Omega-3 Long-Chain Polyunsaturated Fatty Acids for Extremely Preterm Infants: A Systematic Review

Peiyin Zhang, Pascal M. Lavoie, Thierry Lacaze-Masmonteil, Marc Rhainds and Isabelle Marc

Pediatrics 2014;134;120; originally published online June 9, 2014;
DOI: 10.1542/peds.2014-0459

Summary

- NEC still has a considerable impact mortality of ELBWI, even though low incidence in Japan(1.6%)
- The exact reason underlying the low incidence of NEC are poorly understood.
- Some of the traditional, experimental management practices in Japan may account for low incidence of NEC
- The difference of human milk composition (and enterobacterial flora), attributed to the unique lifestyle habits of Japanese people may contribute to the low incidence of NEC
- Owing to the insufficient evidence in the regard, further investigation is warranted

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International Neonatal Consortium

Thank you for your attention!

CRITICAL PATH INSTITUTE

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

International Neonatal Consortium

NEC Society

Jennifer Canvasser, MSW
Founder & Executive Director

Micah, the day before he developed NEC.

Micah's NEC led to bowel resection and renal failure.

Nine months later, Micah lost his battle.

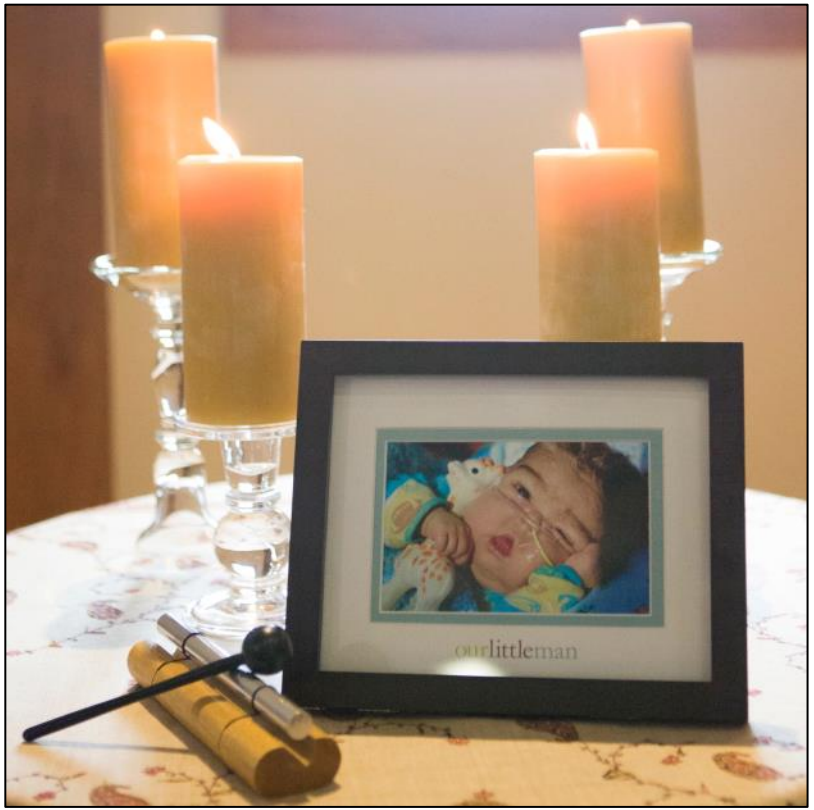
How to increase awareness, funding & prioritization of NEC?

Family-Patient Engagement

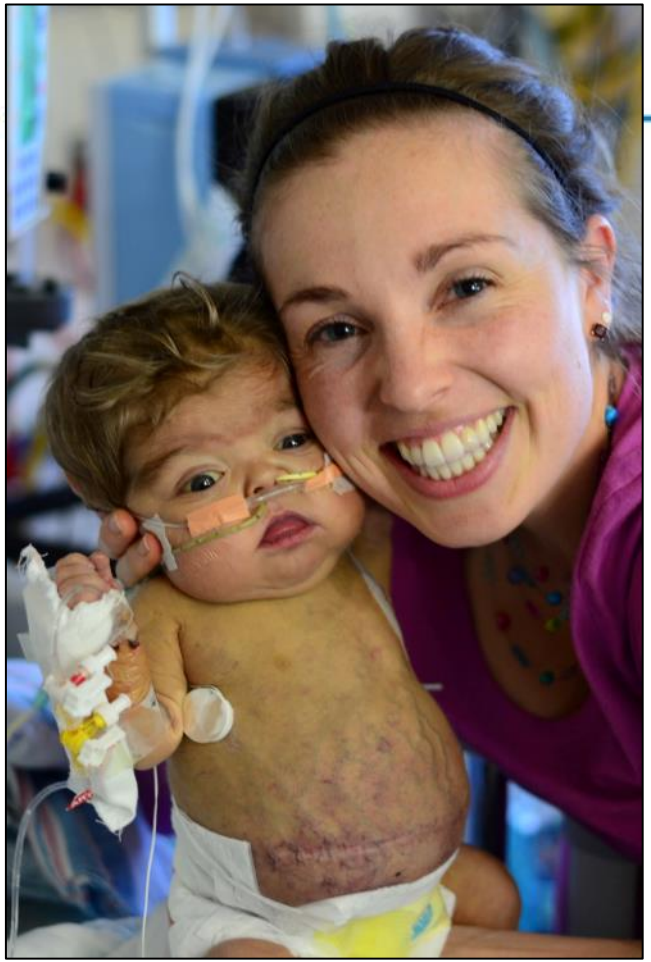
- In the NICU
- In the efforts to drive change
- In mainstream conversations

Engagement in the NICU

Engagement in efforts to drive change



Making NEC a mainstream conversation



Save the date!

NECROTIZING ENTEROCOLITIS SYMPOSIUM

A Transdisciplinary Approach to Improved NEC Outcomes

The NEC Society, in partnership with UC Davis, is proud to present the first national conference on necrotizing enterocolitis (NEC), made possible by a PCORI Engagement Award. The Symposium will bring together the leading clinicians, researchers, parent advocates and others involved in the study and advancement of knowledge in neonatal NEC, a disease that has shown little advancement in prevention or treatment options in over five decades. CME credits will be available.

April 6 & 7, 2017
On the UC Davis Campus

Contact: Jennifer Canvasser
jennifer@NECsociety.org
www.NECsociety.org

Registration details coming soon!

ATTENDEES

- Physicians
- Nurse practitioners
- Nurses
- Family advocates
- Physician Assistants
- Pharmacists
- Dietitians/Nutritionists
- Social workers
- Policymakers
- Nonprofit organizations and other stakeholders

TOPICS

- Defining NEC
- Early detection of NEC
- Innate immunity and NEC
- Human milk and NEC
- NEC prevention checklist
- NEC registry and biorepository
- Parent education and involvement
- Increasing funding for NEC research
- NEC clinical trials
- Probiotics and NEC
- Animal models of NEC
- Treatment options for NEC
- NEC and neurodevelopment
- Transfusion associated NEC

UCDAVIS CHILDREN'S HOSPITAL

NEC SOCIETY

MADE POSSIBLE BY A PCORI ENGAGEMENT AWARD

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Session VI: Necrotizing Enterocolitis

Paolo Manzoni

CRITICAL PATH INSTITUTE

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Strategies to Reduce Necrotizing Enterocolitis: Use of Lactoferrin and Probiotics

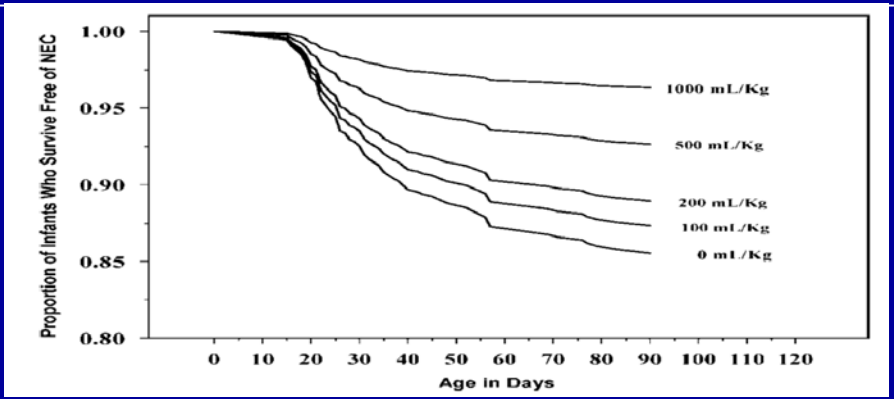
Azienda Ospedaliera Città della Salute e della Scienza di Torino

COMITATO SCIENTIFICO
FONDAZIONE CRESCERE INSIEME AL SANT'ANNA-ONLUS

Disclosure

- I have nothing to disclose related to this presentation

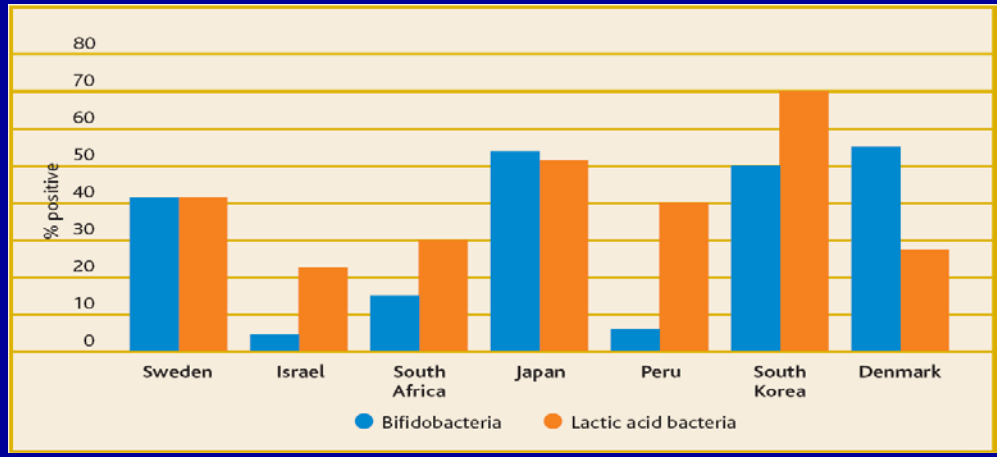
The background: Human Milk prevents NEC



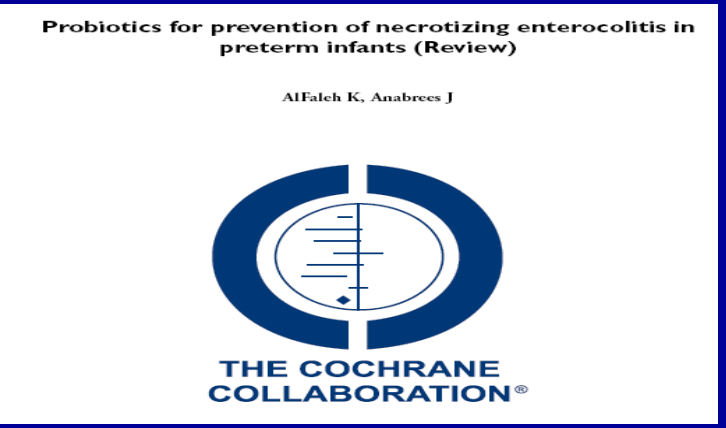
Human fresh Milk prevents NEC: the higher the intake, the higher the protection

Meinzen-Derr J, et al J Perinatol 2009

- **Human fresh milk contains probiotics, regardless of geographic areas and feeding .**
- **An infant fed with 800 ml /day of maternal milk will ingest 10^5-10^7 bacteria every day**



Probiotics and prevention of NEC



COCHRANE 2014

update of the 2011 review

- Only RCTs including < 37 wks g.a. and/or < 2500g bw.
- Twenty-four eligible RCTs
- **High variability** of enrolment criteria, baseline risk of NEC in the control groups, timing, dose, formulation of

	RR	95% CI	Nr. of studies	Nr. of infants
Prevention of severe NEC (> or = stage II)	**0.43**	**0.33-0.56**	**20**	**5529**
Prevention of overall mortality	**0.65**	**0.52-0.81**	**17**	**5112**
Prevention of nosocomial sepsis	**0.91**	**0.80-1.03**	**19**	**5338**

Analysis 1.1. Comparison 1 Probiotics versus control (all infants), Outcome 1 Severe necrotising enterocolitis (stage II-III).

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics versus control (all infants)

Outcome: 1 Severe necrotising enterocolitis (stage II-III)

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Al-Hosni 2012	2/50	2/51		1.02 [0.15, 6.96]
Bin-Nun 2005	1/72	10/73		0.10 [0.01, 0.77]
Braga 2011	0/119	4/112		0.10 [0.01, 1.92]
Costalos 2003	5/51	6/36		0.59 [0.19, 1.78]
Dani 2002	4/295	8/290		0.49 [0.15, 1.61]
Demirel 2013	6/135	7/136		0.86 [0.30, 2.50]
Fern ndez-Carrocera 2013	6/75	12/75		0.50 [0.20, 1.26]
Kitajima 1997	0/45	0/46		0.0 [0.0, 0.0]
Lin 2005	2/180	10/187		0.21 [0.05, 0.94]
Lin 2008	4/217	14/217		0.29 [0.10, 0.85]
Manzoni 2006	1/39	3/41		0.35 [0.04, 3.23]
Manzoni 2009	0/151	10/168		0.05 [0.00, 0.90]
Mihatsch 2010	2/91	4/89		0.49 [0.09, 2.60]
Mohan 2006	2/37	1/32		1.73 [0.16, 18.20]
ProPrems 2013	11/548	24/551		0.46 [0.23, 0.93]
Rojas 2012	9/372	15/378		0.61 [0.27, 1.38]
Roug'x00e9' 2009	2/45	1/49		2.18 [0.20, 23.21]
Samanta 2009	5/91	15/95		0.35 [0.13, 0.92]
Sari 2010	6/110	10/111		0.61 [0.23, 1.61]
Stratiki 2007	0/38	3/31		0.12 [0.01, 2.19]
Total (95% CI)	**2761**	**2768**		**0.43 [0.33, 0.56]**

Total events: 68 (Probiotics), 159 (Control)

0.005 0.1 1 10 200

Favours probiotics Favours control

RR = 0.43 [0.33-0.56]. NNT 30

Probiotic preparations containing either lactobacillus alone or in combination with bifidobacterium were found to be effective.

No reports of systemic infection with the probiotic supplemental organism.

Probiotics for prevention of necrotizing enterocolitis in preterm infants (Review)

Al Falch K, Anabrees J

THE COCHRANE COLLABORATION®

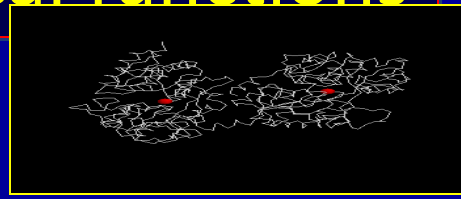
Summary of the current evidence about Probiotics for prevention of NEC and Mortality

- ✓ Probiotics (as a category) can significantly prevent / improve:
  1. NEC
  2. all-cause Mortality prior to discharge
  3. time needed to reach full feeds
- ✓ "The dramatic effect sizes, tight confidence intervals, extremely low *P* values, and overall evidence indicate that additional placebo-controlled trials are unnecessary if a suitable probiotic product is available" (*Deshpande et al , Pediatrics 2010*)
- ✓ The evidence is so striking that the last 2014 Cochrane Review states:
  1. "This updated review of available evidence strongly supports **a change in practice**"
  2. "Whenever a probiotic product is available, its administration for prevention of NEC is recommended"

Gaps in knowledge - QI Actions about Probiotics for prevention of NEC (as of today

- ✓ **Which probiotic strain(s)? Single strains, or Mixtures?**
  - → in most of the NEC studies, ***Lactobacillus spp*** and ***Bifidobacterium spp*** have been used
  - → mixtures proved effective in most cases
  - → A mixture choice (with Lactobacilli and Bifidobacteria) clearly mimics the probiotic's content of human milk
- ✓ **What dosages?**
  - → At least 3 x 10^6 CFU/day
- ✓ **When to start? which duration?**
  - → start as soon as possible to prevent pathological colonization in the gut
  - → It seems reasonable to go ahead till full feeds with human milk are tolerated
- ✓ **What are the interactions with human and formula milk?**
- ✓ **Are they fully safe ?**
  - → Generally yes. So far, only scattered, anecdotical cases of probiotic sepsis in preterms

LACTOFERRIN → Overview of its biological functions



- ✓ LF is the major whey protein in mammalian milk
- ✓ High [77%] structural homology between :
  - Bovine LF → extracted and purified by cow's milk
  - Human LF → recombinant engineering: *thalactoferrin*
- In the stomach, pepsin digests and releases a potent peptide antibiotic called lactoferricin from native LF.
- ✓ Human and Bovine LF share the same:
  - LACTOFERRICIN (N-terminal, 11-aminoacidic peptide with antimicrobial activity) *(Lupetti 2004)*
  - Orally administered LF remains active even after stomach passage
  - High intestinal uptake and gut actions *(Lonnerdal 2011)*

Concentrations of **LACTOFERRIN** decrease in mature human milk vs. colostrum

This decrease typically occurs in all mammalians

Milk	*Concentrations of lactoferrin*
Woman	2 (mature milk) – 6 (colostrum) mg/ml
Cow	0,2-0,5 mg/ml
Rat	<50 mcg/ml
Rabbit	<50 mcg/ml
Dog	<50 mcg/ml
Goat	0,2 mg/ml
Pig	0,2 mg/ml

Why LACTOFERRIN might also prevent NEC? *the rationale*

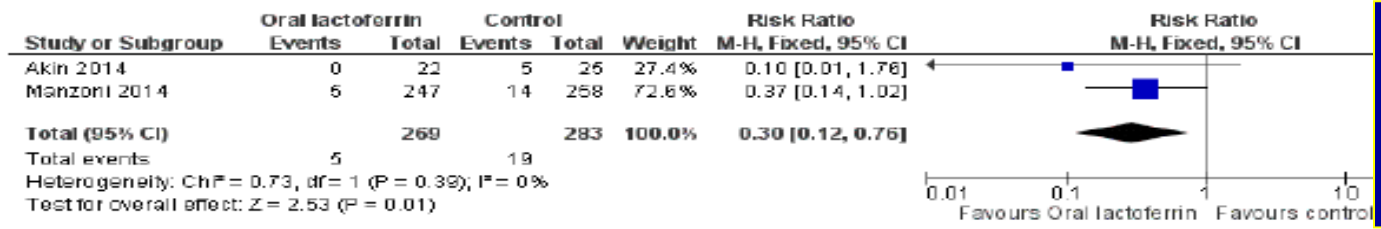
- LF prevents Late-Onset Sepsis in VLBWs *(Manzoni et al, JAMA 2009)*
- Lactoferrin and lysozyme in breast milk are synergistic, and kill bacteria.
- The antimicrobial characteristics of LF may facilitate a healthy intestinal microbiome → LF is bifidogenic, promoting Bifidobacteria and Lactobacilli microflora in the gut → these probiotics prevent NEC *(Alfaleh et al, Cochrane 2014; Deshpande et al, Lancet 2007)*
- LF has trophic and pro-proliferative activity on the nascent enterocytes, regulating gut permeability *(Buccigrossi et al, Ped Res 2007)*
- LF enhances anoikis (apoptosis) of infected enterocytes in the gut *(Sherman et al, Med Hypoth 2005)*
- The immuno-modulatory activates of LF activate dendritic cells (DC) and DCs then induce a Th1 helper cell population that resists neonatal infection.
- Lactoferrin has anti-inflammatory actions that may mitigate the proinflammatory state that is present in the gut before the onset of necrotizing enterocolitis (NEC).
  - LF attenuates oxidation by suppressing free radical activity, and decreasing levels of oxidative products *(Raghuveer et al, Ped Res 2002)*

THE COCHRANE COLLABORATION®

Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants (Review)

Pammi M, Abrams SA

Figure 2. Forest plot of comparison: 1 Lactoferrin alone versus placebo, outcome: 1.2 NEC ≥ stage II.



Effect on NEC

- 2 RCTs retrieved (all with BLF)
- ~~552 VLBW~~ infants analysed. Moderate heterogeneity.
- R.R. 0.30
- NNT 20
- Current available evidence graded as “low-to-moderate quality”

THANK YOU FOR YOUR ATTENTION !!

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Pre congress Courses: September 21st – 22nd 2016

www.iccn2016.eu

- Backup slides

LACTOFERRIN trial for prevention of NEC

Manzoni P, Meyer M, Stolfi I, et al . Early Hum Development 2014 .

- After the end of the JAMA study, 7 of 11 Centres [6 in Italy , 1 in New Zealand] agreed on continuing recruitment for an 18-month additional period , with a target enrolment of 800 patients, to achieve significance for the outcome “NEC”.
- Design, Study Protocol, Enrollment criteria and timing, Randomization 1:1:1, LF and LGG dosages were unchanged

	LF N=251	*PLACEBO N=259*	*R.R.*	*95% C.I.*	*p-value*
Severe NEC (>2nd stage)	**2.0%**	**5.4%**	0.37	0.14-1.00	**0.05**
Overall Mortality	2.0%	6.9%	0.28	0.11-0.76	**0.007**
NEC and/or Death	4.0%	10.1%	0.39	0.19-0.80	**0.008**

Absolute risk reduction = 3.41 percent. **NNT (Number Needed to Treat) = 30**

	LF + LGG N=242	*PLACEBO N=259*	*R.R.*	*95% C.I.*	*p-value*
Severe NEC (>2nd stage)	**0%**	**5.4%**	0.00	---	**<0.001**
Overall Mortality	3.8 %	6.9%	0.53	0.24-1.16	**0.11**
NEC &/or Death	3.8%	10.1%	0.37	0.18-0.77	**0.006**

Absolute risk reduction = 5.41 percent. **NNT (Number Needed to Treat) = 19**

Gaps in the current knowledge

- **Dosages** → likely higher than 100 mg /kg , but how higher? Fixed or pro-kg dosage?
- **Dosing/Schedule** → once a day? Or many times a day (mimicking the human milk?)
- **Duration** → in preterms, how long? And in infants, how long and starting when?
- **Interactions with human milk** → better effects when added to HM or to Formula ?
- **Interactions with probiotics** → better effects when added to BB or LB strains?
- Short-term and long-term **safety ?**
- Any effect on **other outcomes of prematurity** [e.g. ROP, BPD] ?
- **Generalizability** of the bovine LF findings
- Generalizability also to Human Recombinant Lactoferrin ***(Thalactoferrin)***

Proposed guideline for the use of probiotics in preterm neonates based on the evidence available

- → A combination of Lactobacillus and Bifidobacterium is preferred.
- → The dose should be at least 3×10^9 organisms per day
- → Starting when the neonate is ready for enteral feeds
- → Continued until 35 weeks' corrected age or discharge

[Deshpande GC, Rao SC, Keil AD, Patole SK: Evidence-based guidelines for use of probiotics in preterm neonates. BMC Med 2011;9:92.]

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Session VI: Necrotizing Enterocolitis

Josef Neu

CRITICAL PATH INSTITUTE

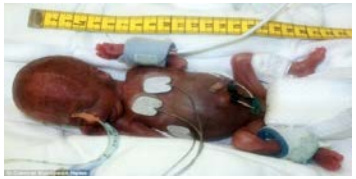
EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

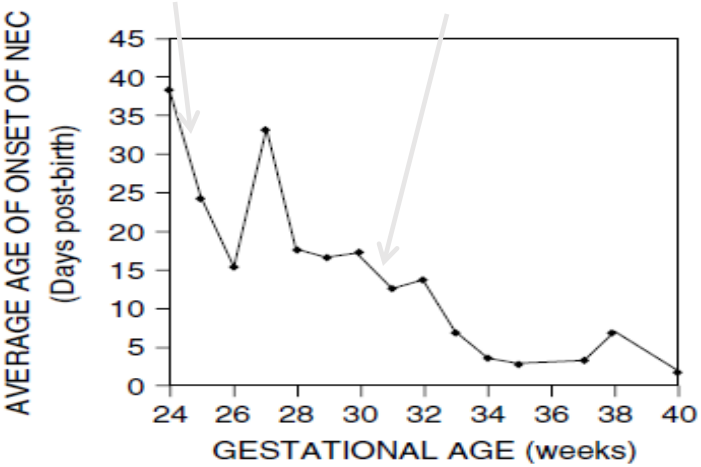
Being led astray: 50 years---not much progress

- **Lumping of several diseases called “NEC” into the same data set. Would we do this for diabetes or cancer?**
  - **Spontaneous intestinal perforations**
  - **Ischemic bowel associated with heart disease, polycythemia**
  - **Food protein intolerance**
  - **“classic” form seen most commonly in preterms**
- **Animal models that do not represent the disease that we see in most babies who develop NEC.**

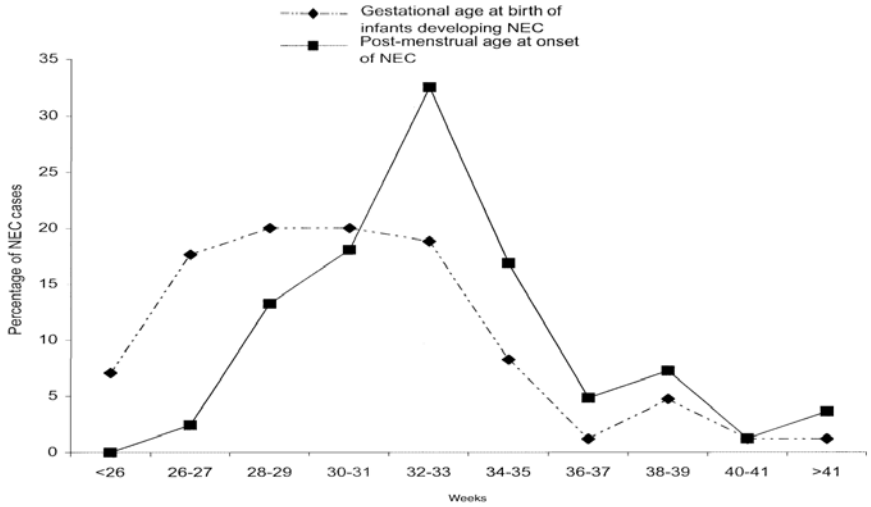
Is there a Clear Definition of NEC? Bells is Broken

- Stage 1-Too non-specific and the term should not be used.
- Stage 2-Radiographic signs can be “fuzzy”.
- Stage 3- Free air on radiograph could signify intestinal necrosis or Spontaneous Intestinal Perforation (SIP)



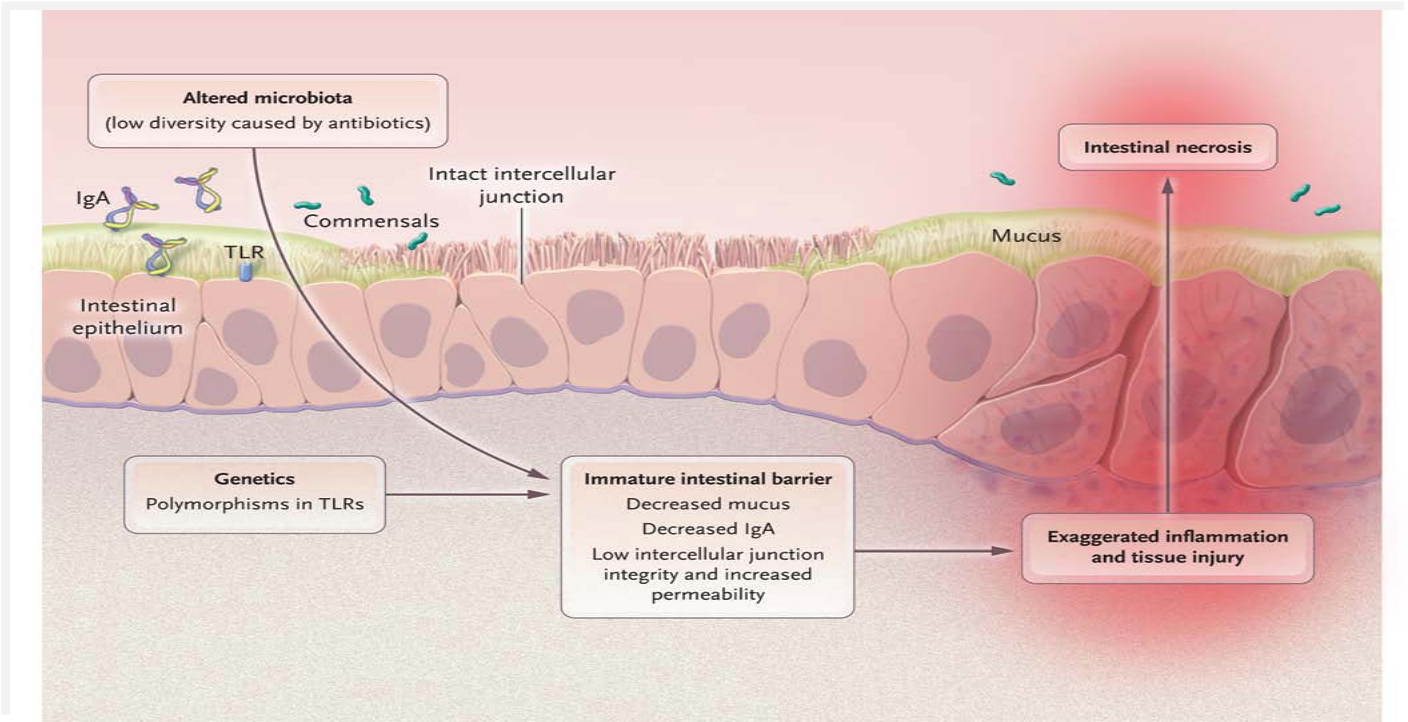


Neu, J. Acta Paediatrica, 2005 94 (Supple 449): 100-105

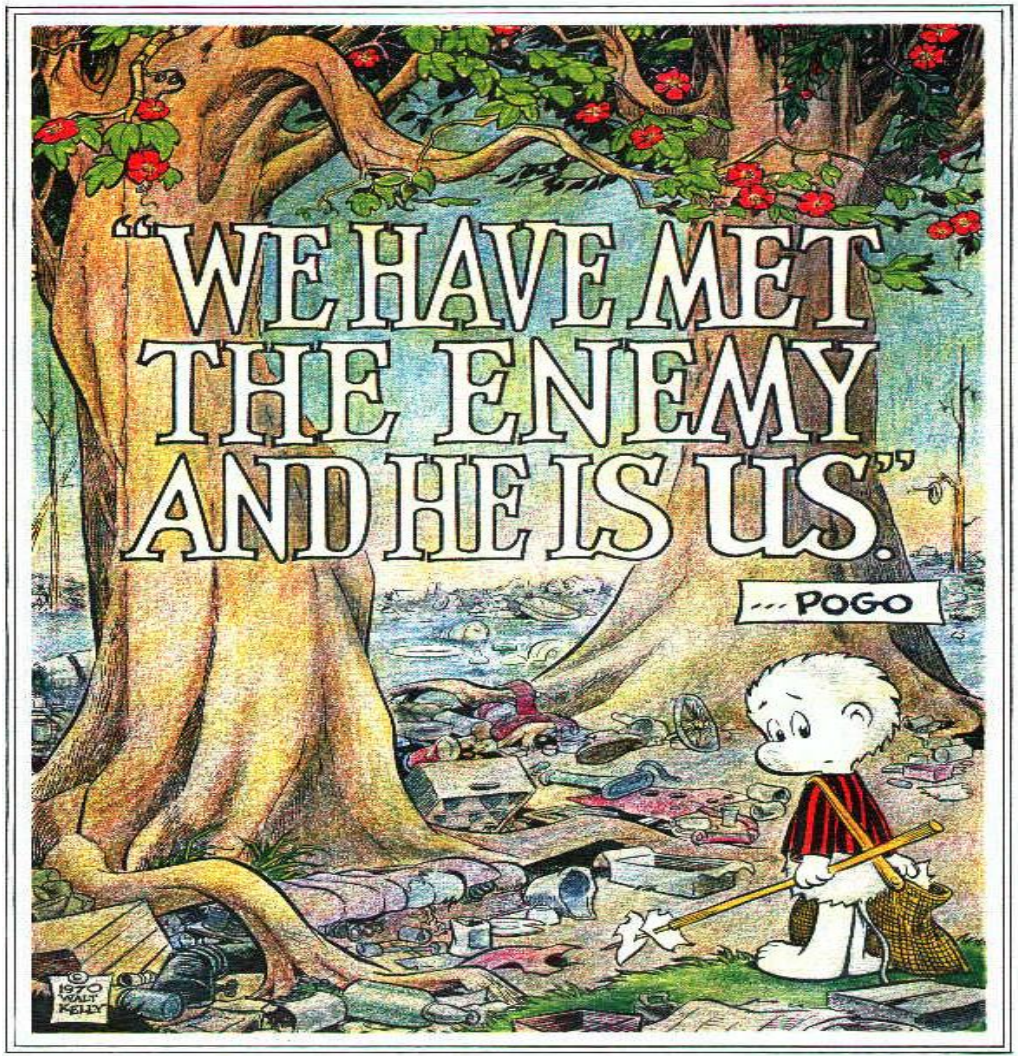


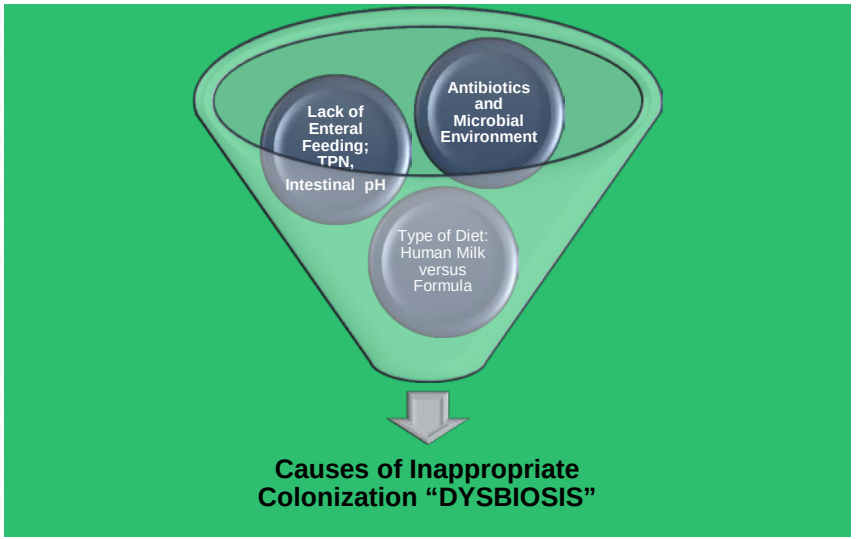
A.R. Llanos, *et al.*, *Paediatr Perinat Epidemiol* ,2002 **16 (4):** 342–349.

Pathophysiologic Overview at the Barrier



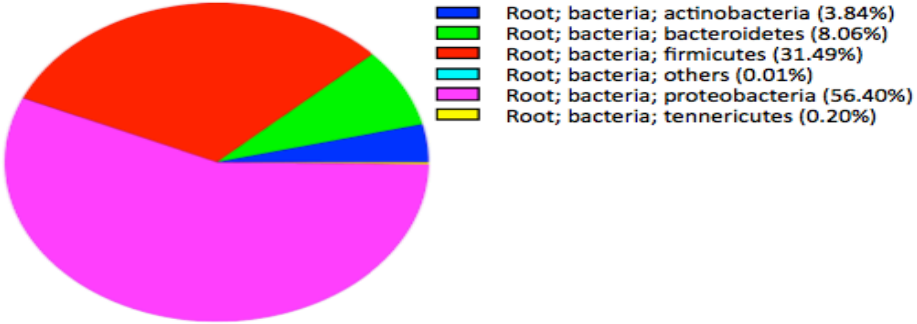
Neu, J., Walker, WA. NEJM. 2011


"WE HAVE MET THE ENEMY AND HE IS US."
—POGO
© 1970 WALT KELLY

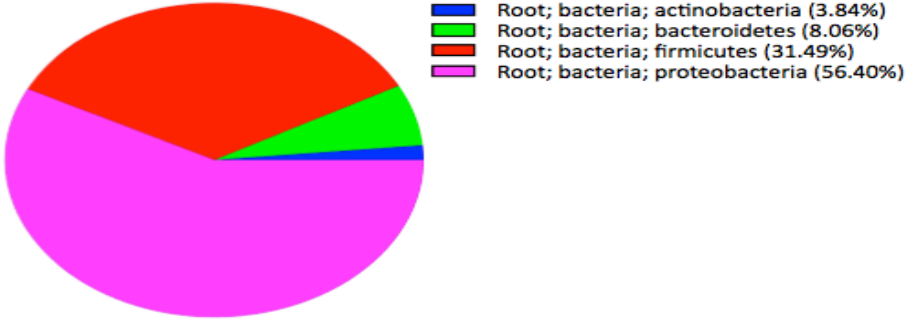

Lack of Enteral Feeding; TPN, Intestinal pH
Antibiotics and Microbial Environment
Type of Diet: Human Milk versus Formula
Causes of Inappropriate Colonization "DYSBIOSIS"

FECAL MICROBIOTA: NEC
Mai V, Young C. PLOS One, May 2011

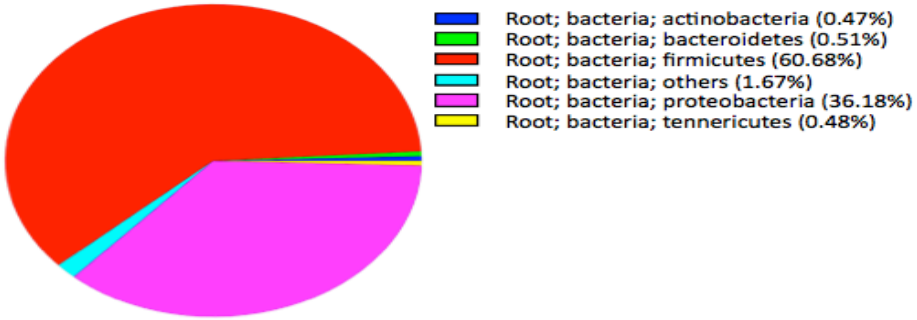
Controls, one week before diagnosis



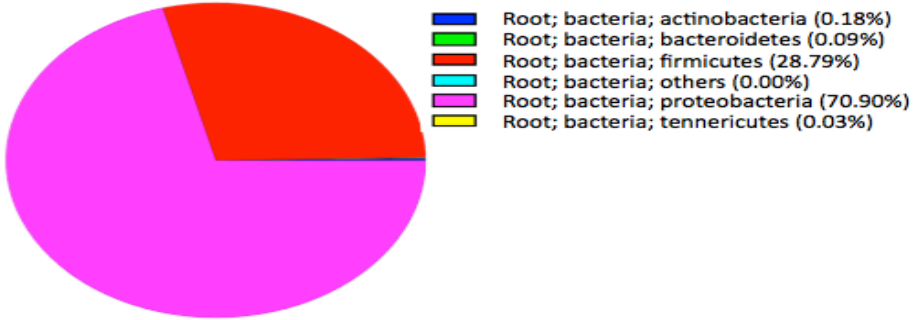
Controls, <72h of diagnosis



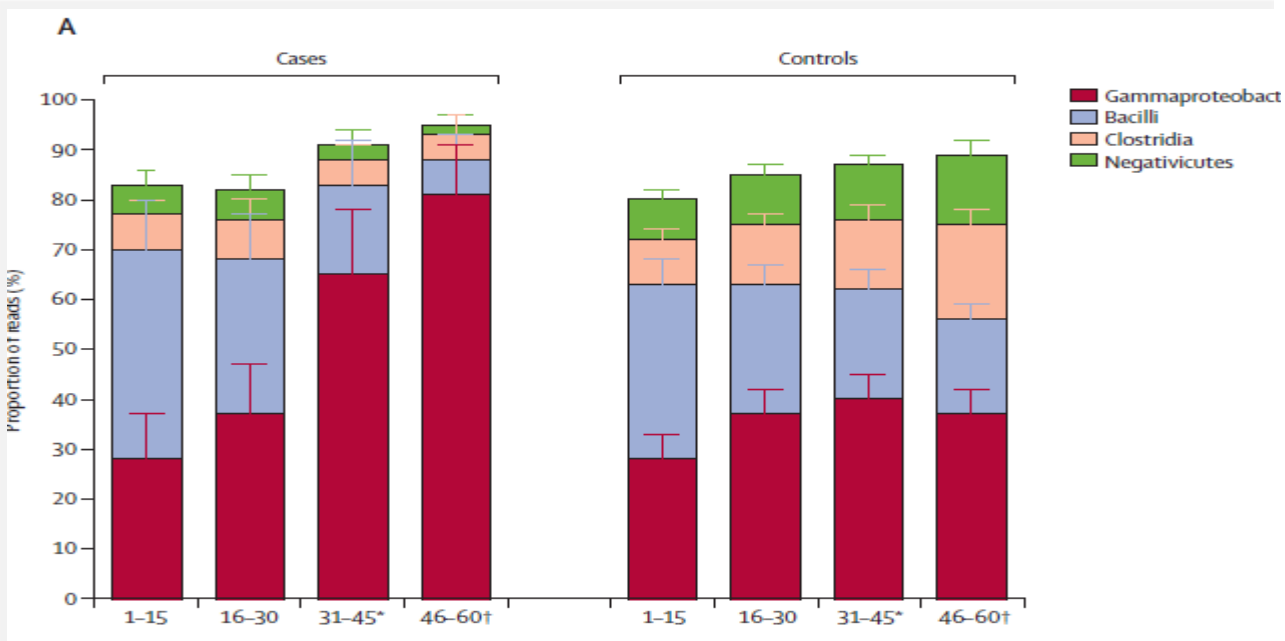
Cases, one week before diagnosis



Cases, <72h of diagnosis



Abundance of Proteobacteria



Warner, B. et al. Lancet March 8,2016

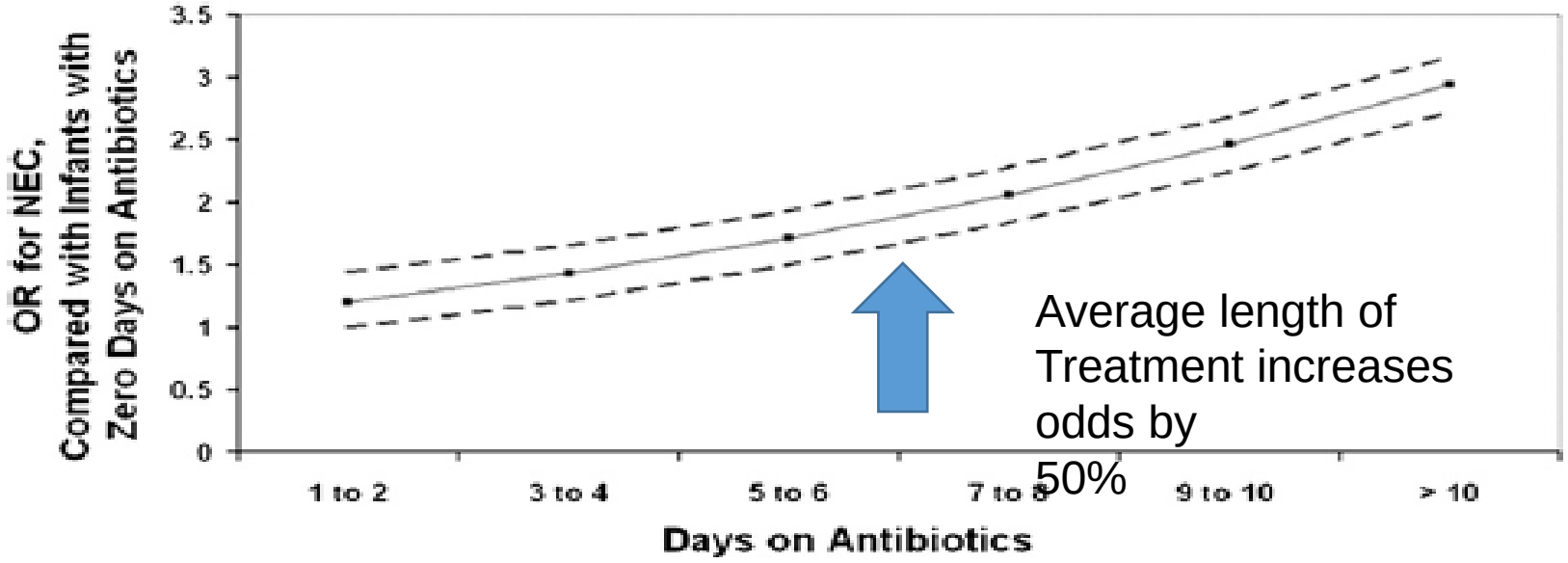
Most Commonly used Drugs in the NICU: Majority of VLBW infants are Exposed to Antibiotics

Top 10 Medications Prescribed in the NICU

Ampicillin	186 799
Gentamicin	171 388
Ferrous sulfate	90 152
Vitamin (multivitamin)	64 329
Cefotaxime	55 455
Caffeine citrate	48 814
Furosemide	47 278
Vancomycin	44 218
Beractant (Survanta)	36 410
Metoclopramide	27 541

Odds Ratio of NEC with Increased Days on Antibiotics

Alexander, V.N. J. Pediatrics, Sept. 2011



Gastric Acid Inhibition

Ranitidine is Associated With Infections, Necrotizing Enterocolitis, and Fatal Outcome in Newborns

WHAT'S KNOWN ON THIS SUBJECT: Although still off-label for newborns, the use of inhibitors of gastric acid secretion continues to increase. Acid-suppressive drugs could facilitate the onset of infections in adults and children. Evidence for efficacy is weak in newborns, particularly if preterm.

WHAT THIS STUDY ADDS: This is the first prospective study demonstrating an association between the use of ranitidine and infections, necrotizing enterocolitis, and fatal outcome in very low birth weight newborns. Caution is advocated in using ranitidine in newborns.

AUTHORS: Gianluca Terrin, MD, PhD,[a] Annalisa Passariello, MD, PhD,[b,c] Mario De Curtis, MD, PhD,[d] Francesco Manguso, MD, PhD,[e] Gennaro Salvia, MD,[f] Laura Lega, MD,[g] Francesco Messina, MD,[h] Roberto Paludetto, MD,[b] and Roberto Berni Canani, MD, PhD[b,i]

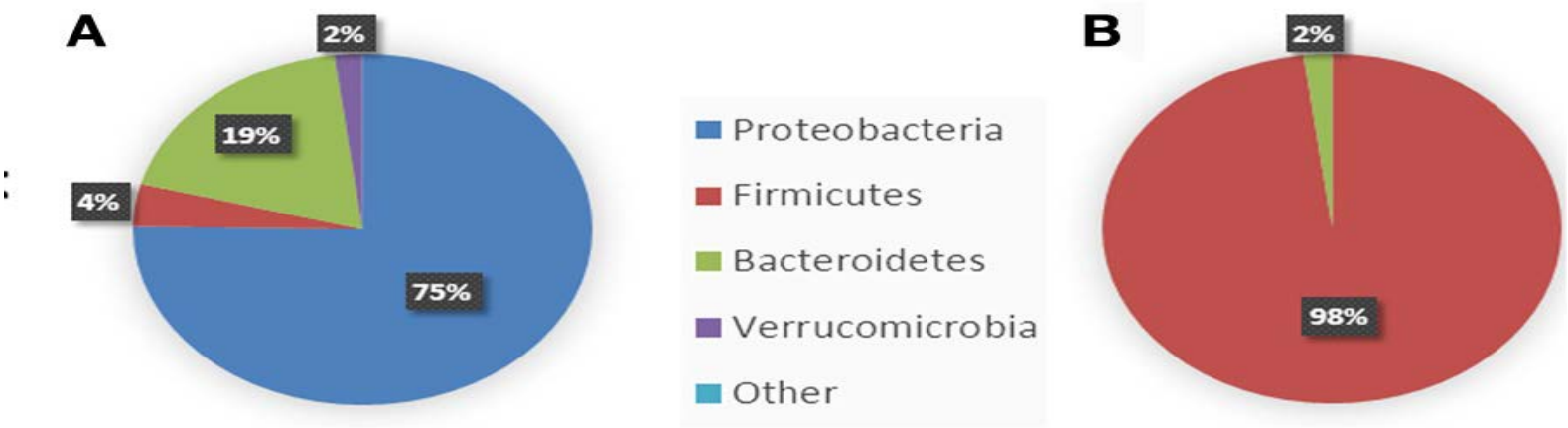
[a]Department of Women's Health and Territorial Medicine, University La Sapienza, Rome, Italy; [b]Department of Pediatrics, University Federico II, Naples, Italy; [c]Neonatology Unit, Monaldi Hospital, Naples, Italy; [d]Department of Pediatrics, University La Sapienza, Rome, Italy; [e]Gastroenterology Unit, Cardarelli Hospital, Naples, Italy; [f]Neonatology Unit, Fatebenefratelli Hospital, Naples, Italy; [g]Neonatology Unit, Meyer Pediatric Hospital, Florence, Italy; [h]Neonatology Unit, V. Betania Evangelic Hospital, Naples, Italy; and [i]European Laboratory for the Investigation of Food Induced Diseases, Naples, Italy

Pediatrics, 2012, 129. e-40-45

Effect of Total Parenteral Nutrition (TPN) in Mice

Unfed + TPN

Fed



Demehri, FR., et al. Cellular and Infection Microbiology, Dec. 2013

Morbidities: Early vs. Late Feeding

Table 3. Univariate Analysis of Neonatal Morbidities by Group.

Outcomes (%)	Early (n = 79)	Late (n = 51)
NEC	6.3	10.0
ROP	16.7	52.1**
CLD	21.5	69.4**
PVL	0.0	6.0*
IVH	24.1	24.0
Comorbidities	8.0	25.0**

* Early vs. Late $p<0.05$;

** Early vs. Late $p<0.0001$

Necrotizing Enterocolitis (NEC); Retinopathy of Prematurity (ROP); Chronic Lung Disease (CLD); Periventricular Leukomalacia (PVL); Intraventricular Hemorrhage (IVH); Cormorbidities = The presence of 2 or more neonatal outcomes.

Konnikova, et al. PLOS One 2015

Microbial Dose from Human Milk

- Assume intake of 800 ml/day
- Assume 10^{5-6} bacterial cells/ml
- This will provide 10 $^{7-8}$ bacterial cells (personalized?) daily, close to the dose in most probiotic studies.

Recommendations

- Define and Delineate “NEC”
- Proximal components of pathophysiology (environment and intestinal immaturities)are important. Once the cascade has started, it is difficult to stop.
- Focus on prevention—”primum non nocere”.
  - Feed (fresh human milk), limit antibiotics and other drugs known to alter microbes.
- Proximal components of pathophysiology and early recognition of risk are important.

Voting Slide – NEC

Considering both impact and feasibility, which of the following projects is your **first** choice?

1. Identification and utilization of biomarkers for the early diagnosis of NEC; are there candidates available and what additional investigation is needed?
2. Identification and utilization of biomarkers for the response to treatment of NEC; or possible prognostic indicators.
3. Detailed review and meta-analysis of current methods to prevent and treat NEC in high-risk neonates leading to prioritization and study of leading candidates.
4. Epidemiologic study of NEC across the globe.
5. Determination and clarification of NEC diagnosis: are there different categories that should be considered?
6. “Walk-in Option A” (offered up by audience)
7. None of the above

Voting Slide – NEC

Considering both impact and feasibility, which of the following projects is your **second** choice?

1. Identification and utilization of biomarkers for the early diagnosis of NEC; are there candidates available and what additional investigation is needed?
2. Identification and utilization of biomarkers for the response to treatment of NEC; or possible prognostic indicators.
3. Detailed review and meta-analysis of current methods to prevent and treat NEC in high-risk neonates leading to prioritization and study of leading candidates.
4. Epidemiologic study of NEC across the globe.
5. Determination and clarification of NEC diagnosis: are there different categories that should be considered?
6. “Walk-in Option A” (offered up by audience)
7. None of the above

Concluding Remarks

- Mark Turner, INC Co-director

Evening Workgroup Sessions

- Seizures, BPD, Data
- 4-8 pm
- Marriott West India Quay
  - Tamarind - BPD
  - Barbados – Seizures
  - Trinidad - Data

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Thank you!

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